EARLY LIFE FACTORS ASSOCIATED WITH STUNTING AND OVERWEIGHT AT 12 MONTHS IN INFANTS ENROLLED IN THE MOTHER AND CHILD IN THE ENVIRONMENT (MACE) STUDY, DURBAN

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ABSTRACT

Introduction: Developing countries are experiencing a double burden of malnutrition, with persistently high levels of stunting and micronutrient deficiencies combined with an increasing prevalence of childhood overweight and obesity. South Africa is no exception with both stunting and overweight prevalence above 20% in children under five years of age. The Global Targets on Nutrition call for a 40% reduction in stunting and no increase in childhood overweight by 2025. In order to develop effective interventions to achieve these targets, an understanding of the determinants of childhood stunting and overweight is required. Longitudinal birth cohort studies have provided evidence that a variety of maternal and infant factors are implicated in the development of childhood overweight and obesity. These studies have been conducted predominantly in developed countries with very little evidence coming from a developing country setting where overweight and stunting coexist, sometimes in the same child.

The ongoing MACE longitudinal birth cohort study, conducted in the city of Durban, was designed to determine the effect of pollution exposure on childhood respiratory development. Longitudinal anthropometric and dietary intake data of infants as well as maternal and household information has been collected. This data, collected between 2013 and 2017, were used in the current MSc Dietetics study to investigate factors associated with overweight and stunting at 12 months of age in participating infants.

Objectives:

1. To describe the prevalence of stunting and overweight at 12 months of age in infants enrolled in the MACE study.
2. To describe the maternal and household characteristics of the study infants and to determine their association with the growth outcomes of stunting and overweight at 12 months.
3. To describe birth anthropometrics, and patterns of growth from 0-6, and 6-12 months, and to determine their associations with growth outcomes at 12 months.
4. To describe infant feeding practices at 6 and 12 months and to determine their association with growth outcomes at 12 months.

Methods: Secondary analysis was performed using anthropometric, infant feeding, and maternal and household information collected by MACE study staff from 290 infants at 12 months of age. An additional infant feeding questionnaire, adapted from the World Health Organization (WHO) Infant and Young Child Feeding (IYCF) Indicators was administered to a subset of 94 mothers attending the 12 month follow up visit. Anthropometric data were

entered into the WHO Anthro programme and used to determine prevalence of stunting and overweight in the study infants at 12 months and to describe growth patterns in the first year of life. Anthropometric information of 144 study infants who had reached the age of 24 months by the end of the data collection process was also included. Statistical techniques were used to determine the association of maternal and household factors, infant growth patterns, and feeding practices, with the outcome variables of stunting and overweight at 12 months of age.

Results: Prevalence of stunting at 12 months was 5.9% while there was a combined overweight and obesity prevalence of 21.7%. By 24 months both stunting and overweight had increased to 10.4% and 26.6% respectively. The households were predominantly low income with half reporting an annual income of R2000 or less but virtually all had access to tap water and electricity. Maternal HIV prevalence was 35%. Only type of housing was associated with growth outcomes; infants living in informal housing were significantly more likely to be severely stunted or overweight than those living in formal, detached houses ($p=0.048$). By 6 months of age over half of the infants were receiving infant formula, with HIV positive mothers significantly more likely to formula feed than HIV negative mothers ($p<0.0005$). Complementary feeding was started before 6 months by 50% of mothers. Assessment of complementary feeding practices at 12 months using the IYCF Indicators showed a lack of dietary diversity and only 30% of infants achieved the Minimum Adequate Diet (MAD) Indicator. Regular intake of inappropriate foods was common. There was however no association of feeding practices with growth outcomes. There was a significant association of growth patterns with growth outcomes at both 12 and 24 months. Infants who were stunted at 12 and 24 months had lower birth weights and lengths and slower linear growth between 6 and 12 months. Infants with higher birthweight and greater weight gains, especially in the 6-12 month period, were more likely to be overweight at both 12 and 24 months. BMI at 12 months was strongly predictive of BMI at 24 months.

Conclusion: In this urban longitudinal birth cohort, stunting prevalence was fairly low while overweight prevalence was high. Low socioeconomic status (SES) was associated with both severe stunting and overweight. The link between birth weight and length and stunting and birth weight and overweight emphasises the need for optimal nutrition and care during pregnancy to prevent both outcomes. Poor infant feeding practices were common, but unexpectedly there was no association with growth outcomes. However, the fact that growth during the 6-12 month period is most strongly associated with later growth outcomes indicates that further investigation of complementary feeding practices, collecting more detailed

information from a larger sample, is warranted. Follow up of the study infants at 4-5 years to determine if early growth patterns remain significant in later childhood is recommended.

PREFACE

This dissertation was written between January 2015 and October 2018, under the supervision of Dr Nicola Wiles, Professor Rajen Naidoo and Professor Frederick Veldman using data collected from mothers and their infants enrolled in the Mother and Child in the Environment (MACE) study between 2013 and 2017.

Signed: _____ Date:

Penelope Anne Jarvie (candidate)

As supervisors of the candidate we agree to the submission of the dissertation.

Signed: _____ Date:

Dr Nicola Wiles (Supervisor)

Signed: _____ Date:

Professor Rajen Naidoo (Supervisor)

Signed: _____ Date:

Professor Frederick Veldman (Supervisor)

DECLARATION OF ORIGINALITY

I, Penelope Anne Jarvie, hereby declare that:

i. The research reported in this dissertation, except where otherwise indicated, is my original research.

ii. This dissertation has not been submitted for any degree or examination at any other university.

iii. This dissertation does not contain another person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from those persons.

iv. This dissertation does not contain another author's writing unless specifically acknowledged as being sourced from other authors. Where other written sources have been quoted, then:

a. Their words have been rewritten but the general information attributed to them has been referenced.

b. When their exact words have been used, their writing has been placed inside quotation marks, and referenced.

c. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References section.

Signed: _____ Dated:

Penelope Anne Jarvie (candidate)

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1.1 Importance of the study

Low and middle-income countries (LMICs) are increasingly facing a double burden of malnutrition (Black *et al*., 2013). In 2014 the number of stunted children globally was 159 million while 41 million children under five were overweight or obese (UNICEF *et al.*, 2015). While stunting rates are decreasing, childhood obesity is increasing, with the prevalence in Africa doubling in the last 20 years (WHO, 2013). In recognition of this problem the World Health Organisation (WHO), in its 2025 Global Targets for maternal, infant and young child nutrition, called for a 40% reduction in the number of children under 5 who are stunted and no increase in the proportion of children who are overweight (WHO, 2012).

South African children have high levels of stunting and overweight/obesity while micronutrient deficiencies, particularly Vitamin A deficiency, remain a problem (Shisana *et al.*, 2013). The South African National Health and Nutrition Examination Survey (SANHANES) from 2012 revealed a national prevalence of both stunting and overweight at above 20% in children under five years of age (Shisana *et al*., 2013), with stunting rates showing little or no signs of improvement from earlier surveys (Said-Mohamed *et al*., 2015). Overweight prevalence appears to be increasing (Kruger *et al*., 2012; Labadarios *et al*., 2005) although determining trends in both stunting and overweight is made difficult because different growth standards and age ranges were used across the surveys (Said-Mohamed *et al*., 2015; Bosman *et al*., 2011).

Both stunting and overweight have short and long term consequences for the individual. Further, at a national level they have an impact on the economy through decreased productivity and increased health care costs (Black *et al*., 2013). The short term consequences of stunting include increased morbidity and mortality, with a severely stunted child having four times the risk of death compared to a non-stunted child (Black *et al.*, 2008). For those children that survive, the consequences can be far reaching and can leave them trapped in a cycle of poverty and malnutrition, that is often transferred into the next generation (Dewey & Begum, 2011).

Childhood overweight can delay motor development in infancy which can lead to reduced physical activity thereby creating a vicious cycle (Slining *et al.*, 2010). In later childhood

overweight can lead to lack of confidence and poor self-esteem which can persist into adulthood (Rossouw *et al.*, 2012) The main concern however, is its association with adult obesity and NCD risk which is especially a concern in LMICs with limited health care budgets (WHO, 2016a; Black *et al*., 2013). Childhood stunting and overweight are linked and they can coexist in the same community, household and even in the same child. Poor growth in the first few years of life may also lead to a short but overweight adolescent at risk of chronic diseases in adulthood (Black *et al.*, 2013; WHO, 2013).

Identifying the determinants of stunting and overweight is vital for the development of effective prevention strategies. The importance of maternal nutrition and nutrition in the first two years of life, the period known as the first 1000 days, for the prevention of stunting, and other forms of undernutrition, was highlighted in the Lancet Special Series on Maternal and Child Undernutrition in 2008 (Black *et al.*, 2008). Five years later, the 2013 Lancet Series on Maternal and Child Nutrition reemphasised the importance of the first 1000 days, extending it into the female adolescent period. Furthermore, the 2013 Series acknowledged the growing problem of overweight in LMICs and the importance of good nutrition during this crucial time for preventing both stunting and overweight (Black *et al.*, 2013). The 2013 Series also emphasised the need to include nutrition sensitive- as well as nutrition specific interventions[1] in order to address the underlying social determinants of malnutrition (Black *et al*., 2013).

Complex social determinants are a feature of both forms of malnutrition. Poor socioeconomic conditions, low levels of maternal education and repeated infections, particularly diarrhoea, are closely linked to stunting and explain why nutrition specific interventions alone have had disappointing results (de Onis *et al.*, 2013). Childhood overweight, while associated with low socioeconomic status (SES) in high income countries (HICs) (Olds *et al*., 2011), was

[1] "Nutrition specific interventions- address the immediate determinants of fetal and child nutrition and development- adequate food and nutrient intake, feeding, caregiving and parenting practises, and low burden of infectious diseases eg micronutrient supplementation, breastfeeding promotion.
Nutrition sensitive interventions-address the underlying determinants of fetal and child nutrition and development—food security; adequate caregiving resources at the maternal, household and community levels; and access to health services and a safe and hygienic environment—and incorporate specific nutrition goals and actions" (Ruel&Alderman, 2013)

previously linked to higher SES in LMICs (Black *et al*., 2013) but this appears to be changing. The most recent South African DHS of 2016 illustrates this with the highest prevalence of both stunting and overweight in the lowest quintile of SES (DoH *et al*., 2017).

Birthweight and rate of growth in infancy and early childhood have also been implicated in poor growth outcomes. Low maternal BMI and short stature are associated with restricted foetal growth and low birthweight (LBW) in their offspring which, together with poor growth in the first 2 years of life, are major determinants of stunting (Black *et al.*, 2013). On the other hand, maternal pre-pregnancy BMI >30kg/m^2 is associated with increased risk of obesity in the child (Black *et al.*, 2013). The Avon Longitudinal Birth study, conducted in the United Kingdom, linked childhood obesity to eight factors including parental obesity, high birthweight and rapid weight gain in the first year of life (Reilly *et al.*, 2005). The importance of rapid weight gain in the infancy period as a predictor of later obesity was confirmed in a number of subsequent studies (Druet *et al*., 2011; Taveras *et al*., 2009; Ong & Loos, 2006), including a 2015 South African study that linked it to a lack of breastfeeding (Ramokolo *et al*., 2015).

Poor infant and young child feeding practices, which include lack of exclusive breastfeeding and poor complementary diets, together with a food system that fails to deliver sufficient amounts of nutritious foods, can lead to both stunting and overweight (WHO, 2013). Many LMICs have experienced a rapid nutrition transition; from a traditional diet based on unrefined grains and legumes to one that is comprised largely of cheap, highly processed, energy dense but nutrient poor foods. Healthier, nutrient dense foods such as vegetables, fruit, lean meats and fish are expensive and often not available in rural areas (Igumbor *et al.*, 2012; Popkin *et al*., 2011). This contributes to poor complementary diets characterised by low dietary diversity and high intakes of inappropriate snack foods such as “crisps” or fizzy drinks (Faber *et al*., 2016). Although a number of studies have investigated the association of breastfeeding with stunting and overweight, the role of poor complementary feeding practices has been less well explored.

Longitudinal birth cohort studies are important tools to investigate early life risk factors for later disease, including later childhood stunting and overweight (Lawlor *et al*., 2009). The high cost of conducting these studies, together with logistical issues, means that there have been very few LMIC birth cohort studies conducted (Batty *et al*., 2007). There is therefore a dearth

of information on the determinants of stunting and overweight in countries where both forms of malnutrition are present. Ongoing at the time of this MSc study, the Mother and Child in the Environment (MACE) longitudinal birth cohort study, conducted in Durban, KwaZulu-Natal province, provided a valuable opportunity to explore the association of a range of potential risk factors with stunting and overweight. Although the MACE study was designed to determine the effect of ambient and environmental pollution exposure on childhood respiratory development, anthropometric data at a number of time points, together with information on sociodemographic factors and infant feeding practices was collected. This data, with additional complementary feeding information collected by the researcher, were used to investigate early life determinants of overweight and stunting in the study infants.

The longitudinal data provided by a birth cohort study allows for the investigation of a number of research questions:

- What is the prevalence of stunting and overweight in this cohort of urban children at 12 months of age?
- Do childhood stunting and overweight share common sociodemographic risk factors?
- Given the high prevalence of antenatal HIV infection in KZN, is there an association between HIV exposure and growth outcomes at 12 months?
- Are there specific times in early infancy when linear growth faltering or rapid weight gain are particularly significant for later stunting and overweight?
- Are poor infant feeding practices such as lack of breastfeeding, early introduction of solid foods, lack of dietary diversity and consumption of inappropriate snack foods associated with both stunting and overweight?

It was anticipated that this study would provide important information regarding the anthropometric status of infants living in an urban/peri urban community. Furthermore, the study would provide information on patterns of growth in the first year allowing for the identification of critical time periods most predictive of later stunting or overweight. This information can inform growth monitoring practices in the wider community. The results of this study will help to describe infant feeding practices in this population and identify those practices associated with poor growth outcomes. It is anticipated that the findings of this study may assist in the development of effective strategies to address the double burden of malnutrition.

1.2 Statement of the problem

The purpose of this study was to determine the prevalence of stunting and overweight in the MACE study infants at 12 months of age. Furthermore, it aimed to make use of the longitudinal data provided by a birth cohort study to explore associations between early life factors and the growth outcomes at 12 months. Although not in the original objectives, due to the longitudinal nature of the study, 24 month data became available for 144 infants and was included in the study.

1.3 Research objectives

This study set out to achieve the following objectives using infants enrolled in the MACE study:

1.3.1 To describe the prevalence of stunting and overweight at 12 months of age in infants enrolled in the MACE study.

- To describe the prevalence of stunting and overweight at 24 months of age in those infants enrolled in the MACE study who had 24 month data.

1.3.2 To describe the maternal and household characteristics of the study infants and to determine their association with the growth outcomes of stunting and overweight at 12 months.

1.3.3 To describe birth anthropometrics, and patterns of growth from 0-6, and 6-12 months, and to determine their associations with growth outcomes at 12 months.

- To determine the association of patterns of growth with growth outcomes at 24 months, in those infants with 24 month data.

1.3.4 To describe infant feeding practices at 6 and 12 months and to determine their association with growth outcomes at 12 months.

1.4 Hypotheses

It was proposed that:

1) Poor breastfeeding practices were associated with stunting and overweight.
2) Lack of dietary diversity was associated with stunting and overweight.

1.5 Inclusion and exclusion criteria

1.5.1 MACE study inclusion and exclusion criteria

The inclusion criteria for the MACE study were as follows:

- Pregnant women of less than 18 weeks' gestation.
- Residents within a 5km radius from the clinic and monitoring station who would remain in this area for the full duration of the pregnancy, and for the follow-up period of 5-6 years. The children, from the pregnancy of interest, would reside in the communities for the duration of follow up.
- Women who tested positive or negative for human immunodeficiency virus (HIV) during their routine antenatal testing were included in the study.

The exclusion criteria for the MACE study were as follows:

- Participating females with any complications during pregnancy including hypertension, diabetes, placenta praevia or genital tract infections, or other complications that would result in adverse growth effects on the foetus.

1.5.2 MSc Dietetics study inclusion and exclusion criteria

For the purpose of this study the inclusion criteria were as follows:

- Pregnant women and their infants who met the MACE study inclusion criteria.
- MACE study infants who had attended the 12 month follow up between 2013 and 2017.

For the purpose of this study the exclusion criteria were as follows:

- Pregnant women excluded from the MACE study
- Infants born preterm, defined as < 37 weeks gestation.

1.6 Definitions

Complementary feeding: "the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk" (PAHO/WHO, 2003).

Exclusive breastfeeding: "the infant receives only breast milk. No other liquids or solids are given, not even water, with the exception of oral rehydration solution, or drops/syrups of vitamins, minerals or medicines" (WHO, 2001).

HIV exposed infant: infant born to an HIV positive mother (DoH, 2015a).

HIV positive infants: positive DNA-PCR test (DoH, 2015a).

HIV positive mothers: positive rapid test (DoH, 2015a).

Low birthweight infants: birthweight < 2500g.

Mixed feeding: "infant receives both breastmilk and any other food or liquid including water, non-human milk and formula before 6 months of age." (WHO, 2001).

Overweight children: WHO- BMI for Age > +2 Z-score (WHO, 2006).
IOTF-BMI for Age > age specific cut off (Cole, 2000).

Obese children: WHO- BMI for Age > +3 Z -score (WHO, 2006).
IOTF- BMI for Age > age specific cut off (Cole, 2000).

Stunted children: Length/Height for Age < -2 Z- score (WHO, 2006).

Severely stunted children: Length/Height for Age < -3 Z score (WHO, 2006).

Wasted children: Weight for length < -2 Z score (WHO, 2006).

Severely wasted children: Weight for length < -3 Z score (WHO, 2006).

Z score: The number of standard deviations a data point is from the mean. (WHO, 2006).

1.7 Abbreviations

ARV:	Antiretroviral
ART:	Antiretroviral Therapy
BAZ:	BMI for age Z score
BMI:	Body Mass Index
DEXA:	Dual-energy X-ray absorptiometry
DHS:	Demographic and Health survey
HAART:	Highly Active Antiretroviral Therapy
HAZ:	Height for age Z score
HIC:	High income country
HIV:	Human Immunodeficency Virus

HFS:	High fat snack
IRF:	Iron rich food
IYCF:	Infant and Young Child Feeding
IOTF:	International Obesity Task Force
KZN:	KwaZulu-Natal
LFA:	Length for age
LAZ:	Length for age Z score
LBW:	Low birthweight
LMIC:	Low and middle income country/ies
MACE:	Mother and Child in the Environment
MAD:	Minimum adequate diet
MDD:	Minimum dietary diversity
MMF:	Minimum meal frequency
PMTCT:	Prevention of Mother to Child Transmission
SES:	Socioeconomic status
SSB:	Sugar sweetened beverage
TDF:	Tenofovir
WFA:	Weight for age
WAZ:	Weight for age Z score
WFL/H:	Weight for length/height
WFLZ:	Weight for length Z score
WHO:	World Health Organization
UNICEF:	United Nations Children's Fund

1.8 Assumptions

The following assumptions were made:

- All MACE fieldworkers had been trained thoroughly.
- All anthropometric measurements were accurately taken by the trained fieldworkers.
- The fieldworkers asked the questions in a standardised manner.
- The participants were honest with their answers.
- The birth anthropometric details and HIV status information that were collected from clinic records were reliable.

1.9 Summary

South Africa has a double burden of malnutrition with national surveys reporting rates of both childhood stunting and overweight above 20%. The consequences of both forms of malnutrition can be far reaching and have an impact at an individual and national level. Stunting and overweight both have a complex set of determinants. Identifying these determinants is important in order to develop effective prevention strategies. Longitudinal birth cohort studies are the most powerful research tools to investigate potential determinants but very few have been conducted in LMIC settings where there is a high prevalence of both stunting and overweight. The MACE birth cohort study provided an opportunity to use longitudinal data to investigate the association of a number of potential maternal, sociodemographic and infant factors with growth outcomes at 12 months of age. It was envisaged that the findings of this research could be used in the development of appropriate intervention strategies within the larger population.

1.10 Dissertation overview

This dissertation consists of six chapters. The first chapter discusses the importance and relevance of the study and presents the study objectives. The second chapter reviews the literature regarding the global and South African prevalence of childhood stunting and overweight and the trends over time. The consequences, determinants and prevention strategies are also discussed. The third chapter discusses the methodology used in the study and the results of the statistical analyses are presented in the fourth chapter. The fifth chapter discusses the results, placing them in the context of the literature presented in chapter two. Chapter six concludes the dissertation, discusses the limitations of the study and makes recommendations for further research.

1.11 Referencing style

This dissertation has been written using the Experimental Agriculture referencing style.

2. LITERATURE REVIEW

2.1 Introduction

Low and middle income countries (LMICs) are still faced with problems related to undernutrition such as stunting and micronutrient deficiencies (Black *et al.*, 2013). However, in recent years there has been a significant increase in childhood overweight. The WHO Global Targets on maternal, infant and young child nutrition highlight this double burden of both over- and under nutrition in LMICs. Out of six global targets to be achieved by 2025, the first is a reduction of stunting in children under five by 40%; while the fourth target calls for no increase in childhood overweight (WHO, 2012). These targets have also been incorporated into the Sustainable Development Goals (SDGs), set by the United Nations in 2015, which includes, among others, a call for an end to malnutrition in all forms by 2030, and the prevention and control of non-communicable diseases (WHO, 2015).

Both stunting and overweight have short- and long term consequences with effects persisting even into the next generation (Black *et al.*, 2013). They both have foetal and early life determinants. Prevention requires a multi-dimensional approach including nutrition interventions that target the first 1000 days from conception to two years of age (WHO, 2016a; Bhutta *et al.*, 2013)

This review will describe the prevalence and trends of stunting and overweight, both globally and in South Africa, as well as their consequences. Their determinants will be explored, focusing on the postnatal period of the first 1000 days. Interventions targeting this stage of life will also be discussed.

2.2 Childhood stunting and overweight: global trends and distribution

While stunting affects approximately four times as many children globally than overweight or obesity, the rate is declining, whereas childhood overweight is increasing (UNICEF *et al.*, 2015). In 2014 the number of stunted children under five years of age was 159 million, which represents an estimated 23.8% prevalence, a decline of 37% since 1990. In the same time period the number of children under five who were overweight or obese increased from 31 million to 41 million, an increase in prevalence from 4.7% to 6.1% (UNICEF *et al.*, 2015).

In the past, childhood obesity was a problem only associated with high-income countries (HICs) but due to the nutrition transition[2], it is increasingly becoming a problem in LMICs (Black *et al.*, 2013). In many communities in these countries stunting and overweight coexist, sometimes even in the same child (Dietz *et al.*, 2015). According to WHO (2013) "Both stunting and overweight can be linked to a food system that fails to deliver quality food", with energy dense but nutrient poor foods cheap and widely available (Igumbor *et al*., 2012). Global patterns of stunting and overweight will now be discussed in more detail.

2.2.1 Stunting

While both stunting prevalence and the numbers of stunted children are declining, Asia still has the highest number of stunted children, due to its large population (de Onis *et al.*, 2013). India alone is home to more than one third of the world's stunted children. Latin America and the Caribbean have also seen declines in the number of stunted children but in Africa the number of stunted children has actually increased as a result of a very limited drop in the prevalence combined with an increase in the number of young children in the region (Black *et al.*, 2013; de Onis *et al.*, 2013). This is illustrated in Figure 2-1, which shows regional trends in the prevalence of stunting as well as the numbers of stunted children from 1990 projected to 2025.

[2] Nutrition transition- " Large shifts have occurred in diet and physical activity patterns particularly in the last two decades of the 20th century. Modern societies seem to be converging on a diet high in saturated fats, sugar, and refined foods but low in fibre-often termed the "Western diet"- and on lifestyles characterized by lower levels of activity" (Popkin&Gordon-Lewis,2004).

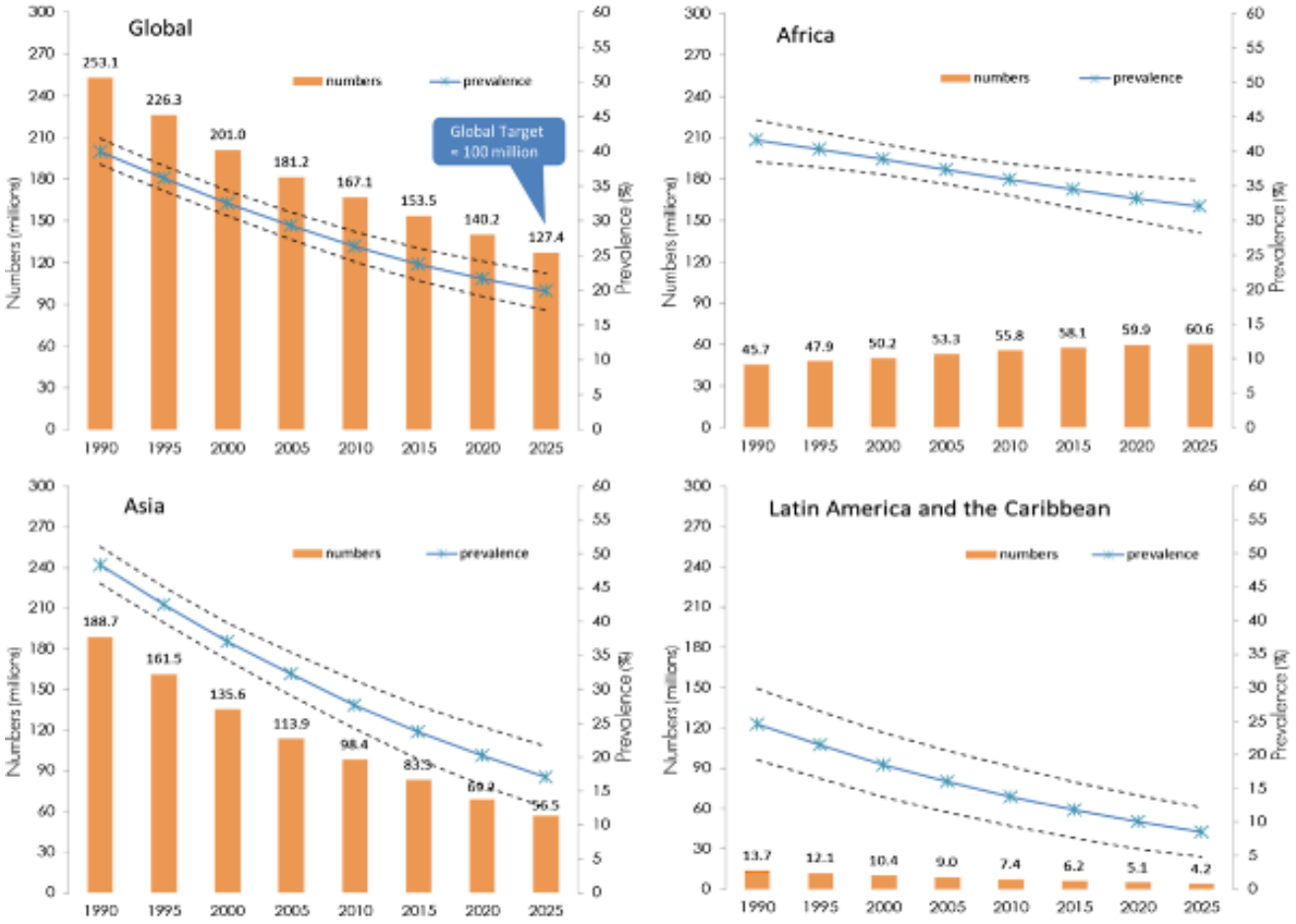

Figure 2-1: Regional trends in stunting- prevalence and numbers: 1990-2025 (de Onis et al., 2013)

Within countries there is great inequality in stunting rates. Global Demographic and Health Survey (DHS) data shows that children in the poorest quintile of households have 2.47 times the stunting prevalence of children in the richest quintile while prevalence in rural children is 1.45 times that of urban children (Black *et al.*, 2013). Within the genders, boys have only a slightly higher prevalence of stunting than girls (Black *et al.*, 2013).

2.2.2 Overweight and obesity

Globally overweight and obesity in children 2-19 years of age has increased by 47% from 1980 to 2013 (Ng *et al.*, 2014) and 41 million children under the age of five years (6.1%) are now overweight or obese. This is an increase of 10 million since 1990 (UNICEF *et al.*, 2015). The prevalence of childhood overweight in HICs is 11.7% which is approximately double that of most LMICs at 6.1%. However, in terms of numbers, many more children in LMICs are affected and the rate of increase is of particular concern (WHO, 2016a; de Onis *et al.*, 2010).

In the WHO Africa Region, the number of overweight children nearly doubled from 5,4 million to 10,3 million between 1990 and 2010 and it is estimated that 75% of overweight children now live in Asia and Africa (UNICEF *et al.*, 2015).

By contrast in HICs, although at very high levels, overweight and obesity prevalence in children and adolescents appears to be stabilising as shown in Figure 2-2 (Ng *et al.*, 2014). This was corroborated by the NCD Risk Factor Collaboration which found a similar trend of recent stabilising of obesity prevalence in HICs in children and adolescents aged 5-19 years (Abarca-Gómez *et al.*, 2017). Younger children appear to be following the same pattern. With the exception of England, a review of data from 18 European Union countries found no evidence of increasing prevalence in infants and pre-school children (Cattaneo *et al.*, 2010). In the United States, prevalence of obesity in 2-5 year olds actually decreased from 13.9% to 8.4% between the NHANES of 2003-2004 and that of 2011-2012 (Ogden *et al.*, 2014). However, data from the most recent NHANES of 2015-2016 showed a sharp increase in prevalence of obesity in 2-5 year olds from the 2011-2012 and 2013-2014 cycles (Skinner *et al*., 2018).

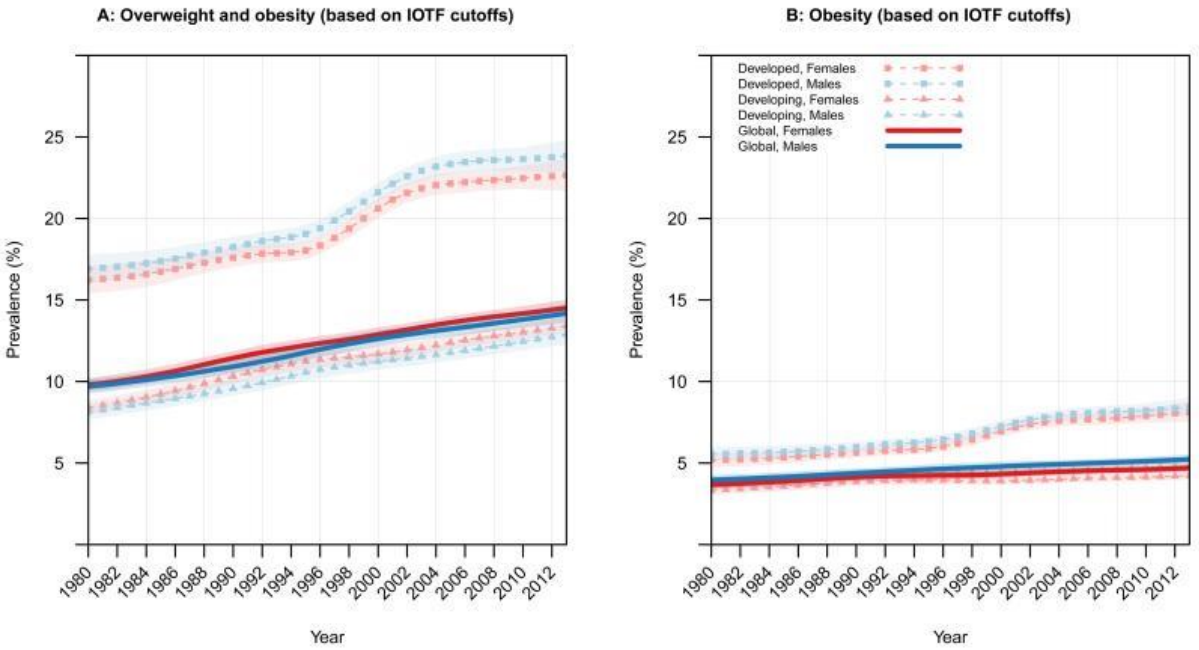

Figure 2-2: Age standardised prevalence of overweight and obesity, and obesity alone, ages 2-19 years, by sex, 1980-2013 (Ng *et al.*, 2014)

In HICs prevalence of childhood overweight is higher in poorer communities with immigrant children at increased risk (Olds *et al.*, 2011). Analysis of NHANES data over time shows a consistently higher prevalence of obesity in Hispanic and African American children and

adolescents (Skinner *et al*. 2018). In LMICs there is a slightly higher prevalence in richest versus poorest quintiles and urban versus rural populations. Gender differences in prevalence rates tend to be small in children under the age of five years (Black *et al.*, 2013).

Having outlined the global trends and distribution of childhood stunting and overweight, this review will now focus on patterns of malnutrition in South Africa.

2.3 Childhood stunting and overweight: South African trends and prevalence

South Africa's double burden of malnutrition is well illustrated by the results of the SANHANES-1 which reported stunting and overweight prevalence above 20% in children under the age of 5 years (Shisana *et al.*, 2013). The anthropometric status of South African children has been documented in a number of national and regional surveys over the years. The results of national surveys are presented in Table 2-1 and those of regional surveys in Table 2-2. Percentages that are emboldened in the table represent the prevalence of combined overweight and obesity. The tables are followed by a discussion of the trends in stunting and overweight prevalence.

Table 2-1: Trends in childhood stunting and overweight in South Africa: National surveys (1994-2016)

Study details	**Description of study**	**Sample size**	**Stunting prevalence (< -2 HAZ)**	**Cut offs used**	**Overweight and obesity prevalence**	**Cut offs used**
SAVACG 1994 (Labadarios and Van Middelkoop, 1995)	National; cross sectional 6 months -72 months	10871	22.9%	NCHS 1977	Not reported	
NFCS 1999 (Labadarios *et al.*, 2005)	National; cross sectional 1-9 years	2613	21.6% 25.5% (1-3 years)	NCHS 1977	**17.1%** 12.1% overweight 5% obese	NCHS 1977 Weight for length/height)
NFCS 1999 Secondary analysis using WHO 2006 Growth Standards (Bosman *et al.*, 2011)	National; cross sectional 12-60 months	1512	17.1% 20.1%	NCHS 1977 WHO 2006	**18.9%** 13% overweight 5.9% obese **30.1%** 20.6% overweight 9.5% obese	NCHS 1977 (weight for length/height) WHO 2006 (weight for length/height)
NFCS 2005 (Kruger *et al.*, 2012)	National; cross sectional 1-9 years	2157	20.7%	WHO 2006	**14%** 10% overweight 4% obese	WHO 2006 BMI for Age
SANHANES-1 2013 (Shisana *et al.*, 2013)	National; cross sectional 0-5 years for stunting; 2-5 years overweight and obesity	1291	**21.5%** 26.5% (0-3 y) 17% (0-9y)	WHO 2006	**22.9%** 18.2% overweight 4.7% obese	IOTF BMI for Age cut-offs
DHS 2016 (DoH *et al.*, 2017)	National; cross sectional 0-5 years	1404	27%	WHO 2006	**13%**	WHO 2006 (weight for length/height)

Table 2-2: Trends in childhood stunting and overweight in South Africa: Regional studies (1990-2016)

Study details	Description of study	Sample size	Stunting prevalence	Cut offs used	Overweight Prevalence	Cut offs used
Birth to Twenty cohort: 1991-1992 (Norris *et al.*, 2009)	Longitudinal birth cohort Johannesburg/ (urban)	1221(12 months) 997 (24 months) 1162(60months)	6.5% 12 months 17.3%24 months 5.6% 60 months 7.8% 12 months 26.5%24months 9.1% 60 months	NCHS 1977 WHO 2006	7.5% 12 months 6.1% 24 months 3.5% 60 months 10% 12 months 12% 24 months 1.3% 60 months	NCHS 1977 Weight for age WHO 2006 Weight for age
Central Region Limpopo: 2001 (Mamabolo *et al.*, 2004) (Mamabolo *et al.*, 2005)	Prospective birth cohort Rural villages in Limpopo	156 (12 months) 162 (36 months)	34.6%12 months 48% 36 months	NCHS 1977	12.3% 12 months 46% 36 months 22% ow 24% obese 19% ow and stunted	NCHS 1977 Weight for length/height IOTF
HST/MRC survey 2003 (Lesiapeto *et al*, 2010)	Cross sectional baseline survey 0-60 months children OR Tambo and Alfred Nzo districts,, Eastern Cape; Umkanyakude and Zululand districts, KZN	2485	23.5% 28.6%	NCHS 1977 WHO 2006	9.5% 13.1% 16.1%	NCHS 1977 Weight for length/height WHO 2006 Weight for length BMI

Table 2.2 continued.

Study details	**Description of study**	**Sample size**	**Stunting prevalence**	**Cut offs used**	**Overweight prevalence**	**Cut offs used**
MRC (Faber and Benade, 2007)	Cross sectional survey Rural KZN 6-12 months infants	505	16%	Epi Info	**23%**	Epi Info Weight for length
Agincourt ; 2007 (Kimani-Murage *et al*, 2010)	Cross sectional survey Agincourt sub district, Mpumalanga Province Rural Children 1-4 years	671	18%	WHO 2006	**8%** 7% overweight 1% obese	IOTF BMI for age
Community Based Nutrition Security Project University of Stellenbosch 2011 (du Plessis *et al* , 2016)	Cross sectional survey Vulnerable communities, Breede Valley sub district, Western Cape Province 0-23 months infants	312	28.8%	WHO 2006	**21.8%**	WHO 2006 Weight for length
Drakenstein Child Health Study 2012-2014 (Budree *et al*, 2017a; Budree *et al* 2017b	Longitudinal birth cohort study Multi ethnic population, low resource district surrounding Paarl, Western Cape Province 12 months infants	342	13%	WHO 2006	**8%** 6% overweight 2% obese	WHO 2006 BMI for age

2.3.1 Stunting

From the figures presented in Tables 2-1 and 2-2, South Africa can be classified as having a stunting prevalence of moderate public health importance (Gorstein *et al.*, 1994). National surveys in Table 2.1 consistently show stunting prevalence in the 20-23% range, except for the DHS of 2016 which showed a prevalence of 27% in children under five years of age. Regional surveys have far greater variation in stunting prevalence ranging from 13% to 48% but mostly falling in the 20-30% range. Stunting prevalence shows variation across age groups and, given the inequity in South Africa, across location, socioeconomic status and race (Said-Mohamed *et al.*, 2015).

2.3.1.1 Variation of prevalence with age

The variation in prevalence with age can be explained by the fact that human growth occurs in in four phases; foetal, infancy, later childhood and puberty so there is some potential for compensatory growth after the foetal and infancy phases (Luo & Karlberg, 2000). Prevalence of stunting will therefore be higher in infants and younger children who have experienced growth failure in the foetal and infancy stage but have not yet reached the later childhood growth phase. This rapid drop off in height followed by improvement in older children may be due to improved resistance to infections by the time children reach the childhood growth phase (Prentice *et al.*, 2013). Repeated infections combined with poor infant and young child feeding and caring practices are the proximal causes of stunting (Black *et al.*, 2008). There is also potential for catch up growth in adolescence due to reported delayed maturation and extended pubertal growth phase in children living in LMICs (Prentice *et al.*, 2013).

The cross sectional studies in Tables 2-1 and 2-2 consistently showed this variation with age. There was far higher stunting prevalence in the 0-3 years age group compared to older age groups in both the National Food Consumption Survey (NFCS) of 1999 and the SANHANES-1. Stunting prevalence in SANHANES-1 decreased from 26% in the 0-3 year age group to 9.4% in the 7-9 year age group. The Agincourt study found the highest prevalence of stunting in 1 year old infants (32%), decreasing to 20% in the 2 year olds. The lowest prevalence of stunting was 5% in the 5-9 year old children.

Longitudinal studies provide the best illustration of stunting variation with age. The trend of stunting peaking at age two to three years and then decreasing can be seen in the data from the Birth to Twenty longitudinal birth cohort study in Table 2.2. Stunting prevalence increased from 1 year (7.8%) to 2 years (26.5%) but decreased again by 5 years down to 9.1% which indicates that some catch up growth is possible after two years. The Limpopo birth cohort study also showed an increase of stunting prevalence from 34.6% at 1 year to 48% at 3 years. International longitudinal studies in LMICs report similar findings. The COHORTS (Consortium of Health Oriented Research in Transitioning Societies) collaboration included data from longitudinal birth cohorts in five LMICs; Brazil, Guatemala, India, Philippines and South Africa. The Birth to 20 cohort was the South African study in COHORTS. Height for age Z scores (HAZ) decreased rapidly from birth to 24 months and then improved modestly by four to five years of age in all the cohorts except India (Stein *et al.*, 2010). Rural Gambian children also had the same pattern, with the lowest HAZ at 24 months before gradually increasing (Prentice *et al.*, 2013).

2.3.1.2 Variation of prevalence with gender, location, socioeconomic status (SES) and race

Most of the South African surveys did not find a gender difference in prevalence of stunting. Although boys had a higher prevalence of stunting than girls in all age categories in SANHANES-1, the differences were not significant, but in the DHS 2016 boys had a prevalence of 30% vs 25% in girls. There was also no significant difference in children under 5 in the Agincourt study while the HST/MRC study in rural KZN and Eastern Cape did find an increased risk of stunting in boys.

Rural informal areas had the highest prevalence of stunting, followed by urban informal areas. This is illustrated in Table 2-2 by the high stunting prevalence in the rural Limpopo birth cohort of 48% by 3 years. The rural KZN and Eastern Cape communities in the HST/MRC study also had a high under 5 prevalence of 28.8%. Both areas experienced high levels of poverty and lack of infrastructure, with the HST/MRC study reporting that 75% of households did not have access to tap water and only 26% of households had electricity.

Provincially stunting prevalence varies considerably between different surveys, making any conclusions difficult. SANHANES-1 reported lower prevalence in Gauteng and KZN and the

highest prevalence in Eastern Cape, Free State and Northern Cape. In contrast Gauteng had the highest prevalence in the DHS with Northern Cape the lowest. As stunting is strongly linked to poor socio economic status it is no surprise that DHS reported a stunting prevalence of 36% in children of the lowest quintile compared to 13% in children of the highest wealth quintile. In SANHANES-1, White and Indian groups were excluded due to low numbers while Coloured children had the highest prevalence of stunting compared with black African children.

2.3.1.3 Trends in stunting prevalence from 1994 to 2016

Interpreting trends in stunting over time by comparing results from different studies is complicated by:

a) The use of different growth references. The United States National Centre for Health Statistics (NCHS) standards (Hamill *et al.,* 1977) were used prior to the release of the WHO Growth References in 2006 (WHO, 2006). Stunting prevalence increases when using the WHO References as can be seen from secondary analysis performed on the 1999 NFCS, Birth to Twenty and HST/MRC studies.

b) A lack of consistency in age groups measured. The SAVACG study included 6 months-72 months (6 years) infants and children, the NFCS (1999 and 2005) 1-9 year olds, SANHANES -1 used 0-14 years and the DHS used 0-5years. Prevalence of stunting using the SANHANES-1 0-9 years data gives a stunting prevalence of 17% which is a 3.7% decrease from the NFCS 2005 of 20.7%. However, using the data from children under five years in SANHANES -1, which is in line with global and regional reporting, gives a stunting prevalence of 21.5%. As was discussed previously, stunting prevalence peaks at around 24 months so studies including children from older age groups, for instance 1-9 years, will tend to decrease stunting prevalence when compared with studies that only include children up to five years or younger.

Despite these issues regarding comparisons between studies, South African stunting prevalence appears to have shown little, if any, improvement over time (Said-Mohamed *et al.,* 2015) and remains above 20% for children under five at a national level.

2.3.2 Overweight and obesity

South Africa is also experiencing high levels of childhood overweight and obesity with the recent SANHANES-1 reporting overweight and obesity prevalence of 22.9 % in children aged 2-5 years (Shisana *et al.*, 2013). When referring to Tables2-1 and 2-2 it is difficult to discern a clear pattern in trends of prevalence over time due to the same issues encountered when describing stunting trends. These include lack of consistency in the growth references, and age categories, used across studies. The impact of these factors will be discussed in more detail in this section in order to evaluate the prevalence of overweight and obesity and trends over time as presented in tables 2-1 and 2-2.

2.3.2.1 Defining overweight and obesity

The lack of a standard definition of childhood overweight and obesity is of concern as it makes meaningful interpretation of prevalence trends very difficult. The various growth references/standards used in the studies in Tables 2.2 and 2.2 are shown in Table 2.3.

Table 2-3: Overweight and Obesity Definitions

Growth Reference	Definition of Overweight	Definition of Obesity
NCHS 1977 (Hamill *et al.*, 1977) Children 2-18 years	>+2 Z score Weight for length/height	>+3 Z score Weight for length/ height
IOTF (Cole *et al.*, 2000) Children 2-18 years	Age specific BMI cut offs Corresponding to BMI 25kg/m2 at 18 years	Age specific BMI cut offs Corresponding to BMI 30 kg/m2 at 18 years
WHO 2006 (WHO, 2006) Children 0-5 years	>+2 Z score Weight for length/height or BMI for Age	>+3 Z score Weight for length/height or BMI for Age

Prior to 2000, surveys used the NCHS 1977 growth reference tables (Hamill *et al.*, 1977). These, however, were developed using only American children. Many countries also had their own growth references which were used in national overweight prevalence studies, making comparisons between studies very difficult. As childhood overweight is a serious public health problem, it is important that trends are monitored, which was difficult with no standard definitions and cut offs. In order to provide an internationally comparative standard the

International Task Force on Obesity (IOTF) published age and sex specific BMI cut off points for overweight and obesity for children from 2-18 years (Cole *et al.*, 2000). These cut offs were created to correspond with BMI at 18 years of 25 and 30kg/m^2 for overweight and obesity respectively. They were developed using data from almost 200 000 individuals aged from birth to 25 years in 6 countries; Brazil, Great Britain, Hong Kong, the Netherlands, Singapore and the United States (Cole *et al.*, 2000).

In 2006 the WHO released the WHO Child Growth Standards for children 0-59 months (WHO, 2006). These were also internationally representative comprising data from children in 6 countries; Brazil, Ghana, India, Norway, Oman and the USA. They differed from previous growth references in that they were prescriptive; children had to be healthy, predominantly breastfed for at least 4 months and live in optimal environmental conditions. The standards therefore describe how children should grow when there are no constraints to their growth (de Onis, 2006).

It must be noted that use of the WHO standard produces very difference prevalence rates of overweight and obesity in children under 5 years when compared to those obtained using the IOTF reference. This was demonstrated by comparing the prevalence of overweight and obesity from a national study in the Czech Republic using both the IOTF and WHO definitions. According to WHO charts the prevalence of overweight in girls at 5 years was 3.4% while it was 15.3% using IOTF cut offs (Monasta *et al.*, 2011). The WHO justified a more cautious approach to defining overweight and obesity in younger children on a number of grounds. Firstly, younger children are still growing; secondly, the population used in constructing the standard was healthy and unhealthy weights for length/height were excluded; and thirdly, the potential risks of young children being placed on restrictive diets (De Onis and Lobstein, 2010). A concern with the WHO standard is that no BMI cut-offs that are associated with increased risk of overweight and obesity later in life have been identified in young children. This is unlike the IOTF cut offs which are based on an association with BMI of 25 and 30kg/m^2 at 18 years (Monasta *et al.*, 2011).

The European Childhood Obesity Group recommended that, although it is not an ideal situation, both references should be used in prevalence studies in the absence of consensus on a common definition of obesity. They further recommend that the definitions and terms must

be clearly stated so that it is clear if the term overweight includes obesity (Rolland-Cachera, 2011). As the IOTF reference is from 2-18 years, studies involving children younger than 2 years must use the WHO standard (Cattaneo *et al.*, 2010). A further consideration is the use of weight for length/height or BMI for Age to define overweight and obesity; prevalence is slightly higher using BMI.

2.3.2.2 Review of South African studies of childhood overweight and obesity prevalence

The studies presented in Tables 2-1 and 2-2 will now be discussed, examining the effect of different growth standards, age categories and other factors on reported prevalence.

Variation in prevalence according to growth reference used

An analysis of South African national trends over time as depicted in Table 2-1 shows the importance of comparing "apples with apples". The SANHANES-1, using the IOTF cut-offs, found that almost one quarter of 2-5 year olds were overweight or obese (Shisana *et al.*, 2013). This appears to be an increase from the 1999 and 2005 NFCS which reported combined overweight and obesity prevalence of 17.1% and 14% respectively in 1-9 year olds (Labadarios *et al.*, 2005; Kruger *et al.*, 2012). However, a reanalysis of the 1999 NFCS 1-5 year olds data using WHO 2006 references (weight for length) found a much higher overweight and obesity prevalence of 30.1%. Using the same one to five year old data but with the NCHS 1977 (weight for length) standards the prevalence was far lower at 18.9% (Bosman *et al.*, 2011). The DHS 2016 showed a decrease of over 10% from SANHANES-1 in the prevalence of overweight in children under five years (DoH *et al.*, 2017). This could perhaps be attributed to the use of WHO 2006 weight for length/height as opposed to the IOTF BMI cut offs used in SANHANES-1. The DHS also had a study population aged 0-5 years as opposed to the 2-5 years in the SANHANES-1 and included relatively small sample sizes. As can be seen, the use of different standards makes establishing a trend very difficult and it would appear that overweight prevalence has more than halved from the 1999 NFCS to the 2016 DHS, which is highly unlikely.

Regional studies, as with stunting, have great variation in overweight prevalence from approximately 10% to just over 20%, with the Limpopo cohort being an outlier at 46%. The HST/MRC study in the Eastern Cape and KZN illustrates the problem of different growth

references. Overweight prevalence was 9.5% with NCHS 1977 standards; 13% and 16% with WHO 2006 weight for length and BMI for Age respectively.

Variation in prevalence according to age of study population

As with stunting, overweight and obesity prevalence also shows a pattern of peaking at around 2 years of age and decreasing again by 5 years. This is illustrated by the Birth to Twenty longitudinal cohort. At the age of 2 years, 12% of children were overweight but this had decreased to 1.3% at 5 years. The Agincourt study, although not longitudinal, showed a decrease in combined overweight and obesity prevalence from 10% in 2 year olds down to 7% in the 5 year old children. Overweight prevalence starts to increase again in girls from about the age of 10 years (Kimani-Murage *et al.*, 2010). The SANHANES-1 had the highest prevalence of overweight and obesity in the 2-5 years age group, with rates increasing again in 10-14 year old girls (Shisana *et al.*, 2013).

Variation in prevalence according to gender, location and SES

There is little difference between overweight prevalence in boys and girls in the under five years age group but older girls in both the SANHANES-1 and Agincourt study had more than double the prevalence of boys (Shisana *et al.*, 2013; Kimani-Murage *et al.*, 2010). Urban areas, both formal and informal, had higher prevalence of overweight and obesity compared to rural areas in SANHANES-1 (Shisana *et al.*, 2013). When looking at the impact of SES, the 2016 DHS had a very interesting finding of highest overweight prevalence in the poorest quintile and lowest prevalence in the highest quintile (DoH *et al.*, 2017), a pattern typically seen in HICs. This was contrary to the Agincourt study which found a positive relationship between overweight and socio-economic status (SES), similar to other LMICs (Kimani-Murage, 2013).

The prevalence of combined stunting and overweight

The combination of overweight and stunting in the Limpopo longitudinal study is concerning as 19% of three year old children were both stunted and overweight (Mamabolo *et al.*, 2005). Secondary analysis of the 1999 NFCS showed that stunting was the most significant risk factor for a child being overweight with a stunted child being twice as likely to be overweight than a non-stunted child (Steyn *et al.*, 2005). A secondary analysis using NFCS 2005 data of children from Gauteng and Mpumalanga found a strong correlation between stunting and obesity with 64.8% of obese children also being stunted. Only 13.6% of children in the normal and underweight group were stunted (Symington *et al.*, 2016). Another South African survey of

952 rural Kwa-Zulu Natal schoolgirls aged 9-12 years found that 9.2% were stunted while 8.5% were overweight and 3.8% obese. Of particular interest was the fact that 22.9% of obese learners and 11.1% of overweight learners were stunted (Tathiah *et al.*, 2013).

The association between stunting and overweight was also demonstrated in a study looking at data from four countries; Russia, China, Brazil and South Africa (Popkin *et al.*, 1996). Children who were stunted were at significantly greater risk of being overweight with adjusted risk ratios ranging from 1.7 in Brazil to 7.8 in Russia. The Birth to 20 study also found significantly higher BMIs in stunted versus non stunted infants at two years of age, however this was not associated with higher subcutaneous fat scores. The authors argued that the higher BMIs in these children did not reflect overweight or obesity but were as a result of the proportionally greater contribution of height to the BMI calculation as height squared is used as the denominator (Cameron *et al.*, 2005). This study also showed no link between stunting at 2 years and overweight at 9 years but this will be discussed further in the next section, 2.4.1.2.

Trends in overweight prevalence 1994-2016

Taking into account the confounding effects of variations in cut offs used and age of the study sample, there does appear to be an increasing trend in overweight and obesity prevalence in children under the age of 5 years. The DHS survey prevalence of 13%, a decrease of 10% from the SANHANES-1 study prevalence, should perhaps be viewed as an outlier until confirmed by other national surveys.

In summary, stunting and overweight in childhood are both highly prevalent in LMICs, and South Africa is no exception. The next section of this review will explore the consequences, both short and long term.

2.4 Consequences of childhood stunting and overweight

Childhood stunting and overweight have short and long term consequences both at an individual and community/country level (Black *et al.*,2013; de Onis *et al.*, 2013). This section will describe the effect of stunting on child mortality and both human capital outcomes and adult NCD risk. The increased risk for development of adult obesity and NCDs as a result of childhood overweight will also be discussed.

2.4.1 Stunting

Stunting has both short and long term consequences. The short term consequences are increased morbidity and mortality, with a severely stunted child having 4 times the risk of death compared to a non-stunted child (Black *et al.*, 2008). For those children that survive, the consequences can be far reaching and can leave them trapped in a cycle of poverty and malnutrition (Dewey & Begum, 2011).

2.4.1.1 Human capital outcomes

In the longer term, stunting in childhood results in impaired cognitive development and reduced adult stature which both impact on earning ability and human development, often referred to as human capital (Victora *et al.*, 2008). Analysis of data from the COHORTS collaboration linked childhood stunting to poorer educational outcomes (Victora *et al.*, 2008). The impact on cognitive development has been shown in South Africa using data from the National Income Dynamics Survey (NIDS). Stunted children started school later, completed fewer years of schooling and were more likely to have failed some grades (Casale, 2016). The effects on cognitive development appear to be irreversible as even those children in the Birth to 20 Cohort who were no longer stunted at 5 years, after having been stunted at 2 years of age, performed significantly worse on cognitive tests than children who had never been stunted. Their performance was very similar to children who had remained stunted (Casale & Desmond, 2016). This is of particular concern when considering the very high rates of stunting in 0-3 year old children (26.5%) in SANHANES-1 (Shisana *et al.*, 2013). Stunting appears to affect the brain in a number of ways including causing structural changes and reduced exploratory behaviour due to lack of energy as a result of undernutrition (Brown & Pollitt, 1996).

Adult stature is most strongly linked to birth length and conditional length at 12 months according to analysis of the COHORTS data (Stein *et al.*, 2010). While educational achievement is generally linked to earnings, a lower adult stature and decreased lean body mass in men may also impact on earning potential in areas where physical labour is a major source of income (Victora *et al.*, 2008).

The cycle of poverty and malnutrition is perpetuated intergenerationally as women who are stunted as children and have short stature in adulthood are themselves more likely to give birth to a child with intrauterine growth restriction (IUGR). Maternal mortality and morbidity are

also increased in women of short stature (Victora *et al.,* 2008). These findings of an intergenerational effect of stunting were confirmed in the 2013 Lancet Maternal and Child Nutrition Series (Black *et al.*, 2013).

The impact of stunting on development of human capital is illustrated very clearly in the Guatemala cohort that forms part of the COHORTS collaboration. While the other four cohorts were observational, the Guatemala study (The Institute of Nutrition of Central America and Panama (INCAP) Oriente Longitudinal Study) was experimental and followed children from four villages between 1969-1977. Pregnant and lactating women and children aged 0-7 years in two of the villages received *Atole,* a high protein, high energy supplement, while the children from the other two received *Fresco,* a non-protein low energy supplement. Both supplements contained added micronutrients (Martorell *et al.,* 1995). Children who received *Atole* under the age of 3 years were taller than the *Fresco* group but no difference was seen if supplementation occurred between 3 and 7 years. A number of follow up studies were conducted when the children were teenagers and adults and the results showed clear advantages for the *Atole* group who were taller, performed better in school and in reading comprehension and intelligence tests and earned higher wages than the *Fresco* group. These benefits were only seen if the children had received the supplements before the age of 3 years. An intergenerational effect was also seen as the babies born to women who had received *Atole* as children were taller (especially boys) and heavier than offspring of women from the *Fresco* group (Dewey & Begum, 2011). This illustrates the long term negative effects of stunting and how interventions can change a vicious cycle to a virtuous cycle.

2.4.1.2 Risk of overweight in childhood and adulthood

The relationship between stunting and overweight was discussed previously with some studies reporting higher prevalence of overweight in stunted versus non stunted children (Popkin *et al.,* 1996; Mamabolo *et al.,* 2005; Symington *et al.*, 2016; Steyn *et al.,* 2005). However, data from the longitudinal Birth to 20 study showed that although children who were stunted at 2 years of age had higher BMIs than non-stunted children, skinfold thickness measurements showed no differences in their fat distribution or centripetal fat ratio (CFR). The authors suggested the higher BMIs were due to distortions related to the mathematical formula for calculating BMI; that is "underheight" as opposed to overweight (Cameron *et al.*, 2005). The issue of "underheight" was also discussed in a paper in the Lancet Obesity Series and the

authors recommended including measures of adiposity rather than just BMI in anthropometric surveys conducted in LMICs (Lobstein *et al.*, 2015).

It is unclear whether early childhood stunting increases the risk for overweight in later years. In the Birth to 20 study, being stunted at 2 years of age did not increase risk of overweight or adiposity once these children reached 9 years of age although this could still be a possibility in adolescence (Cameron *et al.*, 2005). The Agincourt study showed high rates of stunting of 2 year old children together with high prevalence of overweight and obesity in female adolescents in the same rural community but this was a cross sectional study so did not provide evidence that the overweight adolescents had been stunted earlier (Kimani-Murage *et al.*, 2010). A follow up of boys from the Pelotas birth cohort in Brazil at the age of 18, when they had to enlist in the army, found no relationship between stunting at 2 or 4 years and overweight at 18 years. In fact undernutrition in early life seemed to have a protective effect against overweight later in life (Gigante *et al.*, 2007). The 2008 Lancet Series, which used data from the COHORTS collaboration, including that from the Pelotas cohort, did not find any strong association between height for age in early childhood and overweight in adults. In the Guatemalan cohort there was an association between previous stunting and adult central adiposity but this was not seen in the Indian cohort (Victora *et al.*, 2008).

2.4.1.3 Risk of NCDs in adulthood

The evidence linking childhood stunting to increased risk of non-communicable diseases in later life is not as strong as that linking it to human capital outcomes. There is evidence however that adult short stature is linked to metabolic syndrome and increased risk of Type 2 Diabetes (Bosy-Westphal *et al.*, 2009). While cohorts in high income countries have shown associations between low birthweight and development of chronic disease, the association is much weaker in LMICs, although COHORTS did find an association of low birthweight with high blood pressure in later life (Adair *et al.*, 2009). High glucose concentrations, blood pressure and harmful lipid profiles were only associated with childhood undernutrition once adult BMI and height were adjusted for in the Lancet 2008 Undernutrition Series analysis. This indicates a link with rapid weight gain after infancy (Victora *et al.*, 2008). This observation was confirmed by further analysis of COHORTS data confirming that growth, particularly linear growth, in infancy up until 2 years has a beneficial effect on human capital outcomes with little or no negative effect on chronic disease markers. In contrast, rapid weight gain after 2 years,

especially after mid childhood, does not benefit development of human capital but negatively affects cardiovascular disease risk in studies from developing countries (Adair *et al.*, 2013).

The 2008 Lancet Maternal and Child Undernutrition Series was focused on the prevalence and consequences of undernutrition and interventions to address it (Black *et al.*, 2008). The 2013 Maternal and Child Nutrition Series highlighted the emergence of the double burden of malnutrition in LMICs. While stunting in particular remained highly prevalent, many countries were seeing an increase in the prevalence of overweight and obesity, particularly in females and young children (Black *et al.*, 2013).

2.4.2 Overweight

2.4.2.1 Short term consequences

The short term effects of childhood overweight include delayed motor development in infancy which can lead to reduced physical activity thereby creating a vicious cycle (Slining *et al.*, 2010). In later childhood, overweight can lead to lack of confidence and poor self-esteem which can persist into adulthood (Rossouw *et al.*, 2012). Childhood obesity has also been linked to asthma and reduced lung function at the age of 18 (Ziyab *et al.*, 2014).

As a result of increasing overweight and obesity in childhood and adolescence, conditions like Type 2 Diabetes, high blood pressure and abnormal lipid profiles are being diagnosed at increasingly younger ages (WHO, 2016a). Children in the Agincourt study aged from 7-15 years already had a high prevalence of pre-hypertension, impaired fasting glucose and abnormal lipid levels which were all associated with adiposity (Pedro *et al.*, 2014). A Chilean study found biochemical markers of increased CVD risk in obese four year olds (Corvalán *et al.*, 2009).

2.4.2.2 Risk of overweight and NCDs in adulthood

While the short term effects of overweight are of concern, the bigger threat is that overweight in childhood is linked to adult obesity, diabetes and other non-communicable diseases (NCDs) (WHO, 2016a; Black *et al.*, 2013). The increasing prevalence of childhood overweight and obesity in LMICs has the potential to create an enormous NCD burden in years to come.

As was discussed previously, in South African surveys, overweight prevalence tends to peak at around 2 years before decreasing in mid childhood. Prevalence increases again in female adolescents (Kimani-Murage *et al.*, 2010; Shisana *et al.*, 2013). The significance of being overweight in the first 2 years of life in predicting risk of being overweight in adolescence and adulthood is not entirely clear. Birthweight; size at 20 months and 43 months; and most significantly, a rapid weight gain from birth to 20 months and 20 to 43 months were all associated with adolescent overweight and obesity in children from the Pelotas cohort. However, half of all children who were overweight in early childhood did not become overweight adolescents and conversely only 20% of overweight adolescents were overweight in childhood. (Monteiro *et al.*, 2003). A study using data from the Isle of Wight birth cohort identified four different trajectories of BMI in children from birth to 18 years; normal; early transient overweight (overweight at 1 year before decreasing); early persistent obesity (obese by 4 years, never decreasing); and delayed onset (at 10 years) overweight (Ziyab *et al.*, 2014). This indicates that a number of children who are overweight at 1 year would no longer be overweight in later childhood and also that many overweight older children and adolescents were not overweight in early childhood. These findings are similar to those of the Pelotas cohort.

Most birth cohort studies have been conducted in developed countries. They generally show a relationship between rapid weight gain in early life and later risk of overweight or obesity and other risk factors for NCDs. A systematic review of 21 studies from developed countries looked at rate of weight gain and risk of obesity at ages ranging from 5 years to 32 years. The review found strong evidence of a statistically significant association between rapid weight gain up to 2 years of age and subsequent obesity. Infants and children who had rapid weight gain, defined as >+0.67 Z score change in weight for age, were 2-3 times more likely to be obese when older (Ong and Loos, 2006). These findings were confirmed by a subsequent review of systematic reviews on the topic which found rapid weight gain in infancy was consistently associated with increased risk of obesity later in life (Monasta *et al.*, 2010). In a Swedish prospective birth cohort study rapid weight gain in both early infancy (0-6 months) and later childhood (3-6 years) was associated with an increased BMI, fat mass and waist circumference in young adulthood. Rapid weight gain in the infancy phase, which occurred in 25% of the cohort, also predicted taller adult height (Ekelund *et al.*, 2006).

The timing of weight gain and whether it is accompanied by linear growth appears to be important for long term risks in children living in developing countries. Data from COHORTS showed that rapid weight gain after 2 years and into mid childhood was associated with adult overweight, increased adult fat mass, raised blood pressure and dysglycaemia. In contrast weight gain combined with linear growth in the first 2 years was associated with adult fat free mass and was not associated with cardio metabolic risk factors (Adair *et al.*, 2013). This is significant as in developing countries there is a need to balance obesity prevention in early childhood with preventing morbidity and mortality associated with poor growth (Lobstein *et al.*, 2015; Adair *et al.*, 2013). The evidence suggests that promoting healthy growth, in particular linear growth, in the first 24 months has benefits that outweigh the risks (Adair et al., 2013). This is in contrast to the findings from developed countries, probably due to a number of factors, including different causes of LBW, less catch up growth in developing countries and the fact that many of the countries in COHORTS had not yet experienced the nutrition transition (Victora et al., 2008). Rapid weight gain in mid childhood and beyond, however, especially if not accompanied by gains in length, increases risk of obesity and NCDs (Adair *et al.*, 2013).

Childhood stunting and overweight are both important public health problems in LMICs that can have lifelong effects. The determinants of both conditions are complex and, in some cases, shared.

2.5 Determinants of childhood stunting and overweight

Childhood stunting and overweight can largely be prevented, however appropriate interventions need to be based on an understanding of the underlying causes, as well as the size of their effect in order to prioritise allocation of resources (Danaei *et al.*, 2016).

2.5.1 Stunting

Stunting starts *in utero* as it has been estimated that 20% of childhood stunting has its roots in poor foetal growth (Black *et al.*, 2013). A recent global risk factor analysis found that foetal growth restriction, that is, born term but small for gestational age (TSGA), was the single biggest risk for childhood stunting, accounting for approximately 25% of stunted children (Danaei *et al.*, 2016). The intergenerational effects of stunting have already been discussed

where women of short stature have been shown to be at increased risk of delivering babies who have experienced IUGR. The focus of the remainder of this literature review will be on the period from birth to two years of age.

After delivery, a combination of exposure to repeated infections as a result of poor socioeconomic conditions, poor feeding practices and lack of care all contribute to the further drop off in HAZ that is typically seen by two years in LMICs (Black *et al.*, 2013). According to the well known UNICEF conceptual framework on malnutrition, these immediate causes have their roots in basic and underlying causes of malnutrition that is, the political economy, food and agriculture policies, water and sanitation and education (Onyango, 2013; Black *et al.*, 2013). The large disparities in stunting prevalence between the highest and lowest quintiles of socioeconomic status in almost every LMIC illustrates how important these more distal determinants are (Black *et al.*, 2013). As discussed previously, the South African DHS reported stunting rates of 36% vs 13% in lowest vs highest SES quintiles (DoH *et al.*, 2017).

The global risk factor analysis mentioned previously found that, after being born TSGA, the second biggest risk factor for stunting was unimproved sanitation followed by diarrhoea. The analysis grouped 18 known risk factors for stunting into five clusters: maternal nutrition and infection; teenage motherhood and short birth intervals; foetal growth restriction and preterm birth; child nutrition and infection; and environmental factors. Environmental factors included unimproved sanitation, unimproved water source and use of biomass fuels, for example paraffin, for cooking and heating. These factors together had the second largest impact on stunting globally, with the child nutrition and infection cluster only coming in fourth behind maternal nutrition and infection (Danaei *et al.*, 2016). A similar risk factor assessment study in India, which accounts for over a third of the worlds stunted children, also found child nutrition to be less of a risk factor than maternal and socioeconomic factors. Maternal height, BMI, education and household wealth were strongly related to stunting. Dietary diversity was also important (Corsi *et al.*, 2016).

These two studies show that stunting is far more than just a nutrition problem and is driven by underlying social, political and environmental factors that affect household food security and access to safe and hygienic living conditions as well as women's empowerment and education (Black *et al.*, 2013). However this review will now focus on the immediate causes of stunting

which include exposure to infections, poor feeding practices and lack of care (Black *et al.*, 2013).

2.5.1.1 Exposure to infections

While infections like malaria, pneumonia and measles can cause acute wasting and, to a lesser extent, stunting, diarrhoea has been most strongly linked to stunting (Black *et al.*, 2013). A pooled analysis using data from 5 countries showed that each episode of diarrhoea increased the odds of stunting at 24 months and that 25% of stunting was as a result of 5 or more episodes of diarrhoea (Checkley *et al.*, 2008).

Environmental enteropathy, also known as tropical sprue, occurs in children living in unsanitary conditions and causes damage to the structure and function of the small intestine, including villous atrophy and inflammation. While it is often subclinical, it is thought to result in increased intestinal permeability and malabsorption of nutrients which can lead to poor growth (Black *et al.*, 2013; Stewart *et al.*, 2013).

2.5.1.2 Poor feeding practices

The South African Infant and Young Child Feeding (IYCF) Policy recommends exclusive breastfeeding in the first six months followed by introduction of complementary foods with continued breastfeeding up until 2 years and beyond (DoH, 2013b). Exclusive breastfeeding, although very important for child survival (Jones *et al.*, 2003) has not been shown to have great impact on length in infants (Black *et al.*, 2013). A systematic review and meta-analyses of 35 studies found no significant effect of breastfeeding promotion interventions on length/height z scores (Giugliani *et al.*, 2015). Complementary feeding practices however, have a greater impact on linear growth (Black *et al.*, 2013; Marriott *et al.*, 2012; Jones *et al.*, 2014). This was demonstrated by Ruel & Menon (2002) using data from DHS in five Latin American countries. Feeding practices were significantly associated with HAZ in all seven datasets. This association was particularly strong in children aged 12-36 months and persisted even after controlling for potential confounders such as SES and maternal education in four out of the five countries.

Poor complementary feeding practices can be divided into three main aspects according to Stewart *et al.* (2013): namely 1) poor quality food, 2) inadequate practices and 3) unsafe food and water.

1. **Poor quality foods:** this includes lack of dietary diversity, lack of intake of animal protein foods and intake of foods with anti-nutrient properties for example phytates (Stewart *et al.*, 2013). In LMICs there is increasing consumption of unhealthy snack foods by infants and young children which displaces intake of more nutritious foods. Analysis of DHS data from 18 Asian and African countries found that 20% of infants aged 6-8 months were already consuming sugary snacks in one third of the countries studied. Sugar consumption increased with age with up to three quarters of Asian children and nearly a half of African children consuming these snacks in the second year of life. All the countries had a stunting problem in the under 5 population with prevalence ranging from 19%-58%. It was noted that sugary snack consumption was invariably more common than consumption of more nutritious foods such as eggs, vitamin A rich fruits or fortified infant cereals and recommendations were made to investigate the association of unhealthy snack food consumption with both stunting and overweight (Huffman *et al.*, 2014).

   Dietary diversity is increasingly used as an indicator of complementary diet quality and specifically micronutrient content (Allen, 2012). Secondary analysis of data from the 1999 South African NFCS found that dietary diversity was strongly related to both micronutrient adequacy of the diet and HAZ (Steyn *et al.*, 2006). This link between dietary diversity scores and micronutrient density of the diet was confirmed in a Madagascan study (Moursi *et al.*, 2008). A more recent South African study also reported increasing nutrient density of protein and a number of micronutrients with increasing dietary diversity (Faber *et al.*, 2016). The Latin America study described previously used a composite child feeding index that incorporated a dietary diversity score with six food groups (Ruel & Menon, 2002). A study using DHS data from 11 countries in Africa, Latin America and South Asia also used dietary diversity scores and found an association with HAZ. The dietary diversity score in this study was adapted to include seven food groups. The association of dietary diversity with increased HAZ remained after controlling for socioeconomic status. This independent association is important to determine as dietary diversity is strongly associated with socio economic status, which has well established links with child health and nutrition outcomes (Arimond and Ruel, 2004). This independent relationship of dietary diversity with HAZ was also found in a Zambian study (Mallard *et al.*, 2014).

Intake of animal protein foods is considered important as these foods are good sources of high quality protein as well as rich, bioavailable sources of a range of micronutrients including iron and zinc (Lobstein *et al.*, 2015; Allen, 2012). Studies in LMICs have shown that children who consume more animal protein foods are taller, heavier and have better cognitive outcomes than those who consume smaller amounts (Allen, 2012). Iron rich food intake at 6 months was positively associated with HAZ at 18 months in a Zambian birth cohort study (Mallard *et al.*, 2014). In addition to iron, adequate zinc intake is important for prevention of stunting (Krebs, 2007; Black *et al.*, 2013). Complementary feeding diets in developing countries are typically high in grains that are not only poor food sources of zinc but also contain large amounts of phytates which inhibit its absorption (Krebs, 2007; Stewart *et al.*, 2013).

Dairy products are also associated with better linear growth. Milk is a poor source of iron but a good source of calcium and zinc as well as bioactive factors. It stimulates insulin-like growth factor and insulin and contains growth stimulating peptides (Allen, 2012). An Indonesian study found a link between iron-fortified infant food consumption at 9 months and linear growth at 12 months. The iron fortified foods (formula, cereals and rusks) also contained significant amounts of milk powder. In contrast, consumption of iron rich flesh foods was not associated with linear growth, although this could be due to the fact that meatballs and sausages with a high cereal content were the main sources of meat. The authors postulated that the association of the iron-fortified foods rather than flesh foods with LAZ could be due to the growth promoting effect of the milk powder (Diana *et al.*, 2017). Intake of animal protein foods is often very low in poor communities due to the cost and lack of refrigeration facilities in the home. Additional factors limiting inclusion in the diet of infants and toddlers may be cultural or religious beliefs (Allen, 2012).

2. **Inadequate practices**: includes feeding too little food, not feeding often enough or feeding overly diluted foods which have low energy density. It also includes lack of responsive feeding or not feeding during and after illness (Stewart *et al.*, 2013). This aspect is also linked to lack of care and will be discussed in further detail in the next section, **2.5.1.3**.

3. **Unsafe food and water**: includes unsafe food preparation and storage practices as well as lack of access to safe water. These factors all increase the risk of diarrhoea, which, as

discussed previously, is a major risk factor for stunting (Stewart *et al.*, 2013). Aflatoxin contamination is particularly a problem with maize and groundnuts and has been linked to stunting, possibly through a mechanism of gut inflammation (Smith *et al.*, 2012).

Two of the aspects described, namely quality of complementary foods and feeding practices are assessed by the WHO Infant and Young Child Feeding Indicators (hereafter referred to as the IYCF Indicators) (WHO, 2010) which are based on international guidelines for both breastfed and non-breastfed infants (WHO, 2005; PAHO/WHO, 2003). The IYCF Indicators use a dietary diversity score, intake of iron rich or iron fortified foods and frequency of feeding to assess adequacy of complementary feeding. Breastfeeding indicators are also included (WHO, 2010).

The association of the IYCF Indicators with growth outcomes has been evaluated with mixed findings. A pooled analysis of data from 14 countries found lower risk of both stunting and underweight if the indicators for timely introduction of solid foods, minimum acceptable diet (MAD), intake of iron rich foods (IRF) and dietary diversity (MDD) were met (Marriott *et al.*, 2012). A later review used data from 9 countries but did not pool the data. It found lack of consistency across countries with some countries showing a positive relationship between MDD indicators and MAD and HAZ but others showing no association (Jones *et al.*, 2014). Both reviews found a negative association with continued breastfeeding at 12-15 months and child growth. Possible reasons for this unexpected finding could be reverse causality, that is, smaller, more vulnerable infants are breastfed for longer; or overreliance on breastfeeding at the expense of complementary diet quality (Marriott *et al.*, 2012; Jones *et al.*, 2014).

A recent analysis of DHS data from 39 countries used the IYCF Indicators 7 food groups to assess dietary diversity and also looked at consumption of animal source foods (ASF). It was found that increasing dietary diversity and ASF consumption was associated with lower risk of stunting and the authors recommended that MDD be set at 5 rather than 4 food groups (Krasevec *et al.*, 2017). The IYCF Indicators have also been used in South African studies, but these will be discussed later in this section in section **2.5.3.**

2.5.1.3 Lack of care

Lack of care which includes not taking the child for immunisations, neglect or lack of psychosocial stimulation has also been linked to stunting and is strongly correlated with maternal education (Black *et al.*, 2013). There is also an overlap with feeding practices, that is, responsive feeding, preparation of safe, nutritious foods, and frequency of feeding. Lack of maternal education was consistently associated with poor complementary feeding practices in five South Asian countries (Senarath *et al.*, 2012). Achieving IYCF Indicators in a study in Bangladesh was positively associated with maternal education. Mothers who had no education were at higher risk for not achieving any of the IYCF Indicators than those who had secondary or higher education (Kabir *et al.*, 2012). The Zambian study mentioned previously found a significant effect of maternal education on child growth that was independent of household wealth. The effect was mainly seen through the provision of a diverse diet but other ways in which maternal education improves child growth include increased autonomy and confidence in decision making and better access to and use of health and nutrition information and services (Mallard *et al.*, 2014).

2.5.2 Overweight

As with stunting, childhood overweight and obesity starts in the womb and a number of studies have implicated high pre-pregnancy BMI (Ziyab *et al.*, 2014; Weng *et al.*, 2012) or excessive gestational weight gain (Oken *et al.*, 2007) as risk factors for overweight in offspring. This is of particular concern in South Africa with a combined overweight and obesity prevalence in women of 64% (Shisana *et al.*, 2013). Overweight and obesity during pregnancy as well as gestational diabetes result in the foetus developing in an abnormal metabolic environment, increasing the risk of adverse metabolic changes and childhood obesity. This can continue into adolescence and adulthood thereby continuing obesity through the generations (Black *et al.*, 2013; Catalano, 2003). Maternal undernutrition has also been linked to later risk of overweight due to the "mismatch" pathway. Altered gene expression (epigenetic effects) can occur which enables survival in a nutrient poor environment but increases the risk of obesity later when there is a plentiful supply of energy dense foods as is typical in many LMICs which are undergoing or have undergone a nutrition transition (WHO, 2016a; Barker, 2012; Popkin *et al.*, 2011).

Maternal smoking has been identified as a risk factor for overweight in the offspring (Ziyab *et al.*, 2014; Weng *et al.*, 2012) with a meta-analysis of seven studies showing a 47% increased risk of childhood overweight in offspring of mothers who smoked compared to children of non-smokers (Weng *et al.*, 2012). This could be a result of rapid postnatal weight gain following foetal growth restriction but may also be due to the fact that maternal smoking is associated with other poor lifestyle choices (Weng *et al.*, 2012).

A prospective cohort study in the United Kingdom found that three year olds who watched more than eight hours of television per week and slept for less than 10,5 hours a day were at increased risk of developing obesity at 7 years of age (Reilly *et al.*, 2005). A systematic review and meta-analysis of thirty prospective studies found that, in addition to maternal overweight and smoking, high birthweight and rapid weight gain in the first year of life were associated with later childhood overweight and obesity. Feeding practices such as breastfeeding and later introduction of solid foods were found to be protective (Weng *et al.*, 2012).

The interactions of all these potential risk factors for childhood obesity are illustrated in Figure 2-3, which was developed by Monasta *et al.* (2010) from a review of systematic reviews on early life determinants of obesity. The authors acknowledged that causality was very difficult to prove as there are many potential confounders particularly with regards to socioeconomic status. They called on researchers to focus their interventions on these potential determinants as this could help confirm if there is a real effect and stressed the urgency of developing effective interventions to combat the increasing burden of overweight and obesity (Monasta *et al.*, 2010).

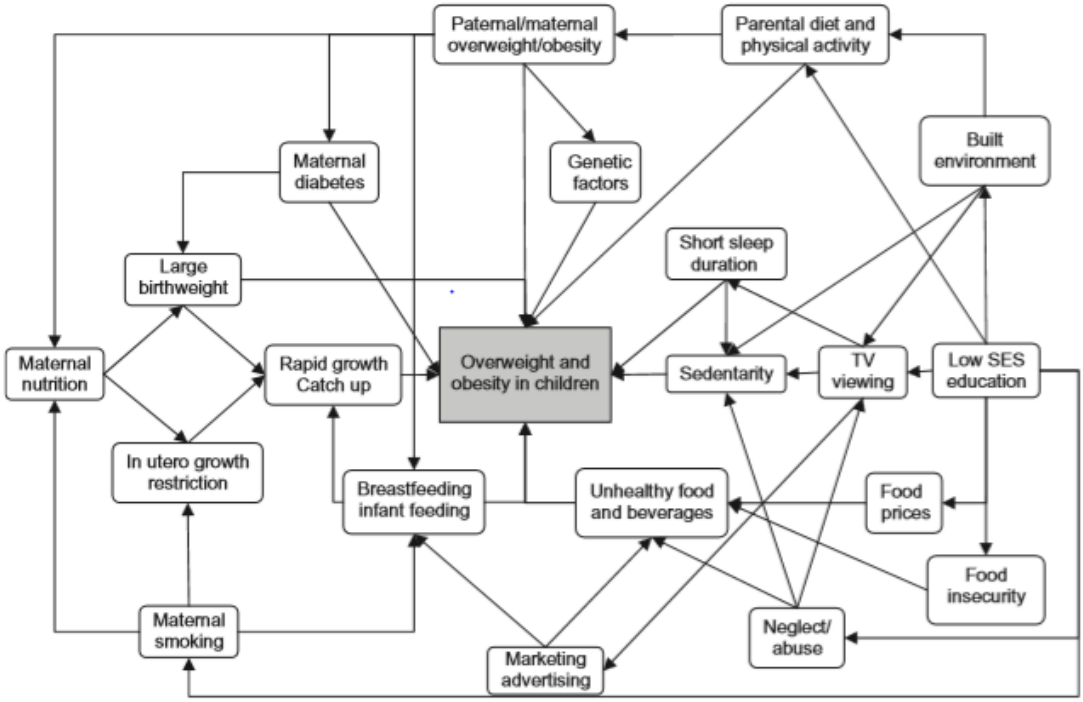

Figure 2-3: The complex web of potential determinants of overweight and obesity in children (Monasta *et al.*, 2010)

This review will now focus on the potential determinants of overweight and obesity that have been identified in infancy and early childhood: birthweight, rate of growth and feeding practices.

2.5.2.1 Birth weight

Systematic reviews have confirmed findings from individual studies of an association between high birth weight and risk of overweight in early childhood (Weng *et al.*, 2012; Monasta *et al.*, 2010). The Avon Longitudinal study found that every 100g increase in birth weight increased odds of overweight at 7 years by 1.05 (Reilly *et al.*, 2005). The nationally representative Millennium Cohort study, also conducted in the United Kingdom, reported a 1.4 Odds Ratio of obesity at 5 years of age for every 1kg increase in birth weight (Brophy *et al.*, 2009). Other studies have reported risks related to birth weight categories varying from > 3.85 kg to > 4.25kg (Weng *et al.*, 2012). This association can be complicated by the fact that obesity in later childhood in developed countries has been linked to rapid postnatal catch up growth in babies who have been born small due to intrauterine growth restriction (Monasta *et al.*, 2010; Ong *et al.*, 2000). A review of a number of studies concluded that the relationship appears to be U- or J shaped with both low and high birthweight associated with later obesity and that low

birthweight is more strongly associated with greater fat mass, a risk factor for cardiovascular disease (Yang & Huffman, 2013).

2.5.2.2 Rate of growth

The link between rapid infancy weight gain and adult overweight and obesity and NCD risk was discussed previously in section 2.4.2, Consequences of overweight in childhood. Its role in the development of childhood overweight and obesity will now be explored.

A meta-analysis using data from 10 birth cohorts totalling nearly 50 000 participants found a twofold higher risk of childhood obesity with each +1 increase in weight SD scores between birth and 1 year (Druet *et al.*, 2011). Increased risk was also seen with the >+0.67 SD increase that is commonly used to classify rapid growth (Ong & Loos, 2006) but the 21% of infants who had very rapid growth in the first year (>+1.33 SDS) had an almost fourfold risk of later childhood obesity (Druet *et al.*, 2011). This association was confirmed in a separate systematic review which included findings from six studies (Weng *et al.*, 2012). An association of rapid weight gain in the first two years with later adiposity was also demonstrated in a South African study using a subset of children from the Birth to 20 Cohort. Children who had >0.67 change in weight for age Z scores from birth to two years (20% of the sample) had higher BMIs and subcutaneous fat stores at nine years (Cameron *et al.*, 2003). A small study of 47 infants found that fat mass and central fat distribution at 12 months was more strongly associated with rapid weight gain in the first 6 months than in the 6 to 12 month period (Chandler-Laney *et al.*, 2013).

Other studies have looked at changes in weight for length or BMI and have also identified the birth to 6 months period as being of greater significance. An American study of 559 infants found increased risk of obesity and subcutaneous fat stores at 3 years in children who had experienced rapid increases in weight for length between birth and 6 months (Taveras *et al.*, 2009). Overweight, obese and morbidly obese 5 year olds in another American study had very different infant growth patterns compared to normal weight children. Overweight and obese 5 year olds had consistently higher BMIs than normal weight children from as early as 2-4 months of age and these BMIs were strongly correlated with BMI at 5 years of age (Ludington-Hoe *et al.*, 2013). As mentioned previously, a Chilean study found that greater gains in BMI starting as early as 6 months were associated with obesity and biochemical markers of CVD risk at 4 years of age (Corvalán *et al.*, 2009).

The consistent association of early rapid weight gain with later childhood obesity may be due either to nutrition or the environment or may be a genetic marker for future weight gain trajectory (Druet *et al.*, 2011). The "growth acceleration hypothesis" proposes that early and rapid growth, particularly in the first 6 months of life, programs the infant metabolic profile to be susceptible to obesity in later childhood and adulthood (Young *et al.*, 2012; Singhal, 2007). This association of rapid early growth with later obesity calls for earlier screening to identify high risk infants (Ludington-Hoe *et al.*, 2013).

The majority of these birth cohort studies have been conducted in HICs and the findings of an increased risk of childhood obesity with early rapid weight gain may not hold true in LMICs where there is a need to balance the short and long term risks and benefits of rapid postnatal catch up growth (Druet *et al.*, 2011). Analysis of COHORTS data has supported the beneficial effects of weight gain in the first two years of life on schooling outcomes, particularly in babies with a lower birth weight, but found that later weight gain did not have the same benefits (Martorell *et al*., 2009). The negative effects of rapid growth in later childhood, but not if the growth occurs before the age of two years, on cardio metabolic risk factors has also been discussed previously (Adair *et al.*, 2013). Therefore, in LMICs the emphasis should be on promoting growth, especially linear growth in the first two years. The Chilean and South African studies show that in LMICs that are more advanced in the nutrition transition, early excessive weight gain that is disproportionate to linear growth can lead to overweight and obesity and adverse biochemical markers in later childhood (Corvalán *et al.*, 2009; Cameron *et al.*, 2003).

2.5.2.3 Feeding practices

Feeding practices that have been implicated in the development of childhood obesity are formula feeding/lack of breastfeeding, early introduction of solid foods and inappropriate complementary foods.

a) Breastfeeding

The evidence of a protective effect of breastfeeding against childhood overweight has been mixed in cohort studies. Systematic reviews of the individual studies have shown that it has a moderately protective effect both in childhood and adulthood (Young *et al.*, 2012). A meta-

analysis of 10 studies found a 15% reduction in the odds of childhood overweight with breastfeeding (Weng *et al.*, 2012), similar to the findings of a previous review which found a 22% reduction (Arenz *et al.*, 2004). The benefit appears to be linked to duration, although again the evidence has been mixed with a number of studies finding no effect (Weng *et al.*, 2012). There was a significant reduction in risk of obesity at two years in children in a German birth cohort who were breastfed for more than 6 months compared to those breastfed for less than 3 months (Weyermann *et al.*, 2006). A systematic review found that for every month of additional breastfeeding there was a 4% reduction in risk of overweight (Harder *et al.*, 2005). A randomised controlled trial (RCT) in Belarus, the PROBIT trial, however found that prolonged duration of breastfeeding had no beneficial effect on height or adiposity at 6.5 years (Kramer *et al.*, 2007).

Breastfeeding duration may be particularly important in babies at high risk of obesity due to maternal factors such as high BMI or smoking. An American study found that high risk babies breastfed for less than 2 months were significantly more likely to gain weight rapidly in the first few months than high risk infants who were breastfed for longer periods (Carling *et al.*, 2015). Although breastfed babies who experience rapid early growth are also at risk for later obesity, this risk is less in those " rapid gainers" who are exclusively breastfed for at least 6 months (Young *et al.*, 2012).

As most birth cohorts are conducted in developed countries, there has been very little information on the association between breastfeeding and later risk of overweight and obesity in LMICs. Analysis of data from the previously discussed Pelotas, Brazil, cohort found no association between breastfeeding and obesity at 11 years of age. There was a small reduced risk of overweight in infants breastfed from 1 to 3 months. A limitation of the study was that due to very low levels of exclusive breastfeeding in the study sample in 1993, the effect of exclusive versus partial breastfeeding could not be analysed (Neutzling *et al.*, 2009). A cross-sectional study conducted in three Mexican cities in infants aged 5-24 months reported a protective effect of breastfeeding against overweight and obesity (Jimenez-Cruz *et al.*, 2010). In a Chinese study that compared feeding practices in normal weight vs overweight infants and young children, breastfeeding rates were significantly lower in the overweight group (Jingxiong *et al.*, 2009).

Determining the impact of breastfeeding on childhood overweight and obesity is extremely difficult as there are many potential confounders such as maternal education and socioeconomic status (Carling *et al.*, 2015; Young *et al.*, 2012). A strength of the Pelotas study was that there was very little risk of confounding by socio economic factors because breastfeeding duration was not associated with socioeconomic status and maternal education as is the case in cohorts in developed countries (Neutzling *et al.*, 2009). The findings of no association between breastfeeding and later adiposity in the PROBIT RCT is further evidence that confounding by socioeconomic factors may be responsible for the beneficial effects of breastfeeding reported in observational studies in HICs (Kramer *et al*., 2007).

There is also lack of consistency in the literature regarding the definitions of "exclusive" breastfeeding, "predominant" breastfeeding, "some" breastfeeding and "no" breastfeeding (Young *et al.*, 2012). Exclusive breastfeeding for 4-6 months compared to no exclusive breastfeeding was found to protect against overweight and obesity in later childhood in a study involving children from eight European countries. It is interesting to note that shorter periods of exclusive breastfeeding (1-3 months) did not have a statistically significant effect (Hunsberger *et al.*, 2012). A large Chinese cohort also found a protective effect of exclusive breastfeeding, with children exclusively breastfed for 3-5 months having a 13% reduction in risk of overweight at 4-5 years of age which increased to 27% if exclusive breastfeeding was maintained to 6 months (Zheng *et al.*, 2014).

There are a number of potential mechanisms to explain the protective effect of breastfeeding. Breastfed infants are better able to self-regulate their intake and tend to consume lower volumes than formula fed infants (Yang & Huffman, 2013; Young *et al.*, 2012). This ability to self-regulate could also extend beyond the breastfeeding period, making children more responsive to internal hunger and satiety cues. In contrast, formula fed infants tend to rely on caregivers to decide when feeding is over, "finish the bottle", which can condition them to overeat in later life (Moss & Yeaton, 2014). Infants enrolled in the Infant Feeding Practices Study who were predominantly fed expressed breastmilk from a bottle showed similar lack of self-regulation to those fed formula milk, indicating that it is the act of feeding from the breast that is important in establishing self-regulating behaviour (Li *et al.*, 2010).

In addition, formula has higher concentrations of most macro- and micro-nutrients compared to breastmilk. The combination of increased volume and higher energy density can lead to higher energy intake in formula fed infants (Young *et al.*, 2012). Higher energy intake at 4 months was associated with increased weight gain from birth to 2 years and increased BMI at 3 and 5 years in the Avon Longitudinal study. Each 420kJ/day increase in energy intake at 4 months of age was associated with 46% increased risk for being overweight at 3 years and 25% increased risk at 5 years of age. This association was only found in formula and mixed-fed infants due to the difficulties of assessing energy intake of breastfed infants (Ong *et al.*, 2006). The link between formula feeding and faster weight gain was also shown in the UK Millennium Cohort Study. Infants breastfed for 4 months or more gained weight slower than infants never breastfed or breastfed for less than 4 months (Griffiths *et al.*, 2009).

The higher protein content of formula has been associated with rapid postnatal weight gain in both preterm and term infants and higher fat mass in later childhood (Poskitt & Breda, 2012; Young *et al.*, 2012; Singhal *et al.*, 2010). A recent review of a number of trials highlighted the benefits of feeding a lower protein formula. Infants fed the low protein formula gained weight slower and had significantly reduced risk of overweight than infants fed the standard higher protein formula. Weight gain in the low protein group was similar to that of the breastfed controls and was not inferior to WHO growth standards (Haschke *et al.*, 2016). This has implications for formula composition in the future. Cow's milk protein appears to be specifically associated with risk of obesity (Poskitt & Breda, 2012) possibly through increasing blood levels of insulin and insulin-like growth factor which can promote synthesis of adipocytes (Hoppe *et al.*, 2004). Dairy protein and increased levels of insulin-like growth factor was discussed previously as it is also associated with improved linear growth (Diana *et al.*, 2017; Allen, 2012). Therefore in countries with high rates of stunting and predominantly vegetable diets the addition of animal protein, including dairy, to complementary feeding diets should be encouraged, provided the dairy does not displace breastmilk (Poskitt & Breda, 2012).

Apart from the differences in macronutrient content between breast- and formula milk, there are a number of bioactive factors in breastmilk such as leptin, insulin, ghrelin and adiponectin that may elicit endocrine effects and modulate growth (Weng *et al.*, 2012; Young *et al.*, 2012). The role of the microbiome in the development of obesity is a relatively new field. Breastmilk is a rich source of both probiotics, providing a continuous source of bacteria, and prebiotics, in

the form of oligosaccharides. The microbiome of a breastfed infant differs from that of a formula-fed infant. It is thought that the microflora of a formula-fed infant may be more efficient at extracting energy from carbohydrate and fat thereby contributing to more rapid weight gain (Thompson, 2012).

b) Age of introduction of solid foods

The evidence of a link between the early introduction of solid foods, often defined as < 4 months of age, is also contradictory with conflicting results in a number of studies. Formula fed infants enrolled in Project Viva, a longitudinal birth cohort study, had a six fold increase in the odds of being obese at the age of 3 years if solids were introduced before 4 months. There was no association with obesity and age of introduction of solids in children who were breastfed for at least 4 months (Huh *et al.*, 2011). A large American birth cohort study found that introducing solids before 4 months was associated with a 5-10% increased prevalence of obesity at 2 years and 4 years of age when compared to introducing solids from 4-5 months or 6 months. There appeared to be no increased benefit of delaying solid food until 6 months on obesity rates (Moss and Yeaton, 2014). However in a much smaller Australian study there was a 15% lower prevalence of obesity at 10 years in children who were introduced to solids at 24 weeks (6 months) vs. at 20 weeks or less (Seach *et al.*, 2010). In LMICs the COHORTs collaboration showed an association with early introduction of solid foods and higher weight at 2 years and adiposity in adulthood (Fall *et al.*, 2011). In a large Chinese cohort introduction of solids before 3 months was associated with higher BMI and overweight, but not obesity, at 4-5 years of age. It is interesting to note that early introduction of solid foods was very common with 65% of infants fed complementary foods before 3 months (Zheng *et al.*, 2015).

Other studies have shown no association between age of introduction of solid foods and later overweight. A recent analysis of data from the Infant Feeding Practices Study, an American birth cohort study, found that introduction of solid foods before 4 months was not associated with increased risk of obesity at 6 years when compared to introduction at 4-6 months. Breastfeeding status did not affect the results (Barrera *et al.*, 2016). Early introduction of solids was not associated with rate of weight gain from birth to 3 years in the Millennium Cohort Study once height at 3 years was taken into account (Griffiths *et al.*, 2009). The Pelotas birth cohort study found no association between starting complementary feeding before 4 months of age and obesity at 11 years (Neutzling *et al.*, 2009). In another birth cohort study in a LMIC

(Caleyachetty *et al.*, 2013) there was no association between age of introduction of solids and adiposity (measured as sum of skinfold thicknesses) at 5 years in children in the Mysore region of India. The Mexican cross sectional study mentioned previously compared overweight and obesity rates in infants started on solid food before 6 months and after 6 months and found no significant difference but did not consider the impact of starting before 4 months (Jimenez-Cruz *et al.*, 2010).

The conflicting evidence from these studies makes it very difficult to draw any conclusions. A systematic review on the effect of timing of solid food introduction and risk of later overweight concluded that there was no clear association but that very early introduction (≤ 4 months of age) may increase the risk (Pearce *et al.*, 2013). Potential mechanisms that could increase the risk of obesity include epigenetic modification of metabolic programming by specific foods or a hormonal influence e.g. increased ghrelin levels (Pearce *et al*., 2013). Infants fed solids early tend to have higher energy intakes which leads to rapid weight gain and high weight for length (Thompson & Bentley, 2013) and, as previously discussed, higher BMI in later childhood (Ong *et al.*, 2006).

c) Type of complementary foods

There is surprisingly little information on the relationship of nutrition composition of complementary foods to subsequent risk of obesity. The focus previously has been on breastfeeding duration and timing of introduction of solid foods and there is a need for more research into the effect of composition and quality of the complementary feeding diet (Young *et al.*, 2012).

A systematic review on the types of food introduced and risk of childhood overweight concluded that high energy and protein intake, particularly dairy protein, appear to be associated with higher BMI and body fat mass in later childhood. No specific foods or food groups were linked to childhood BMI. A varied, balanced diet as advised in national complementary feeding guidelines should be followed as this was associated with greater lean mass (Pearce & Langley-Evans, 2013).

The systematic review finding regarding increased risk of later childhood overweight and increased energy intake was based on the Ong *et al.* (2006) study which has been discussed

previously. Higher energy intake at 4 months predicted greater weight gain in the first few years of life and increased childhood obesity risk. Two other cohorts published subsequent to the review provide some further evidence. The U.S. Infant Care and Risk of Obesity birth cohort study found that inappropriate complementary feeding practices led to higher energy intakes (420kJ/day) and higher weight for lengths throughout the study period (birth-18 months), putting the infants at high risk for later childhood obesity (Thompson & Bentley, 2013). A large Chinese cohort study did not measure energy intake but found an association between overweight at 4-5 years and consumption of fish oil in infancy which the authors linked to increased energy intake and rapid weight gain in infancy (Zheng *et al.*, 2015). Although cross sectional, a Chinese study comparing feeding practices in overweight and normal weight infants and young children found that energy intakes were significantly higher in overweight children (Jingxiong *et al.*, 2009).

The link between high protein intake and later obesity risk has been demonstrated in a number of RCTs comparing high and low protein content infant formulas as discussed previously (Haschke *et al.*, 2016). There is also some evidence for a link between high protein content (as % of energy) of the complementary feeding diet and later childhood overweight and body fatness, although there have been conflicting results (Pearce & Langley-Evans, 2013). A German birth cohort study found that high animal protein intake, particularly dairy protein, at 12 months may be associated with higher fat mass at 7 years (Gunther *et al.*, 2007). The authors of the review concluded that higher intakes of protein in the second year of life may be a better predictor of obesity in childhood than intakes earlier in life (Pearce & Langley-Evans, 2013). It must be noted that the levels of protein associated with higher BMI in the 12-24 month period, 2.6-3g/kg/day, are more than double the FAO/WHO recommendations (Yang & Huffman, 2013).

A RCT published subsequent to the above mentioned reviews found that breastfed infants allocated to a predominantly meat complementary feeding regimen between 5-9 months of age had greater linear growth than infants allocated to the traditional infant cereal based regimen. The meat group also had increased WAZ but this was proportional to the gain in length so there was no difference in WFL Z scores between the two groups. Body fat percentage was not assessed. There was also no difference in insulin like growth factor-1 levels. The protein intake of the meat group was double that of the cereal group (2.9 vs 1.4 g/kg/day; 17% of energy vs

9% of energy) but caloric intake was virtually identical. These findings provide evidence that in breastfed infants in a developed country setting, high meat protein intake can support optimal linear growth without increasing adiposity as measured by WFL. The study was, however, very small with only 42 infants, but did collect very detailed dietary information. The findings may not be applicable to formula fed infants (Tang & Krebs, 2014).

The Pearce & Langley-Evans (2013) review found no link between carbohydrate and fat intake and later BMI or adiposity and these findings were confirmed in another review on the association of early nutrition with later obesity (Yang & Huffman, 2013). There was no evidence of a link between early micronutrient status and obesity but this is an area that requires more research (Yang & Huffman, 2013). The association of dietary diversity with overweight was investigated in a very small Canadian longitudinal study. Breastfed infants who were fed homemade complementary foods had greater dietary diversity and reduced adiposity at 12 months (measured by DEXA) compared to those who received commercial infant foods (Mok *et al.,* 2017). Dietary diversity was measured using the WHO IYCF Indicators.

Intake of inappropriate complementary foods such as sugar sweetened beverages (SSBs) and high fat snacks (HFS) has been identified as an increasing problem in LMICS (Contreras *et al.,* 2016; Huffman *et al.,* 2014) and was discussed previously in relation to its role in stunting by displacing more nutrient dense foods, in the diet and, in the food budget (Huffman *et al.,* 2014). It can also promote development of overweight through increasing energy intake and rapid weight gain in infants as well as the long term development of unhealthy eating habits such as preference for sweet and salty foods (Contreras *et al.,* 2016; Huffman *et al.,* 2014). These products are also often high in trans fats and sodium which have both been implicated in the development of NCDs (Huffman *et al.,* 2014). Inappropriate feeding practices, including intake of SSBs, chips and ice-cream were associated with increased energy intakes and weight for length in infants in the U.S. Infant Care and Risk of Obesity Study (Thompson & Bentley, 2013). The Mexican study discussed previously found that consumption of SSBs and HFS more than once a week was strongly associated with both overweight and obesity. The consumption of these foods was the greatest risk for obesity in the study population; greater than maternal BMI, no breastfeeding or early introduction of solid foods. The consumption of SSBs and HFS started before 6 months of age and by the second year of life, 60% of children were consuming these items at least once a week (Jimenez-Cruz *et al.,* 2010).

Fruit juice, which is often considered by caregivers to be a healthy drink for infants and young children, has also been linked to childhood obesity. The American Academy of Pediatrics recently updated its guidelines to recommend that fruit juice not be given to infants younger than one year and limited to not more than 120-180ml per day in children aged 1-6 years (Heyman & Abrams, 2017). It appears that fruit juice may act as a "gateway" drink to juice and SSB consumption in later childhood. This was demonstrated in the Project Viva cohort; higher juice intake at one year was associated with higher juice and SSB consumption and BMI Z scores in later childhood. Early juice intake can establish a preference for sweet beverages which sets a pattern for later SSB consumption (Sonneville *et al.*, 2015).

Apart from the nutritional content of the complementary diet, responsive feeding practices, previously discussed in relation to stunting, have been found to be protective against rapid early weight gain. Excess parental control of the amount of food eaten can override the infant's ability to self-regulate which has implications for eating habits beyond the infancy period (Redsell *et al.*, 2016; Daniels *et al.*, 2012).

In developed countries childhood overweight is more prevalent in children living in households of low SES but this relationship is less clear cut in developing countries. A study using data from a large, nationally representative longitudinal survey in America found that much of the disparity in obesity rates is due to the clustering of poor infant feeding practices in low SES mothers. The three major factors identified were lack of breastfeeding, early (< 4 months) introduction of solids and putting the baby to bed with a bottle. Low socioeconomic status increased the risk of childhood obesity by 20% but this risk could be reduced to 7% if healthy infant feeding practices were followed with predominant breastfeeding being particularly important. This emphasises the importance of supporting healthy infant feeding practices in low socioeconomic settings in developed countries (Gibbs & Forste, 2013).

2.5.3 Determinants of stunting and overweight: the South African perspective

South Africa, as with other LMICs, has a double burden of malnutrition with high levels of both stunting and micronutrient deficiencies together with an increasing prevalence of overweight in children under five years (Shisana *et al.*, 2013). In LMICs stunting and overweight share many determinants. Findings from three prospective cohort studies

(Limpopo, Drakenstein and Promise-EBF) and three cross-sectional studies (KZN MRC, Mpumalanga and MRC/HST) that explored associations between infant feeding, maternal and household factors, and anthropometric status are summarised in Table 2-4. All the studies reported high rates of both stunting and overweight with the exception of the Promise-EBF study which only reported on overweight. This section will discuss the associations with overweight and stunting reported on in the studies presented in Table 2-4 and will include additional relevant information from other South African studies. These studies were not included in the table as they did not investigate associations with growth outcomes but they help to provide a more complete account of IYCF practices in South Africa.

Table 2-4: Determinants of stunting and overweight: South African studies

Study details	Description of study	Prevalence of stunting and overweight	Determinants of stunting	Determinants of overweight
Central Region Limpopo: 2001 (Mamabolo *et al.*, 2004) (Mamabolo *et al.*, 2005)	Prospective birth cohort Rural villages in Limpopo n=162	**12 months** Stunting: 34.6% Overweight and obese:12.3% **36 months** Stunting: 48% Overweight and obese: 46% Stunted and overweight: 19%	Lack of electricity. Larger household size. Student mother. Short maternal stature. Lower length at one year.	Working mother. Rapid weight gain in first year of life.
MRC (Faber & Benade, 2007)	Cross sectional survey Rural KZN; Valley of a Thousand Hills 6-12 months infants n=505	Stunting: 16% Overweight: 23%	Increased CRP levels- may indicate HIV infection. Home gardens associated with increased LAZ.	
HST/MRC survey 2003 (Lesiapeto *et al*, 2010)	Cross sectional baseline survey 0-60 months children OR Tambo and Alfred Nzo districts,, Eastern Cape; Umkanyakude and Zululand districts, KZN n=2485	Stunting: 28.6% Overweight: 16.1%	Male gender. Household wealth index was protective against stunting. Maternal education not associated.	Higher maternal BMI. Household has no regular source of income. Low maternal education- protective against overweight but increased odds of underweight.
Agincourt ; 2007 (Kimani-Murage *et al*, 2010) (Kimani-Murage, 2013)	Cross sectional survey Agincourt sub district, Mpumalanga Province Rural. Children 1-4 years n=671	Stunting: 18% Overweight: 8%	HIV infected infant. Low birthweight. Young maternal age. Mozambican origin.	Older maternal age. Less than secondary certificate of household head. Higher SES. Adequate food security.

Table 2.4 continued.

Study; date of study; authors	Description of study	Prevalence of stunting and overweight	Determinants of stunting	Determinants of overweight
Promise-EBF trial 2006-2008 (Ramokolo *et al.*, 2015)	Longitudinal birth cohort Data from 3 sites (Paarl, Rietvlei and Umlazi) Anthropometric status assessed at 2 years n=641	Overweight: 30% Obesity 8.7%		Not breastfeeding at 12 weeks associated with increased risk of rapid weight gain 12-24 weeks and increased overweight and obesity at 2 years.
Drakenstein Child Health Study 2012-2014 (Budree *et al.*, 2017 a; Budree *et al.*, 2017 b)	Longitudinal birth cohort Multi ethnic population, low resource district surrounding Paarl, Western Cape Province 12 months old infants n= 342	Stunting: 13% Overweight: 9%	Birthweight. Alcohol and tobacco use in pregnancy. SES. Mixed race ethnicity. Longer duration of exclusive breastfeeding was associated with lower HAZ score. Poor infant feeding practices but no association with stunting.	Poor infant feeding practices but no association with overweight. Higher prevalence in black African infants. No association with birthweight.

2.5.3.1 Sociodemographic factors

Although there were no consistent findings, low birthweight and indicators of low SES tended to be associated with stunting. Higher SES was associated with overweight in the Agincourt study but not in others and in the MRC/HST study lack of regular source of income was associated with increased risk of overweight. Maternal alcohol use and smoking were particularly high in the Drakenstein cohort; 18% and 31%% respectively and were associated with both lower birthweight and poor infant growth postnatally (Budree *et al.*, 2017b).

2.5.3.2 HIV exposure

HIV infection was associated with stunting in the Agincourt study (Kimani-Murage, 2013) and was suspected as a cause of the high CRP levels associated with stunting in the rural KZN MRC study (Faber & Benade, 2007); both studies were conducted prior to ARV programme accessibility.

Growth failure is well documented in untreated HIV infection (Venkatesh *et al.*, 2010) but infant HIV infection is less of a factor in stunting in the current era of reduced mother to child transmission and early detection and treatment of infected children as part of the Prevention of Mother to Child Transmission (PMTCT) programme (DoH, 2015a). The growth of HIV uninfected but exposed children was compared to that of HIV unexposed children in the Vertical Transmission Study (VTS) conducted in KZN province. Growth patterns of the HIV exposed but uninfected children were as good as those of HIV unexposed children. This is an important finding as up to 40% of children are HIV exposed in Southern Africa (Patel *et al.*, 2010). However, there were high rates of exclusive breastfeeding in the study as mothers were supported to practise exclusive breastfeeding for 6 months and those few who were formula feeding were carefully counselled and were living in conditions that allowed for safe formula feeding (Patel *et al.*, 2010). Of concern is the fact that outside of the research setting described in the VTS, many HIV positive mothers choose to formula feed their infants. A small study conducted in 2011 in Worcester in the Western Cape reported that none of the HIV positive mothers (self- reported) had initiated breastfeeding (Goosen *et al.*, 2014).

The Drakenstein study conducted between 2012-2015, also in the Western Cape, found that less than half of HIV positive mothers initiated breastfeeding (Budree *et al.*, 2017 a). Exclusive formula feeding was practised by 50% of HIV positive mothers in the Gert Sibande district of

Mpumalanga province with only 35% exclusively breastfeeding at 3-6 months of age (Ladzani *et al.*, 2011). These feeding practices may reflect prior Department of Health policy that provided free infant formula to HIV positive mothers (DoH, 2007). Since 2011, as part of the Tshwane Declaration for the support of breastfeeding in South Africa, free supply of formula was discontinued and exclusive breastfeeding for 6 months and continued up until one year together with provision of maternal ARVs was advised (DoH, 2013b; Tshwane Declaration, 2011). Subsequent guidelines recommend continued breastfeeding up to 2 years, in line with guidelines for HIV uninfected mothers (DoH, 2017a).

The effect of exposure to maternal anti-retroviral treatment on infant growth has been investigated. A systematic review of trials investigating potential effects of maternal ARV therapy on HIV exposed but uninfected children found mixed results. Some studies reported increased rates of prematurity and low birthweight while others did not find an effect. Lower LFAZ were reported in some studies but the authors of the review concluded that the effects were transient. They also pointed out the difficulty of determining whether any effects are due to the drugs themselves or to exposure to the virus in utero (Heidari *et al.*, 2011). A later study investigating the use of Tenofovir (TDF), which is one of the anti-retrovirals used in the South African PMTCT programme, found no increased risk of low birthweight or SGA. There was a slightly lower mean head circumference and LAZ at 12 months in infants exposed to a TDF regimen (Siberry *et al.*, 2012).

2.5.3.3 Breastfeeding practices

Inappropriate breastfeeding practices have been linked to both stunting and overweight. The Promise-EBF prospective cohort study found that infants who were not breastfed at 12 weeks had increased rates of rapid weight gain from 12-24 weeks which predicted increased prevalence of overweight and obesity at 2 years (Ramokolo *et al.*, 2015). This link between rapid weight gain in the first 6 months of life and subsequent childhood obesity was discussed previously as well as the increased risk of rapid weight gain in formula fed infants. The only other study from Table 2.4 to explore the association of breastfeeding practices with anthropometric outcomes was the Drakenstein study which found that longer duration of exclusive breastfeeding was associated with lower HAZ at one year (Budree *et al.*, 2017 a). It is not clear whether this reflected exclusive breastfeeding beyond six months.

The other studies describe a similar pattern of breastfeeding practices in many different regions of South Africa. Typically there are high breastfeeding initiation rates, ranging from 86% in the Drakenstein study to 96% in the rural KZN MRC study (Faber & Benade, 2007), followed by early introduction of other fluids and/or solid foods. Breastfeeding is commonly practised, with over 80% of infants in both the Limpopo and KZN studies still breastfed at 9-12 months of age, but exclusive breastfeeding under 6 months of age is the exception. The South African Infant and Young Child Feeding (IYCF) Policy recommends exclusive breastfeeding for the first 6 months, followed by introduction of complementary foods and continued breastfeeding up to 2 years (DoH, 2013b), but there appears to be little adherence to them. The Limpopo study reported rates of exclusive breastfeeding at 3 months of age of only 10% and by 1 month 64% of infants were already receiving formula and/or solids in addition to breastmilk (Mamabolo *et al.*, 2004). The rural KZN MRC study had similar results with exclusive breastfeeding rates of 11 % at 4 months (Faber & Benade, 2007) while in the Drakenstein study 13% of infants were exclusively breastfed for 6 months or longer with higher rates (26%) in those HIV positive mothers who had initiated breastfeeding (Budree *et al.*, 2017a).

These findings of low exclusive breastfeeding rates in the studies in Table 2-4, echo those of other studies documenting feeding practices in South African infants (MacIntyre *et al.*, 2005; Goosen *et al.*, 2014). The MacIntyre *et al.* (2005) study conducted in Gauteng in 1997-1998 found almost universal breastfeeding, with 99% of infants under 9 weeks of age receiving breastmilk, but less than 5% were exclusively breastfed. When comparing this earlier study with more recent ones, there does appear to be an increase in exclusive breastfeeding rates; the most recent DHS conducted in 2016 reported rates of 32% (DoH *et al.*, 2017) which is similar to the 38.5% of infants in the Breede Valley study (du Plessis *et al.*, 2016), although the authors felt that this reflected over-reporting.

Formula use, apart from being associated with rapid weight gain in early life and increased risk of childhood obesity, can also contribute to poor infant growth. This is particularly a risk in poor communities where over dilution of formula is common due to cost constraints. In the MacIntyre *et al.* (2005) study, 10% of mothers over diluted infant formula while this practise was much higher in the Goosen *et al.* (2014) study at 45% and even higher at 66% in a study conducted in rural and urban KZN (Faber *et al.*, 2016). Apart from the risk for stunting from decreased nutrient intake, formula fed infants are also at increased risk of diarrhoea from

bacterial contamination of formula feeds. The role of repeated episodes of diarrhoea in the development of stunting has been discussed previously. Unsafe preparation practices were documented in the Goosen *et al.* (2014) study, with some mothers reporting that night feeds were stored in a flask and one big bottle was mixed for the whole day. High levels of faecal bacteria as well as significant over dilution of feeds were found in an urban/ peri-urban setting in KZN (Andresen *et al.*, 2007).

2.5.3.4 Complementary feeding practices

Complementary feeding practices in South Africa are also often poor with early introduction of solid foods, lack of dietary diversity and regular consumption of inappropriate high fat/ salt/ sugar snacks and sugar sweetened beverages (du Plessis *et al.*, 2013). These feeding practices have been implicated in the development of both stunting and overweight.

Age of introduction of solid foods

By 1 month of age 44% of infants in the Limpopo study had received solid foods, increasing to 80% of 3 month old infants. Force feeding of infants by pushing food down the infant's mouth was practised by close to 30% of mothers which is completely contrary to the principles of responsive feeding and encouraging self-regulation of intake. This study reported exceptionally high overweight and obesity rates in three year old children (Mamabolo *et al.*, 2004; Mamabolo *et al.*, 2005). Similar patterns of very early introduction of complementary foods were reported by MacIntyre *et al.* (2005) with 37% of infants younger than 9 weeks having already received solids, most of them before they were 7 weeks old. This study was conducted in 1997-1998 but in the Promise-EBF study conducted 10 years later almost two thirds of infants were receiving cereal by the age of 12 weeks and 28% had already received it by 3 weeks of age (Ramokolo *et al.*, 2015) while Faber & Benadé, (2007) reported that 61% of infants had received solid foods before the age of 4 months. The Faber *et al.*, (2016) study conducted in 2011 reported average age of introduction of solids of 3.5 months in rural areas and 4.2 months in urban areas. The more recent Drakenstein study which reported on data collected between 2012 and 2014 found lower rates of early introduction of solid foods; 19% before 4 months (Budree *et al.*, 2017a). This may indicate a recent trend towards later introduction of solid foods, corresponding with the increase in EBF rate reported in the DHS (DoH *et al.*, 2017)

Nutritional adequacy of complementary foods

Lack of dietary diversity with poor micronutrient intake has been widely reported with soft maize meal and other starchy foods making up the bulk of the typical complementary feeding diet in South Africa (du Plessis *et al.*, 2013). Although maize meal in South Africa is fortified with a range of micronutrients, including iron, zinc and folate, the amounts eaten by infants and young children are too small to have a significant impact on micronutrient intake and to improve micronutrient status (Faber, 2005).

The Limpopo study analysed nutrient intakes at 1 and 3 years and found that the diet was energy dense but lacking in micronutrients, particularly iron, zinc and calcium. Carbohydrate comprised 70% of energy intake while only 20% of energy came from fat. Although protein intake was adequate it was mainly in the form of plant protein and therefore low in iron, zinc and lacking some essential amino acids. The high phytate content of the diet also contributes to lower absorption of iron and zinc. This poor nutritional intake is reflected in the nutritional status of the children; apart from the high stunting and overweight prevalence previously discussed, biochemical deficiencies of both iron (33%) and folate (20%) were present (Mamabolo *et al.*, 2006).

This combination of poor micronutrient intake, high rates of stunting (16%) and overweight (23%) at 6-12 months of age, and biochemical deficiencies of iron (35% iron deficiency anaemia), zinc (32%) and vitamin A (20%) was also reported by Faber & Benadé (2007) and Faber (2005) in rural KZN. As with the Limpopo study, intakes of calcium, iron and zinc were particularly low, reflecting the poor intakes of animal and dairy products. In the 24 hour dietary recall period only 17% of infants had consumed animal products, 26% dairy products and 18 % vitamin A rich fruit or vegetables. In contrast, 82% of infants had consumed maize meal porridge. The addition of high energy foods e.g. sugar, margarine, peanut butter to the porridge was common and probably inappropriate given the high rates of overweight in the study population. Intake of micronutrients was significantly higher in infants consuming infant products i.e. infant cereals, jarred foods and infant formula powder, with infant cereal contributing 51% of the iron intake of infants that consumed them. Jarred foods contributed far less to micronutrient intake than cereals.

IYCF Indicators

Four more recent studies have assessed complementary feeding practices using the Infant and Young Child Feeding Indicators that have been discussed previously. The Breede Valley study found that only 44% of children aged 6-23 months achieved adequate dietary diversity; 71% were fed the appropriate number of times in a day and 44% received a minimum adequate diet. Iron rich foods were consumed by 55% of infants and 89% received iron either in the form of food or a supplement (du Plessis *et al.*, 2016). The Drakenstein study had much higher rates of adequate dietary diversity with 75% of infants consuming foods from 4 or more groups by 12 months of age although this figure was much lower at 6 and 9 months. Data on feeding frequency was not collected so the minimum adequate diet indicator could not be assessed. Dairy intake was good with 92% of infants consuming dairy daily at 12 months. Animal food intake was also much higher than in previous studies at 77% but of concern was that this was mainly in the form of processed meats, high in fat and sodium (Budree *et al.*, 2017a). The Faber *et al.* (2016) study found that less than 25% of infants achieved adequate dietary diversity. This study also assessed nutrient intake and once again confirmed that iron, calcium and zinc are problem nutrients but they also reported low intakes of niacin. Higher dietary diversity scores were associated with higher nutrient density of protein and a number of micronutrients as was discussed previously. The nationally representative DHS also used the IYCF Indicators and found that only 23% of infants aged 6-23 months received a minimum adequate diet (DoH *et al.*, 2017).

Consumption of inappropriate foods

The last aspect to consider when discussing the complementary feeding diet is the intake of inappropriate foods. As was discussed previously, this can impact both on stunting, by displacing micronutrient rich foods in the diet, and on overweight, by increasing energy intake and establishing unhealthy taste preferences that track into childhood and later life. The Limpopo study reported that by 3 years of age sweets and crisps were the third and fourth most commonly consumed items, milk did not make it into the top 20 with tea being more often consumed. This is of concern given the inhibitory effect of tannins in Ceylon tea on iron absorption (Mamabolo *et al.*, 2006). The rural KZN study found that savoury snacks were consumed by 42% of 6-12 months old infants most days of the week and biscuits by 27%. Carbonated drinks were consumed on most days by 12% of infants with a further 26% having them once a week. Tea consumption was also common (Faber & Benadé, 2007). The Faber *et*

al. (2016) study highlighted the early introduction of chips/savoury snacks with over 50% of infants already consuming them at least once a week from 6-12 months of age, rising to over 80% in the second year of life. Carbonated beverage, tea and cakes/ biscuits were also regularly consumed from 12 months of age. The Drakenstein study found high intake of inappropriate food consumption. At 6 months 13% of infants were drinking fruit juice daily. By the age of one year over half of infants were drinking carbonated beverages or eating refined sugary foods daily while a third consumed crisps daily (Budree *et al.*, 2017a).

The poor nutritional status of South African children is not surprising when considering the poor breastfeeding and complementary feeding practices described. There is little adherence to the South African IYCF Policy which recommends that mothers exclusively breastfeed their children for the first six months, followed by the introduction of adequate safe and appropriate complementary foods with continued breastfeeding up to two years and beyond (DoH, 2013b).

The determinants of both childhood stunting and overweight in LMICs are rooted in underlying socioeconomic conditions, food systems and health care systems and therefore tackling these issues requires comprehensive interventions that address more than the immediate causes of inadequate feeding practices.

2.6 Prevention strategies for childhood stunting and overweight in LMICs

2.6.1 Global context

Interventions to promote optimal growth are vital due to the difficulty of reversing early growth faltering (Dewey & Begum, 2011) and of treating overweight and obesity in childhood and beyond (Lobstein *et al*., 2015). Both stunting and overweight also have serious consequences in childhood and adulthood and into the next generation making prevention even more imperative. The Lancet 2008 Maternal and Child Undernutrition Series introduced the concept of focusing nutrition interventions on the crucial first 1000 days, from conception to two years of age, as this is the period during which irreversible and lasting damage is done and when interventions can have the greatest impact (Black *et al.*, 2008). While the 2008 Series only addressed undernutrition, including stunting, the Lancet 2013 Maternal and Child Nutrition Series acknowledged the increasing problem of overweight and obesity in LMICs; the double burden of malnutrition. It re-emphasised the importance of focusing interventions on the first

1000 days, even extending it to encompass female adolescence, and highlighted the importance of this time period for prevention of both stunting and overweight (Black *et al.*, 2013).

The double burden of malnutrition was also recognised in the WHO 2025 Global Nutrition Targets. Target number 1 calls for a 40% decrease in the prevalence of stunting while target number 4 is no increase in childhood overweight (WHO, 2012). The recognition of the global problem of childhood overweight and obesity led to the establishment in 2014 by the WHO of the Commission on Ending Childhood Obesity (ECHO). The final recommendations were released in 2016 and have three strategic objectives. The first is to tackle the obesogenic environment and norms. The second is to adopt a life cycle approach to obesity prevention and the third is to provide effective treatment for overweight children (WHO, 2016a). The second objective emphasises the importance of interventions focused on three critical time periods namely preconception and pregnancy; infancy and early childhood (these two time periods therefore encompass the first 1000 days); and later childhood and adolescence (WHO, 2016a). All these global recommendations therefore reinforce the importance in LMICs of interventions in the first 1000 days that promote linear growth while preventing obesity.

Given the complex web of determinants of both stunting and overweight that was described previously, it is obvious that nutrition specific interventions alone will not solve the malnutrition problems in LMICs. The 2013 Lancet series emphasised the importance of including nutrition sensitive programmes for example: water and sanitation; agriculture and food security; female empowerment and access to health care; as well as creating an enabling environment through legislation and policies (Black *et al.*, 2013). This is illustrated in Figure 2-4.

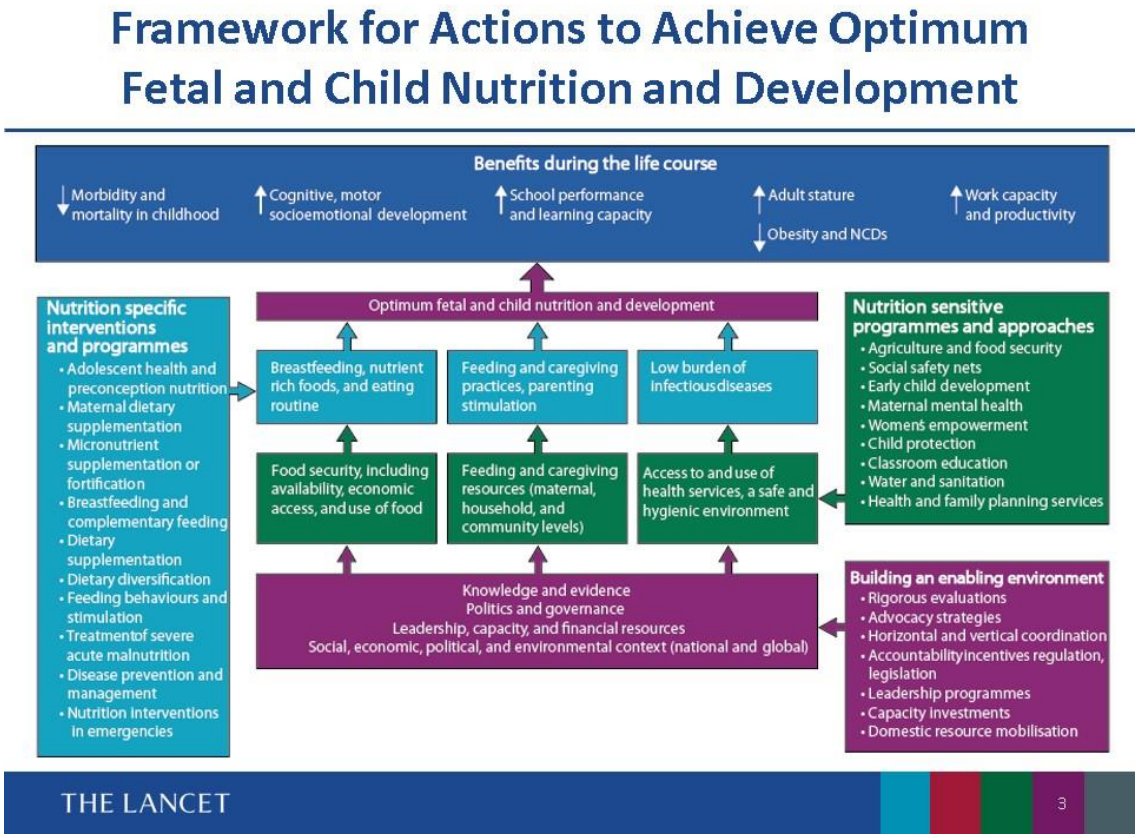

Figure 2-4: Framework for actions to achieve optimum foetal and child nutrition and development (Black *et al.*, 2013)

Brazil is an example of a country that has successfully reduced stunting rates through nutrition sensitive programmes as well as nutrition specific interventions such as breastfeeding promotion (de Onis *et al*., 2011). Stunting rates decreased from 34% in 1986 to 6% in 2006 and the success can be attributed to four main reasons: increased purchasing power of low-income families; improved educational level of mothers; improvements in water and sanitation; and virtually universal basic health care. Mexico achieved similar results through a programme of expanded and better targeted cash grants to poor families and improved access to health care (de Onis *et al.*, 2011).

Nutrition specific interventions aimed at IYCF practices include breastfeeding and complementary feeding. As was discussed previously, while breastfeeding is important for child survival and lays the foundation for appropriate timing of complementary feeding, it does not significantly improve stunting rates (Giugliani *et al.*, 2015; Black *et al.*, 2013). There is mixed evidence regarding the role of breastfeeding in the prevention of overweight/ obesity. It

appears to have a small benefit in observational studies (Weng *et al*., 2012) and a systematic review and meta analyses of breastfeeding promotion intervention studies also reported a modest but significant reduction in BMI z scores (Giugliani *et al*., 2015). In contrast, a systematic review of randomised controlled trials of interventions aimed at reducing risk of overweight and obesity in infancy and early childhood found no positive effect of breastfeeding (Redsell *et al.*, 2016).

Complementary feeding interventions have also had modest effects on stunting. A systematic review on the topic (Lassi *et al*., 2013) confirmed the findings of a previous review (Dewey & Adu-Afarwuah, 2008). The most effective interventions were: providing educational messages about feeding animal source foods, in areas where they are available and affordable; provision of food supplements and increasing energy density of foods in areas where the traditional complementary food has a low energy density. Micronutrient fortification alone had little or no impact on linear growth (Dewey and Adu-Afarwuah, 2008) although more recent research has found that the use of micronutrient powders appears to improve complementary feeding practices and micronutrient intake (Siekmans *et al*., 2017). Nutrition specific interventions on their own are unlikely to have a large effect on stunting and there is a need for studies examining an integrated approach; combining nutrition specific interventions with nutrition sensitive interventions such as water, sanitation and hygiene as well as care for mothers and children (De Onis *et al.*, 2013). The need for integrated interventions to address the complexity of linear growth faltering in impoverished children was well illustrated in a trial conducted in four countries with high stunting prevalence (Krebs *et al*., 2012). Infants were randomised to receive either meat or a micronutrient fortified cereal, starting at 6 months and continued through 18 months. There was no difference in length gain between the two groups but what was most striking was that neither intervention prevented the increase in stunting rates from ~30% at 6 months to ~50% at 18 months (Krebs *et al*., 2012). The lack of an effect of nutrition supplementation interventions on stunting outcomes, together their potential to increase the risk of overweight, was acknowledged by the WHO in guidelines aimed at addressing stunting and wasting in the context of the double burden of malnutrition (WHO, 2017). The guidelines recommend that caregivers of stunted children receive general nutrition counselling, applicable to all children under 5 years, but advise against routine provision of nutrition supplementation. This recommendation has also been incorporated into the KZN DoH Standard Operating Procedures on the Prevention and Management of Malnutrition in KZN (KZN DoH, 2018).

The previously mentioned systematic review on obesity prevention interventions found that complementary feeding interventions, specifically those that included responsive feeding aspects showed some promise in reducing the risk of obesity. Use of lower protein formulas, had a positive effect on weight outcomes as was discussed previously (Redsell *et al.*, 2016).

The ECHO report also highlighted the need for an approach to childhood obesity prevention that goes beyond nutrition specific interventions with its first strategic objective: tackling the obesogenic environment and norms. It recommends that governments need to take action i.e. through food, agriculture and trade policies that ensure availability of healthy, affordable foods; taxation of unhealthy food and beverages (SSB Tax in particular); restriction of marketing and advertising of unhealthy foods to young children and their parents; and taking steps to ensure that the built environment is conducive to physical activity. Other aspects include nutrition education and food labelling (WHO, 2016a). In a paper for the 2015 Lancet Obesity Series, by? (Lobstein *et al.*, 2015), the authors call for stricter controls on promotion of commercially prepared complementary foods. They caution against the increasing use of these foods which replace family foods in the diet and accustom infants' taste buds to highly processed foods and sweetened juices.

2.6.2 South African policies and strategies

The South African Roadmap for Nutrition (DoH, 2013a) and the Strategy for the Prevention and Control of Obesity in South Africa (DoH, 2015b) provide the framework for policies that address both stunting and overweight.

The Roadmap is aligned with the nutrition specific interventions outlined in Figure 2-7 and includes interventions to promote healthy weight gain in pregnancy; optimal infant and young child feeding practices through the IYCF Policy; micronutrient supplementation and fortification programmes for pregnant women and children; and treatment of acute malnutrition (DoH, 2013a). South Africa has focused much attention on breastfeeding promotion. The Tshwane Declaration affirmed that South Africa is a country that protects, promotes and supports breastfeeding (Tshwane Declaration, 2011). Over 70% of public hospitals are MBFI accredited and the Code of Marketing of Breastmilk Substitutes in South Africa was legislated in 2012 (DoH, 2012).

The Strategy for Prevention and Control of Obesity in South Africa incorporates many aspects included in the ECHO Report. It aims to tackle the obesogenic environment through a number of strategies including legislation e.g. the newly introduced tax on SSBs; norms and standards for sugar and fat content of processed foods; nutrition content of foods displayed at restaurants; restrictions on marketing and advertising of unhealthy foods to children as well as taking action to increases access and availability of vegetables and fruits. There is also a goal to facilitate physical activity. As with the ECHO report, a life cycle approach to obesity prevention is an integral part of the Strategy. The maternal, infant and young child nutrition interventions described in the Roadmap are incorporated, namely healthy weight gain during pregnancy, breastfeeding promotion and healthy complementary feeding practices with an explicit focus on obesity prevention. The draft Paediatric Food Based Dietary Guidelines for South Africa were recently field tested and contain clear messages aimed at promoting optimal growth in the complementary feeding period and beyond (du Plessis, 2018). In addition, early detection of overweight at routine growth monitoring visits and inclusion of obesity prevention messages in the updated Road to Health booklet are further strategies along with healthy eating at Early Child Development centres (DoH, 2015b).

Nutrition sensitive interventions include high rates of access to safe water of 93%, although improved sanitation figures are much lower at 66% (WHO, 2016b). Health care is free for pregnant women and children (Chopra *et al.*, 2009). There is a widely accessed social grant system, with the most commonly accessed grant being the Child Support Grant, which has been found to have a positive impact on HAZ (Heinrich *et al.*, 2012).

As can be seen, South Africa has a comprehensive set of both nutrition specific and nutrition sensitive interventions and yet stunting rates remain stubbornly high and overweight and obesity continue to increase. High rates of poverty and unemployment no doubt play a role, with SANHANES-1 reporting that less than half the population (45.6%) is food secure. The nutritional quality of the diet was also poor with 40% of the population having a DDS less than 4 (Shisana *et al.*, 2013). As a result of the nutrition transition there has been a shift away from traditional diets based on unrefined grains and legumes to a diet high in sugar, refined grains and cheap vegetable oils (Popkin *et al.*, 2011). Energy dense but nutrient poor, highly

processed foods are widely available and cheap while healthier foods such as vegetables, fruit, lean meats and fish are expensive and often not available in rural areas (Igumbor *et al.*, 2012).

An independent report evaluating the implementation of nutrition interventions found that nutrition policies are often poorly implemented due to staff shortages and lack of staff training on nutrition. Breastfeeding and complementary feeding both scored very poorly on implementation effectiveness. Poor inter sectoral collaboration between the Departments of Health, Agriculture and Social Development was reported. KwaZulu-Natal was singled out in the report for its significant improvements in stunting rates. It is the only province where nutrition is managed at a Directorate level, giving it a higher profile. Nursing staff in the province had significantly better knowledge on key nutrition messages when compared to other provinces and there was a more appropriate budget for nutrition (du Plessis, 2015; DoH *et al.*, 2014). A recent review of South African interventions aimed at reducing maternal, neonatal and child morbidity and mortality, including some nutrition interventions for example breastfeeding, KMC and Vitamin A supplementation, also came to the conclusion that although policies were good and evidence based they were often poorly implemented and practised (English *et al.*, 2017). The Obesity Strategy was only published fairly recently with the SSB tax finally implemented on 1 April 2018, so evaluation of the effectiveness will take a number of years.

Therefore, poor nutritional outcomes despite good policies can be explained by lack of effective policy implementation against a backdrop of extremely high levels of unemployment, poverty and food insecurity in a country that has undergone a rapid nutrition transition.

2.7 Conclusion

Childhood stunting and overweight are prevalent throughout LMICs and South Africa is no exception. Stunting has both short-term and long-term consequences. Stunted children have increased morbidity and mortality and in the long term, the evidence of the negative effect of childhood stunting on development of human capital is unequivocal. The risks for later development of obesity and NCDs are less clear-cut. Childhood obesity also has short-term consequences, but the long-term risk of increased adult obesity and NCD development is the most serious threat especially in a LMIC setting with limited health budgets and resources.

The determinants for both are complex and begin even before conception. This review, however, focused on the period from birth to two years. Being born too big or too small; rapid weight gain that is disproportionate to length gain, particularly after two years of age; and poor infant feeding practices result in poor growth outcomes. Stunting is closely linked to poor socioeconomic circumstances, lack of access to health care and basic services such as water and electricity. In HICs childhood obesity also affects the poor disproportionately while in LMICs this relationship is less clear but appears to be moving in a similar direction in some countries, including South Africa.

Both stunting and overweight require nutrition specific as well as nutrition sensitive interventions in order to address the underlying determinants. Regarding nutrition specific interventions, feeding practices that promote linear growth in the first two years are important for both stunting and overweight prevention. Breastfeeding is vital for child survival and, while it has little impact on stunting, it is modestly protective against overweight. High protein formulas have been shown to increase rapid weight gain in the first year, which is a risk for later obesity. Dietary diversity and intake of iron rich foods are important aspects of complementary feeding for prevention of stunting. Dairy protein appears to have specific growth promoting effects, however high protein intake in the complementary feeding diet, especially dairy protein, increases the risk for overweight. Intake of energy dense but micronutrient poor foods such as SSBs, chips, sweets, biscuits is increasingly common in infants and young children in LMICs because of the nutrition transition and is a risk for both stunting and overweight. The same optimal IYCF practices, that is: exclusive breastfeeding for 6 months; introduction of diverse, micronutrient-rich foods at six months with continued breastfeeding to two years are important aspects of both stunting and overweight prevention.

Information on the determinants of childhood stunting and overweight is usually obtained from longitudinal birth cohort studies. The majority of these studies have been conducted in HICs and investigate the determinants of overweight only. There have been very few conducted in LMICs such as South Africa which experience high rates of both stunting and overweight. The MACE longitudinal birth cohort study provides an opportunity to investigate the association of socioeconomic conditions, infancy growth patterns and feeding practices with stunting and overweight at 12 months of age.

3. METHODOLOGY

3.1 Introduction

This study aimed to identify risk factors for overweight/obesity and stunting at 1 year of age in infants enrolled in the Mother and Child in the Environment (MACE) longitudinal birth cohort study, Durban. As discussed previously, both overweight and stunting are prevalent amongst South African children (Shisana *et al.*, 2013). Identifying risk factors is important for developing effective prevention strategies and longitudinal birth cohort studies provide opportunities to investigate the association of a wide range of exposures in early life with growth outcomes at a later stage (Lawlor *et al*., 2009). The purpose of this chapter is to describe both the MACE and the MSc (Dietetics) study design. The study population is described as well as the methods and materials used in the collection of anthropometric and questionnaire data. The statistical tests used to analyse the data are discussed. Furthermore, steps taken to address issues of reliability, validity and bias, together with ethical considerations are described.

3.2 Study Design

This MSc (Dietetics) study performed secondary data analysis using anthropometric and questionnaire data collected in the MACE study. This section will therefore firstly describe the MACE study and the strengths and limitations of longitudinal birth cohorts followed by a description of the MSc (Dietetics) study.

3.2.1 MACE study design

The MACE study is an ongoing longitudinal birth cohort study designed to determine the effect of ambient and environmental pollution exposure on childhood respiratory development. A detailed protocol is attached (Appendix A, p140) and only the aspects relevant to this study will be described. The MACE study is monitoring the health and development of a cohort of pregnant women and their offspring attending public sector antenatal clinics in the north and south of Durban, KwaZulu-Natal. It plans to enrol 2000 women and their infants, with follow up of the children until the age of six years. The priority outcomes will be indicators of respiratory development as well as adverse respiratory outcomes.

3.2.1.1 Longitudinal birth cohort studies

Longitudinal birth cohort studies are increasingly used for epidemiological research into early life determinants of later disease (Lawlor *et al*., 2009). A longitudinal birth cohort study follows the same individuals from birth, or pregnancy in some cases, taking repeated measures over time (Western *et al*., 2015). The individuals are usually born around the same time, but this may stretch over a few years, and follow up typically continues for years and often decades (Batty *et al*., 2007).

Pregnancy and birth cohort studies provide one of the most powerful research methods for medical and social research (Western *et al*., 2015). Their advantages include being able to investigate a wide range of exposures and outcomes; and providing longitudinal data, which enables researchers to identify associations between exposures and outcomes. This is in contrast to cross sectional studies which only provide information about subjects at one point in time, making it difficult to determine if the exposure preceded the outcome (Western *et al.*, 2015).

Birth cohort studies are however extremely costly to set up and maintain, with many of the more recent cohorts aiming to enrol 100 000 subjects (Lawlor *et al*., 2009). Further, retention of subjects over long periods of time can be difficult, resulting in large numbers lost to follow up. If this loss of subjects is not random it can skew the results (Song & Chung, 2010; Batty *et al*., 2007). As with other observational studies, cohort studies cannot confirm causality and are limited to identifying associations (Reilly *et al.*, 2005). One of the barriers to confirming causality of a given exposure is that confounding by a number of factors, particularly sociodemographic factors can occur. Confounding is when a variable has an independent effect on both the independent and dependent variable and can lead to false conclusions of causality between the independent and the dependent variable. While these factors are usually controlled for, residual confounding can still occur (Lawlor *et al*., 2009). Cross cohort comparisons can be useful to determine if an association is confounded by comparing results from cohorts conducted under different social conditions. An example is breastfeeding, which has been shown in some cohorts in HICs to have a protective effect against childhood overweight. However, it is uncertain whether this finding is confounded by the fact that in HICs breastfeeding is more common in women of higher SES, which is in itself protective against overweight (Lawlor *et al*., 2009). In comparison to the findings from birth cohorts conducted

in HICs, a birth cohort conducted in Brazil, a LMIC where breastfeeding rates were not linked to SES, found no association between breastfeeding and overweight (Neutzling *et al*., 2009). This indicates that the relationship observed in HIC cohorts was probably due to confounding by SES.

Birth cohorts in LMICs are rare compared to those in HICs and tend to be smaller and run for shorter periods of time. This is due to a number of factors including the high cost of maintaining the cohort, the fact that many births occur at home and not in hospital and difficulties in retaining participants because of greater internal migration in LMICs compared to HICs (Batty *et al*., 2007).

3.2.2 MSc (Dietetics) study design

For the purpose of this MSc (Dietetics) study, the researcher extracted questionnaire and anthropometric data that were collected in the MACE study from 2013 to 2017. The variables of interest were determined, following a review of the literature on the determinants of childhood stunting and obesity. An additional questionnaire, adapted from the WHO IYCF Indicators, was administered by the researcher to caregivers attending the 12 month follow up visit from March 2016 to March 2017. The study had two aims:

1) To determine the prevalence of stunting and overweight/obesity in the study infants at 12 months
2) To determine risk factors for stunting and overweight in the study infants.

Although not included in the original objectives, 24 month anthropometric data became available for 144 infants during the year it took to collect infant feeding data from 94 12 months old infants. This 24 month data was included in the study results in order to report on the change in anthropometric status from 12 months to 24 months.

3.3 Study population

Pregnant women in their first trimester, were enrolled in the MACE study. These women were attending public sector ante natal clinics in the south (Austerville, Merebank, Bluff, Lamontville and Wentworth Hospital antenatal clinic) and north (Newlands East, Newlands West and Kwa-Mashu Clinic B) of Durban. Enrolment started in 2013 and is currently ongoing.

The women were followed up at the clinics and at home during pregnancy, and the infants were followed up at 6 months and 12 months at the MACE study clinics at King Dinizulu Hospital and King Edward Hospital. The MACE study continues to follow up children until 6 years of age but due to time constraints, the MSc (Dietetics) study investigated outcomes at 12 months of age. Anthropometric data at 24 months of 144 infants was included as described in the previous section.

3.4 Study methods and materials

The MSc Dietetics study used anthropometric and questionnaire data collected by the eight MACE study fieldworkers and clinical staff. The researcher collected additional infant feeding information at the 12 month clinic visit from a sub set of infants. This section will describe the methods in more detail.

3.4.1 Anthropometry

Hospital birth weight and lengths were taken from the infants' Road to Health booklets. Infants were weighed and measured at the 6 and 12 month mark by trained MACE study fieldworkers. Weights were taken using an electronic baby scale (Seca 354) and lengths were measured in a supine position using an infantometer (Seca 416). Weight was measured to the nearest 10 g and length to the nearest 0.1 cm. The 24 month data was collected using a Seca 786 mechanical column scale and measuring rod (Seca 224). Children who were less than 24 months were measured in a supine position using the infantometer.

Date of birth, gender, date of clinic visit, weight and length were entered into the WHO Anthro computer programme, version 3.2.2 (WHO, 2011), which generated z scores for weight for age (WAZ), length for age (LAZ) and weight for length (WFLZ) for each child at birth, 6 months, 12 months and 24 months. BMI for age z scores (BAZ) were also generated for 12 and 24 month data. The LAZ and BAZ at 12 and 24 months were used to determine the prevalence of stunting and overweight/obesity in the study. Infants were classified using the WHO guidelines: stunted < -2 LAZ, severely stunted < -3 Z score; overweight >+2 BAZ, obese >+3 BAZ (WHO, 2006). Children with 24 month data were also classified using the IOTF age specific BMI cut offs if they were two years or older at the 24 month visit as the IOTF cut offs can only be used from the age of 2 years (Cole *et al*., 2000). There is lack of consensus regarding which classification method to use for childhood overweight so both the WHO

guidelines and IOTF cut offs were used in order to allow comparisons to be made with other studies (Rolland-Cachera, 2011).

Anthropometric data were additionally used to determine the association of birth weight and length, and patterns of growth, with growth outcomes at 12 and 24 months. Birth WAZ and LAZ were used in the analyses together with patterns of growth from 0-6, 6-12 and 12-24 months. Rapid growth was defined as ≥0.67 change in WAZ or WFLZ between two time points; normal growth was <0.67>-0.67 change and slow or catch down growth as a ≤-0.67 change (Ong & Loos, 2006). This change is equivalent to the crossing of one significant percentile band for example from the 2nd to the 10th centile (Ong & Loos, 2006). Change in LAZ was also measured in the same way in order to determine associations with stunting outcomes.

All flagged z scores were first checked with the original data collection forms and corrected if there had been a capturing error. Out of range measurements that remained were excluded as per the WHO recommendations (WHO, 2011).

3.4.2 Questionnaire data

3.4.2.1 Maternal and household sociodemographic characteristics

MACE study questionnaires were used to collect maternal and household sociodemographic information. Carefully selected fieldworkers drawn from the study area communities were trained and supervised to conduct baseline interviews with participants. The fieldworkers were all matriculated and had undergone training in HIV and TB counselling. They were volunteers at their respective clinics before recruitment into the MACE study. Training included providing techniques and practice in conducting interviews in a consistent and neutral manner. The questionnaires consisted of standardised questions extracted from past and current European birth cohorts, as well as environmental health studies conducted by the MACE research team previously. Questionnaires were tested in a pilot study, conducted in 2011 among 100 women, and revised where necessary. The questionnaires can be found in Appendices B to G (pp170-183).

Selected maternal and household sociodemographic information of interest in the MSc Dietetics study was extracted from the MACE data set for descriptive purposes. From these

variables a number of exposure variables were identified, following a review of the literature, as potential risk factors for stunting and/or overweight. These variables were included in further analysis to test their association with the outcome variables. Further details of these exposure variables are now presented.

Socioeconomic status

Information on income, housing type and number of household members was used from the MACE Enrolment Questionnaire, an abridged version of which is presented in Appendix B (p170), and was captured as categorical data.

Access to tap water and electricity

Information regarding access to water and electricity was obtained from the MACE Enrolment questionnaire (Appendix B, p170).

Maternal education

Information on level of education was obtained from the MACE Enrolment Questionnaire (Appendix B, p170) and captured in categories ranging from having a highest education level of primary school or less up to the level of degree or diploma.

Maternal smoking

Information on antenatal smoking and alcohol consumption was obtained from the MACE 3rd Trimester; Part 2 Questionnaire, an abridged version of which is presented in Appendix C (p174), and was captured as either a "*yes*" or "*no"* variable.

Infant HIV exposure

HIV status in the MACE study mothers was recorded in the Clinical data at Trimester 3 questionnaire (Appendix D, p176) and recorded as "positive", "negative" or "unknown". Mothers were tested by DoH staff with a rapid HIV test during routine antenatal clinic visits as per South African PMTCT Guidelines (DoH, 2015a). Confirmation of maternal status was obtained from the Labour/Postnatal questionnaire (Appendix E, p178) and, if there was discordance between the two questionnaires, maternal HIV status was verified by asking the relevant field worker.

3.4.2.2 Infant feeding practices

The MACE medical staff recorded limited information on infant feeding practices at 6 months (n=233) and 12 months (n=290). An additional questionnaire using the WHO Indicators for Assessing Infant and Young Child Feeding (hereafter referred to as the WHO Indicators) (WHO, 2010) was adapted for use in the South African context. It was administered by the researcher to provide more detailed information on feeding practices at 12 months in a sub set of 94 infants; this being the number of infants who had attended the 12 month visit during the data collection period from March 2016 to March 2017.

MACE questionnaire information

The MACE 6 month follow up questionnaire, an abridged version of which is presented in Appendix F (p181), included questions about type of milk feeds and age of introduction of complementary foods. The information on type of milk feeds was classified as exclusively breast fed (EBF), exclusively formula fed (EFF), fresh milk, or mixed feeds (MF). The age of introduction of complementary foods was captured both as a continuous variable and as categories; < 4 months; 4-5.9 months; 6 months and > 6 months. Information on the type of introductory food was categorised as porridge or infant cereal. Commercial jarred food intake was also recorded. All of these variables were used to describe infant feeding practices at 6 months of age. The 6 month feeding variables were further analysed to determine their association with growth outcomes at 12 months.

The 12 month MACE follow up questionnaire, an abridged version of which is presented in Appendix G (p183), also included a question regarding the type of milk feeds which were categorised in the same way as for 6 month milk feeds. As with the 6 month feeding practices information, 12 month data were used both for descriptive purposes and to test their association with growth outcomes.

Feeding practices at 12 months

The 12 month Nutrition questionnaire was adapted by the researcher for the South African context from the WHO Indicators (WHO, 2010). The questionnaire is attached in Appendix H (p185). The researcher administered the questionnaire to the mothers of children attending the 12 month visits at the study sites at King Edward and King Dinizulu hospitals. The WHO Indicators were selected as they are a tested valid and reliable tool (WHO, 2008a). They are

fairly quick to administer which was an important consideration as there was limited time to conduct the interviews. A further advantage is that the relative simplicity of the tool meant that the MACE fieldworkers could be trained in administering them in the future.

Although the WHO Indicators are made up of eight indicators that include both breastfeeding and complementary feeding, for the purposes of this study, only four of the core indicators were assessed. These core indicators were (i) Minimum Dietary Diversity; (ii) Minimum Meal Frequency; (iii) a composite measure of MDD and MFF, Minimum Adequate Diet; and (iv) Iron Rich Food Consumption. A 24 hour dietary recall was used to collect the data as per the WHO (2010) guidelines. Information on milk feeds (breast, formula or fresh milk/maas) was also collected as it was required in the MDD, MMF and MAD calculations.

The Dietary Diversity Score was calculated by allocating 1 point for any food consumed out of the following seven food groups:

1. Grains, roots and tubers;
2. Legumes and nuts;
3. Dairy products;
4. Flesh foods;
5. Eggs;
6. Vitamin A rich fruit and vegetables;
7. Other fruits and vegetables.

The Minimum Dietary Diversity (MDD) indicator was calculated based on having consumed at least four of the seven food groups in the previous 24 hours (WHO, 2010). Data were captured as a continuous score as well as categorical “met MDD” or “did not meet MDD”.

According to the WHO Indicators, the Minimum Meal Frequency (MMF), for infants aged 12 months is the consumption of solid, semi solid or soft foods three times in the previous 24 hours for breastfed infants and four times for non-breastfed infants (WHO, 2010). Milk feeds are included in the MMF calculation for non-breastfed infants, but not for breastfed infants. Both continuous and categorical data were captured.

The Minimum Acceptable Diet (MAD) Indicator is a combination of the MDD and MMF Indicators. Breastfed infants must have had the MDD and MMF the previous day. Non-breastfed infants must have had the MDD (not including milk feeds) and MMF the previous day as well as two milk feeds (WHO, 2010). Data were captured as having an “adequate” or

"inadequate" diet. Iron Rich Food (IRF) consumption included flesh foods, infant formula and infant cereal. It was captured as a "yes" or "no" variable.

The WHO advises that in countries that are experiencing a nutrition transition, where childhood obesity is a problem, indicators of high sugar and high fat food consumption should be included (WHO, 2010). Therefore, an unquantified food frequency questionnaire formed part of the 12 Month Nutrition Questionnaire (Appendix H, p181) and was used to collect data on fruit juice, sugar sweetened beverage (SSB) and high fat snack (HFS) consumption; hereafter referred to as inappropriate food consumption. The percentage of infants consuming each of the three categories once a week or more was determined for descriptive purposes. This enabled comparison with other South African studies that had collected similar data (Budree *et al*., 2017; Faber *et al*., 2016) A daily consumption score was calculated and entered as continuous data in order to determine the association with growth outcomes. An unquantified food frequency questionnaire was used to collect the high sugar/high fat foods information as it could be missed with a 24 hour dietary recall.

The 12 month Nutrition Questionnaire was used to describe complementary feeding practices in the study infants. It also provided an opportunity to test the association of the WHO complementary feeding indicators, together with inappropriate food intake, with child anthropometric status in a setting where both stunting and overweight prevalence is high.

3.5 Data analysis

The MACE study data of interest in the MSc Dietetics study were extracted from a number of different data sheets and incorporated into a master Microsoft Excel spreadsheet. Anthropometric data was entered into the WHO Anthro programme (WHO, 2011) to generate Z scores for birth, 6 month, 12 month and 24 month data. The Z scores and the 12 Month Nutrition questionnaire data were entered into the master spreadsheet.

Once the data had been checked for errors, it was analysed using the Statistical Package for Social Sciences (SPSS) version 21. Descriptive tests were used to describe maternal and household characteristics, anthropometric status of the study infants, patterns of growth and feeding practices.

Further analysis of the data was conducted to explore associations of the exposure variables with the outcome variables, namely stunting and overweight / obesity at 12 months. Chi square tests of independence were performed to test if any significant associations existed between selected household and maternal factors, and infant feeding practices, and the outcome variables.

The association of birth anthropometric status, and patterns of growth, with growth outcomes at 12 months was explored using both categorical growth outcomes and continuous data. Mean birth and growth pattern data in relation to LFA and BMI categories at 12 months was investigated using analysis of variance (ANOVA). The LFA categories were normal length, stunted and severely stunted. The BMI categories were "wasted", "normal", "at risk of overweight", "overweight" and "obese". Linear regression analysis was applied to explore the relationship using continuous data. The aims of these analyses were:

a) To identify differences in mean birth WAZ and LAZ, and growth patterns, between those of normal height and BMI at 12 months and those who were not.
b) To explore the causal relationship between birth WAZ and LAZ, and growth patterns, and 12 month LAZ and BAZ
c) To identify the time period during which growth most strongly predicts LAZ and BMIZ at 12 months.

Significance was set at a p value of less than 0.05. Table 3-1 provides further details about each objective; the variables relevant to each objective; and the combination of statistical methods applied.

Table 3-1: Data analysis of objectives

Research Objective	Variable	Method of analysis
1. To determine the anthropometric status of the study infants at 12 months. 1.1 To describe the prevalence of stunting and overweight in the cohort. 1.2 To determine the outcome variables, namely stunting and overweight in the study infants.	12 month weight and length. Additional information required: Date of visit, gender, date of birth.	Data entered into WHO Anthro programme; WAZ, LAZ, WFLZ, BAZ generated. Report generated from WHO Anthro of prevalence in cohort of stunting, wasting, overweight and obesity.
2.1 To describe maternal/ household characteristics of the cohort. 2.2 To determine if maternal/household characteristics are associated with stunting and overweight.	Household income Number of household members Type of housing Number of rooms Access to tap water Access to electricity Maternal smoking Maternal alcohol intake Maternal level of education Maternal HIV status	2.1 Descriptive statistics. 2.2 Chi square tests of independence.
3.1 To describe infant anthropometric status at birth and growth patterns from birth to 6 months and from 6 months to 12 months. 3.2 To determine if birth weight and length and patterns of growth from 0-6 and 6 to 12 months are associated with growth outcomes at 12 months.	Birth, 6 month and 12 month weights and lengths.	3.1 Data entered into WHO Anthro programme; WAZ, LAZ, WFLZ generated. Change in WAZ, LAZ and WFLZ from birth to 6 months and 6 months to 12 months calculated. 3.2 ANOVA; Linear regression analysis.
4.1 To describe infant feeding practices at 6 months and 12 months. 4.2 To determine if infant feeding practices are associated with growth outcomes at 12 months.	**6 months (MACE)** BF/FF/mixed. Age intro of CF. Type of food (porridge, cereal, Purity). **12 months (MACE)** BF/FF/milk. **12 month Nutrition Q** Adequacy of CF. Intake of SSB/HFS.	4.1 Descriptive statistics. 4.2 Chi square tests of independence.

3.6 Reliability and validity

In order for research to produce trustworthy results it is important that the instruments used to collect data are both reliable and valid (Roberts *et al*., 2006). Reliability is the ability of an instrument or test to consistently give the same measurement while validity is the ability of the instrument or test to accurately measure what it is intended to measure (Roberts *et al*., 2006).

The reliability and validity of the anthropometric data collected in the MACE study was addressed by training fieldworkers in standard procedures for weighing and measuring children using the WHO guidelines (WHO, 2008b) and by calibration of the electronic scales. The WHO Growth Standards, which were used to classify stunting and overweight, are internationally recognised. They were developed using rigorous methodology and are a technically robust tool that can be used to assess children regardless of ethnicity, socioeconomic status or type of feeding. (WHO, 2006). The growth standards were externally validated through field-testing in four countries, chosen to provide representative samples of both under- and over-nutrition (Onyango *et al*., 2007).

The sociodemographic questionnaire used to collect baseline data was adapted from questionnaires used by the MACE study team in previous studies in the same community. They were pilot tested in 100 subjects and changes made where necessary. Fieldworkers were trained in asking questions in a consistent and neutral manner. MACE medical doctors collected the feeding practices information in the MACE 6 and 12 month follow up questionnaires.

The 12-month Nutrition questionnaire used the WHO Indicators. These are simple, valid and reliable indicators to assess infant and young child feeding practices, developed through testing in 10 countries over a 5-year period (WHO, 2008a). They have been used in a number of recent South African studies (Budree *et al*., 2017; DoH *et al*., 2017; du Plessis *et al*., 2016) which allows for comparison across studies. Collection of consistent and accurate information was ensured as the researcher administered all the questionnaires personally, asking the questions exactly as specified (WHO, 2010).

3.7 Reduction of bias

Bias in research is “any trend or deviation from the truth in data collection, data analysis, interpretation and publication which can cause false conclusions” (Simundic, 2013). In longitudinal birth cohort studies the two main sources of bias are selection bias and confounding bias.

Selection bias can occur at recruitment if the subjects volunteering to participate in the study are not representative of the population. Golding & Birmingham (2008) recommend a number of strategies to maximise recruitment including personal contact of study staff with potential

participants; providing clear information about the study, the benefits and any risks as well as any cost implications; assurance of confidentiality; and the voluntary nature of the study. The MACE study followed all of these recommendations as study fieldworkers were stationed at all the participating clinics, all eligible women were invited to participate and the informed consent form was explained to the mothers in their home language before signing. The informed consent form explained the purpose of the study, what would be required of participants, benefits and risks, confidentiality, reimbursement of travelling costs, and the voluntary nature of the study. This consent form is presented in Appendix K (p189).

Selection bias can also occur if large numbers of participants were lost to follow up and the characteristics of these participants differs significantly from those who remained in the study (Song & Chung, 2010). Retention of participants is always a problem in longitudinal studies, and is particularly difficult in birth cohorts in LMICs (Batty *et al*., 2007). Strategies to enhance retention in the MACE study included regular follow up by field workers, covering of transport costs, meals provided at clinic visits and occasional gift packs.

An important limitation to determining causation between exposure and outcome in any cohort study is potential confounding (Lawlor *et al*., 2009). Socioeconomic status and maternal education are well documented confounding factors in the relationship between feeding practices and growth outcomes (Lawlor *et al*., 2009; Reilly *et al*., 2005). Information on income and maternal education was collected in the MACE baseline questionnaire.

3.8 Roles of the researcher

The researcher identified the variables of interest from the MACE datasets. Data required to measure the variables were extracted from eight separate data sheets and incorporated into one master datasheet. Weights and lengths of each child at birth, 6 months, 12 months and 24 months were entered into the WHO Anthro programme and the Z scores were then entered into the master data sheet. The researcher coded 6 month nutrition information from the MACE 6 month questionnaires. The 12 month Nutrition Questionnaire was developed by the researcher; using the WHO IYCF Indicators and adding a section on inappropriate foods consumption. The questionnaire was administered to mothers attending the 12 month clinic visit by the researcher. This data collection period ran from March 2016 to March 2017. Mothers received feedback on their child's growth and complementary feeding advice from the researcher after

the questionnaire was completed. Information collected in the questionnaire was used to calculate DDS, MF and determine if MDD, MMF and MAD indicators were met. This data were entered into the master spreadsheet together with inappropriate food consumption scores.

3.9 Ethical considerations

Ethical clearance for the MACE study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal; Reference: BF 263/12 (Appendix I, p187) and the KwaZulu-Natal Department of Health (Appendix J, p188). Women enrolled in the MACE study signed individual consent forms (Appendix K, p189). Additional ethical approval for the MSc (Dietetics) study was also obtained from BREC; Reference: BE 479/15 (Appendix L, p193). Confidentiality was maintained through the use of codes for each participant and no names were recorded.

3.10 Summary

This chapter described the MACE longitudinal birth cohort study, which provided most of the data used in the MSc (Dietetics) study. The strengths of longitudinal birth cohort studies as a research tool were discussed together with the limitations. The chapter briefly reviewed the literature supporting the choice of exposure variables. The methodology used to collect and analyse the anthropometric and questionnaire data used in the MSc (Dietetics) study was described. Aspects related to the quality of the data namely, reliability and validity of the tools used, and the steps taken to avoid bias were outlined. The next chapter describes the results of the study.

4. RESULTS

The results of the study research objectives outlined in Chapter 1 are now presented. The household and maternal characteristics are outlined to provide context to the study population. The anthropometric status of the study population at 12 months of age is described. The associations of selected household and maternal characteristics with the outcome variables of stunting and overweight at 12 months are presented. The infant factors that could potentially be associated with the outcome variables, namely growth patterns and feeding practices, are described together with their association with the outcome variables.

4.1 Maternal and household characteristics of the study population

Data were available for 288 mothers. The mean maternal age was 26.0 years (range 15-43 years; SD 6.0) and level of education was relatively high where 55% (n=160) had a matric certificate and a further 15% (n= 43) had a degree or diploma. HIV prevalence was 35.3% (n=102). Participants' primary language were predominantly indigenous African languages: 65.7% (n=189) spoke Zulu or Xhosa at home and 32.5% (n=94) spoke English or Afrikaans. Income levels were low with 66.8% (n=192) of mothers and 30.4% (n=88) of fathers reporting that they had no income or earned less than R2000.00 per year. Less than 5% (n=13) of mothers reported that they had smoked or consumed alcohol during pregnancy. While the average household size ranged between 2 and 18 members, the average size was six. Access to tap water was universal and only three households did not have electricity. Less than 10% (n=25) of the sample lived in informal housing. Details of the maternal and household characteristics are reported in Table 4-1.

Table 4-1: Maternal and household sociodemographic information (n=288)

Variable	n		%	
Maternal education:				
Primary school or less	4		1.3	
Some high school	81		28.4	
Matric	160		55.4	
Degree/diploma	43		14.9	
Lived with spouse	80		28.1	
HIV prevalence	102		35.3	
Alcohol intake during pregnancy	13		4.5	
Smoked during pregnancy	11		3.8	
Home language:				
Zulu	173		60.2	
English	93		32.2	
Xhosa	16		5.5	
Afrikaans	1		0.3	
Type of housing:				
Detached/ semi-detached house	199		69.1	
Flat/ apartment	64		22.2	
Informal house	25		8.7	
Access to tap water	288		100	
Access to electricity	285		98.9	
Annual Income	**Maternal**		**Paternal**	
	n	**%**	**n**	**%**
No income	133	46.4	62	21.5
<R2000,00	59	20.4	26	9
R10 000-R30 000	53	18.3	72	24.9
R30 001- R75 000	24	8.3	54	18.7
R75 001-R150 000	9	3.1	39	13.5
>R150 000	0	0	10	3.5
Refused to answer	10	3.5	26	9

4.2 The anthropometric status of the study infants at 12 months

The sample consisted of 290 infants; 157 (54.1%) males and 133 (45.9%) females. Two study infants had anthropometric data at 12 months but maternal data was missing. The anthropometric status of the study infants, classified using WHO criteria for length for age (LFA) and BMI for age, is presented in Table 4.2. The prevalence of overweight was 14.5% (n=42) and obesity 7.2% (n=21) with a further 22% (n=64) classified as being at risk of overweight according to WHO criteria. The mean BMI for age Z score (BAZ) was 0.81 (SD 1.42). Stunting prevalence was far lower at 5.9 % (n=17) with a mean length for age Z score

(LAZ) of -0.01 (SD 1.24), while four children (1.4%) were wasted. Only one child (0.35%) was both stunted and overweight. Chi square tests revealed no relationship between gender and either stunting (p=0.897) or overweight/obesity (p=0.222).

Table 4-2: Anthropometric classification of study population at 12 months (n=290*)

	n	%
LFA **		
Normal	273	94.1
Stunted (<-2 SD)	15	5.2
Severely stunted (<-3 SD)	2	0.7
BMI ***		
Normal	159	54.8
At risk of overweight(>+1SD)	64	22.1
Overweight (> +2 SD)	42	14.5
Obese (> +3 SD)	21	7.2
Combined overweight and obesity	63	21.7
Wasted (<-2 SD)	4	1.4

* Anthropometric data at 12 months was available for an additional 2 infants who had no maternal data
**Mean LAZ = -.01 (SD 1.24)
***Mean BAZ =.81 (SD 1.42)

The LAZ and BAZ for each child, which were used to determine the prevalence of stunting and overweight categories, were also used to generate the outcome variables of stunting and overweight/obesity in the study population. They are used in the analyses that follow in sections 4.3, 4.4, 4.5 and 4.6.

4.3 Association of maternal and household characteristics with stunting and overweight at 12 months

Chi square tests of independence were used to determine if there was an association between maternal and household characteristics and stunting and/or overweight. There was a significant relationship between type of housing and stunting (Fisher's exact=8.321, p=0.048). Significantly more than expected of those who lived in a flat/apartment compared to houses were stunted and of those who lived in informal housing were severely stunted. Type of housing was also associated with BMI outcomes (Fisher's exact = 15.014, p=0.040). Significantly more than expected of those who lived in informal housing compared to those who lived in houses or flats were at risk of overweight, overweight or wasted. The other variables had no significant association with either stunting or overweight categories or wasting. Table 4-3 presents the cross-tabulation of selected maternal and household characteristics across LFA and BMI categories.

Antenatal smoking was not associated with birthweight (p=0.226) or length (p=0.910). There was also no association between antenatal alcohol intake and birthweight (p=0.183) or length (p=0.449).

Table 4-3: Maternal and household characteristics across LFA and BMI categories at 12 months

	LFA Categories				BMI Categories							
	Stunted		Severely stunted		Wasted		At risk of overweight		Overweight		Obese	
	n	%	n	%	n	%	n	%	n	%	n	%
Annual Income:												
<R10 000(maternal)n=192	12	6.3	2	1	1	0.5	43	22.4	31	16.1	16	8.3
<R10 000(paternal)n=88	4	4.5	0	0	2	2.3	13	14.8	13	14.8	7	7.9
>R10 000(maternal)n=86	2	2.3	0	0	3	3.5	17	19.8	10	11.6	5	5.8
>R10 000(paternal)n=174	9	5	2	1.1	2	1.1	42	24.1	26	14.9	11	6.3
Type of housing:												
House (n=198)	8	4	1	0.5	1	0.5	40	20.3	32	16.2	16	8.1
Flat/apartment (n=64)	7	10.9*	0	0	1	1.6	15	23.4	3	4.7	4	6.3
Informal housing (n=25)	0	0	1	4*	1	4*	8	32*	6	24*	1	4
Maternal education:												
Some secondary (n=85)	7	8.2	1	1.1	0	0	15	17.5	15	17.5	4	4.7
Matric (n=160)	7	4.4	1	0.6	3	1.9	38	23.8	19	11.9	10	6.3
Degree or diploma (n=43)	1	2.3	0	0	1	2.3	10	23.3	7	16.3	7	16.3
Home language:												
Zulu/ Xhosa (n=190)	7	3.7	2	1	2	1	39	20.5	30	15.7	16	8.4
English/ Afrikaans (n=93)	7	7.5	0	0	2	2.2	23	24.7	8	8.7	5	5.4
Maternal HIV status:												
HIV positive (n=99)	5	5	1	1	2	2	24	24.2	14	14.1	7	7.1
HIV negative (n=186)	10	5.4	1	0.5	2	1.1	39	21	27	14.5	14	7.5
Antenatal smoking:												
Yes (n=11)	2	18.2	0	0	0	0	3	27.3	1	9.1	0	0
No (n=277)	13	4.7	2	0.7	4	1.5	56	20.8	39	14.5	21	7.6
Antenatal alcohol:												
Yes (n=13)	1	7.7	0	0	0	0	3	23.1	4	30.8	0	0
No (n=276)	14	5.1	2	0.7	4	1.5	60	21.8	38	13.8	21	7.6

*p<0.05

4.4 Birth anthropometric data and patterns of growth in the first 12 months

This section presents birth anthropometric data and data on rate of growth from birth - 6 months and 6-12 months, followed by an analysis of the association of this data with the outcome variables.

4.4.1 Description of birth anthropometric status and rate of growth from birth-6 months and 6-12 months

a) Birth data

The mean birthweight was 3173 g and the prevalence of term, low birthweight (<2500 g) was 8.2% (n=23). Detailed birth anthropometric data of the sample can be found in Table 4.4. Mean birth weight for age Z score (WAZ) of - 0.2299 was below the WHO reference mean while mean birth LAZ of 0.3461 was above it.

Table 4-4: Birth anthropometric data of study sample (n=288)

	Mean	SD	Minimum	Maximum
Birthweight	3173.39 g	479.76	1910 g	4570 g
WAZ	-0.2299	1.03678	-4.18	2.25
LAZ	0.3461	1.42780	-4.16	4.7

*Birth/maternal data was missing for two subjects

b) Growth from birth to 6 months

Change in WAZ, LAZ and weight for length Z score (WFLZ) between birth and 6 months was calculated and classified as "slow" (≤-0.67); "normal" (-0.67 to +0.67) and "rapid" (≥+0.67) growth. The prevalence of each category of growth in the study sample is presented in Table 4-5.

Table 4-5: Prevalence of growth categories: 0-6 months

	Slow growth		Normal growth		Rapid growth	
	n	%	n	%	n	%
WAZ (n=233)*	26	11.2	84	36.1	123	52.7
LAZ (n=226)**	92	40.9	73	32.4	60	26.7
WFLZ (n=226)**	28	12.4	30	13.3	168	74.2

* 50 infants did not attend 6 month visit and an additional 7 had missing/out of range weights at birth or 6 months
**7 additional infants who attended 6 month visit had missing or out of range lengths at birth or 6 months

Rapid gains in weight occurred in over 50% (n=123) of the sample in the first 6 months of life. Linear growth on the other hand was more evenly distributed across the growth categories with "slow" growth being the most predominant category. This disproportionate weight gain in relation to linear growth is reflected in the almost 75% (n=168) prevalence of "rapid" gain in weight for length in the study sample.

c) Growth from 6-12 months

Change in WAZ, LAZ and WFLZ between 6 and 12 months was calculated and classified as "slow", "normal" and "rapid" growth as before. The prevalence of each category of growth in the study sample is presented in Table 4-6.

Table 4-6: Prevalence of growth categories: 6-12 months

	Slow growth		Normal growth		Rapid growth	
	n	%	n	%	n	%
WAZ (n=233)*	29	12.5	158	67.8	46	19.7
LAZ (n=232)**	64	27.6	98	42.2	70	30.2
WFLZ (n=232)**	64	27.6	107	46.1	61	26.3

* 50 infants did not attend 6 month visit and an additional 7 had missing/ out of range weights at 6 or 12months
**An additional 1 infant who attended 6 month visit had missing length data at 6 or 12 months

Prevalence of "rapid" gain in WAZ decreased considerably in the 6-12 month period, compared to the 0-6 month period, with more than two thirds of infants falling into the "normal" growth category. Change in LAZ was once again spread more evenly among the categories but "slow" growth was now the least common category. The decrease in rapid weight gain accompanied by increased linear growth resulted in a sharp decrease in the prevalence of "rapid" WFLZ gain from over 75% in the 0-6 month period to 26% (n=61). The difference in growth patterns tween birth to 6 months and 6 to 12 months is illustrated in Figure 4-1.

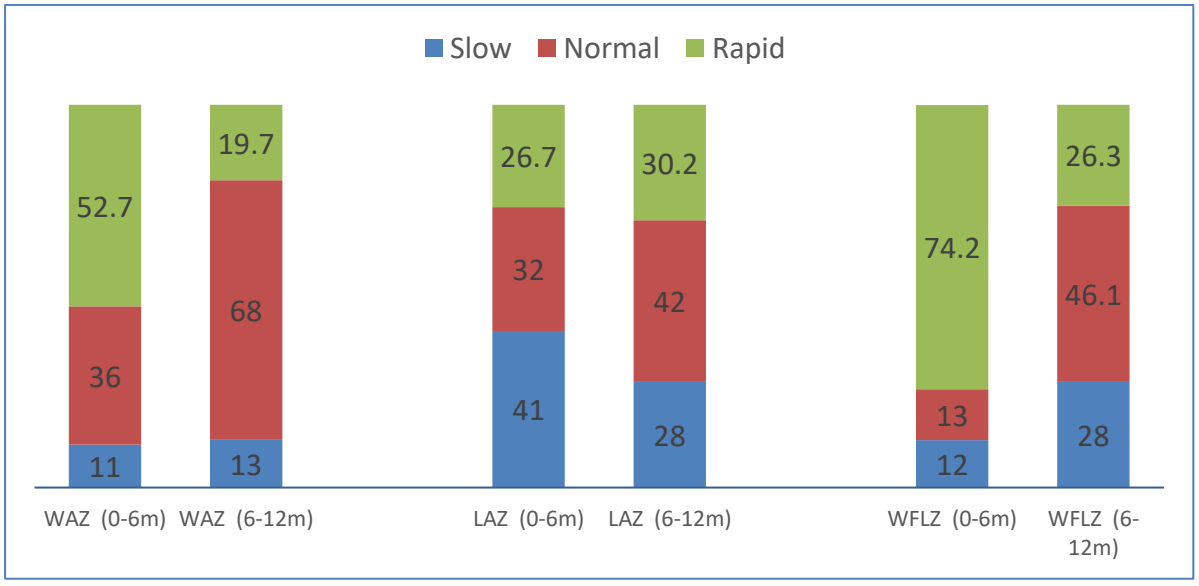

Figure 4-1: Percentage of infants in each growth category: 0-6 and 6-12 months

4.4.2 The association of birth anthropometric data and growth patterns with growth outcomes at 12 months

This sub-section first presents mean birth and growth pattern data in relation to LFA and BMI categories at 12 months using analysis of variance (ANOVA). The LFA categories are normal length, stunted and severely stunted. The BMI categories are wasted, normal, at risk of overweight, overweight and obese. The second part of the sub-section reports the results of the linear regression analysis.

4.4.2.1 Difference in growth patterns across 12 month growth outcome categories

Mean birth WAZ and LAZ and the mean change in LAZ for each LFA category; and change in WAZ, LAZ and WFLZ for each BMI category, at 12 months was determined. Analysis of variance (ANOVA) was applied to test for significant differences in means across categories. Results are reported for LFA categories first, and BMI categories second, for birth, 0-6 and 6-12 months data.

a) Birth data

Mean birth WAZ and LAZ for each LFA category and BMI category together with the results of the ANOVA are presented in Tables 4-7 and 4-8 respectively.

There was a significant difference in mean birth WAZ and LAZ for the different LFA categories. Children who were classified as normal height at 12 months had a significantly higher birth WAZ on average than those classified as stunted (p=0.002). Mean birth LAZ was also significantly higher in children classified as normal height at 12 months compared to those classified as stunted (p=0.002).

Table 4-7: Mean birth WAZ and LAZ across LFA categories at 12 months

	Mean birth WAZ (SD)	**Mean Birth LAZ (SD)**
LFA categories at 12 months:		
Normal	-0.1766 (1.0078)	0.4189 (1.4062)
Stunted	-1.1260 (1.2160)	-0.7127 (1.3316)
Severely stunted	-0.7000 (0.5374)	-1.4350 (1.2374)
Total	-0.2299 (1.0368)	0.3461 (1.4278)
df	2:284	2:281
F-statistic	6.397	6.251
p value	p=0.002	p=0.002

Table 4-8: Mean birth WAZ, birthweight and LAZ across BMI categories at 12 months

	Mean birth WAZ (SD)	Mean Birthweight (SD)	Mean birth LAZ (SD)
BMI categories at 12 months:			
Wasted	-2.215 (1.210)	2325 g (413.32)	-1.5367 (1.5366)
Normal	-0.4112 (0.9756)	3088 g (435.80)	-0.3768 (1.3247)
At risk of overweight	-0.798 (1.1969)	3235 g (576.78)	0.2253 (1.5672)
Overweight	0.1968 (0.7743)	3396 g (392.64)	0.3762 (1.6269)
Obese	0.1514 (0.6441)	3338 g (306.44)	0.5814 (1.1410)
Total	-0.2337 (1.0366)	3173 g (479.76)	0.3384 (1.4245)
df	4:281	4:283	4:278
F-statistic	8.508	8.357	1.599
p value	p<0.0005	p<0.0005	p=0.175

There was a significant difference in mean birth WAZ for the different BMI categories. Children who were classified as wasted at 12 months had a significantly lower birth WAZ and birthweight on average than all other BMI categories. The specific p values for each category are as follows: normal (p=0.012); at risk of overweight (p=0.002); overweight (p<0.0005; obese (p<0.001). Children classified as overweight at 12 months had a significantly higher mean WAZ (p=0.014) and weight (p<0.0005) at birth than those classified as having a normal BMI. There was no significant difference in mean LAZ at birth across BMI categories at 12 months (p=0.175).

b) Change in LAZ, WAZ and WFLZ: 0-6 months

Data were analysed to determine if mean change in Z score between birth and 6 months differed across LFA and BMI for Age categories at 12 months. Results for LFA categories are presented in Table 4-9 and those for BMI categories in Table 4-10.

Table 4-9: Mean change in LAZ 0-6 months across LFA categories at 12 months

	Mean change LAZ: 0- 6 months (SD)
LFA categories at 12 months:	
Normal	-0.2770 (1.6387)
Stunted	-0.8878 (1.7676)
Severely stunted	-0.3450 (0.9405)
Total	-0.2958 (1.6392)
df	2:223
F-statistic	0.752
p value.	p =0.473

There was no significant difference in the mean birth to 6 month change in LAZ across LFA categories at 12 months (p=0.473). Mean change in Z score for all categories except stunting was within in the range for "normal" growth (-0.67 to +0.67 change).

Table 4-10: Mean change in WAZ, LAZ and WFLZ across BMI categories at 12 months

	Mean change WAZ: 0-6 mths (SD)	Mean change LAZ: 0-6 mths (SD)	Mean change WFLZ: 0-6 mths (SD)
BMI categories at 12 months:			
Wasted	0.5050 (0.6537)	0.2167 (0.8594)	0.8750 (0.0354)
Normal	0.4487 (1.1256)	-0.3810 (1.5014)	1.5047 (1.9736)
At risk of overweight	0.9308 (1.1818)	-0.1331 (1.8790)	1.8813 (2.2377)
Overweight	1.0374 (1.070)	-0.6103 (1.5929)	2.2494 (2.0505)
Obese	2.0011 (1.120)	0.2683 (1.9403)	2.6747 (1.9038)
Total	0.7555 (1.1988)	-0.2958 (1.6392)	1.7802 (2.0551)
df	4:228	4:221	4:226
F-statistic	8.732	1.093	2.039
p value	p<0.0005	p =0.361	p =0.090

There was only a significant difference in the mean change in WAZ across BMI categories. The mean change in WAZ from 0-6 months was significantly greater in those children who were classified as obese at 12 months than in those classified as normal (p<0.0005) or at risk of overweight (p=0.019). Mean change in WAZ was above +0.67 for the sample in total at +0.7555 with only children classified as being wasted or normal at 12 months having a mean change in WAZ in the "normal" growth range.

Change in LAZ was in the "normal" growth range for all BMI categories while all BMI categories had a mean change in WFLZ in the "rapid" growth category. Children classified as overweight or obese at 12 months had a mean change in WFLZ greater than + 2.

c) Change in LAZ, WAZ and WFLZ: 6-12 months

Data were again analysed to determine if mean change in Z score between 6 and 12 months differed across LFA and BMI for age categories at 12 months. Mean change in LAZ in each LFA category is presented in Table 4-11. Change in WAZ, LAZ and WFLZ is shown in relation to BMI categories in Table 4-12.

Table 4-11 Mean change in LAZ across LFA categories at 12 months

	Mean change LAZ: 6 -12 months (SD)
LFA categories at 12 months:	
Normal	0.1191 (1.2974)
Stunted	-0.3633 (1.1263)
Severely stunted	-1.9680 (0.3606)
Total	0.0826 (1.3011)
df	2:230
F-statistic	3.150
p value	p=0.045

Infants who were classified as normal at 12 months of age had a significantly greater mean change in LAZ between 6 and 12 months than infants classified as severely stunted at 12 months (p=0.024).

Table 4-12: Mean change in WAZ, LAZ and WFLZ across BMI categories at 12 months

	Mean change WAZ:6-12 months (SD)	**Mean change LAZ:6-12 months (SD)**	**Mean change WFLZ:6-12 months (SD)**
BMI categories at 12 months:			
Wasted	-0.3400 (0.7918)	1.0375 (0.1611)	-1.6175 (0.8611)
Normal	-0.1373 (0.6726)	0.1052 (1.1955)	-0.3821 (0.9011)
At risk of overweight	0.3206 (0.5798)	0.0231 (1.4263)	0.2779 (1.0053)
Overweight	0.5019 (0.7878)	-0.0106 (1.4112)	0.5445 (1.4683)
Obese	1.0817 (1.1240)	0.0422 (1.5987)	1.4228 (1.3510)
Total	0.1406 (0.8015)	0.0826 (1.3011)	0.0067 (1.1952)
df	4:228	4:228	4:228
F-statistic	15.882	0.615	17.779
p value	p<0.0005	p=0.652	p<0.0005

As with the 0-6 month results there were no significant differences in mean change in LAZ 6-12 months between BMI categories at 12 months (p=0.652). There were, however, significant differences in mean change of both WAZ and WFLZ. Infants classified as obese (p=0.003), overweight (p=0.002) and at risk of overweight (p<0.0005) at 12 months had significantly greater mean gains in WAZ between 6-12 months than infants classified as normal BMI at 12 months. Change in WFLZ also differed: infants classified as obese (p<0.0005), overweight (p=0.018) and at risk of overweight (p=0.001) at 12 months had significantly greater mean gains in WFLZ between 6-12 months than infants classified as normal BMI at 12 months. Mean change in WAZ and WFLZ fell within the "normal" rate of growth category (-0.67 to +0.67) for all BMI categories except for obese. Additional analysis showed a mean change in WAZ between birth and 12 months of + 2.97 in infants classified as obese at 12 months. Infants

classified as wasted at 12 months had "slow" WFLZ growth. Overweight (p<0.0005) and obese (p=0.007) infants had significantly greater mean change in WFLZ than wasted infants.

4.4.2.2 Linear regression analysis

Regression analysis was performed to explore the relationships between the independent variables birth anthropometric status; rate of growth from 0-6 months; and rate of growth from 6 to 12 months, and the dependent variables LAZ and BAZ at 12 months. The results of the analysis are presented in Table 4-13 for stunting (LAZ) outcomes and 4-14 for overweight (BAZ) outcomes.

Table 4-13: The relationship between LAZ at 12 months and birth WAZ and LAZ, and change in LAZ

	Beta coefficient	Model summary R square	df	F-statistic	p value
WAZ					
Birth	0.394	0.106	1:285	33.800	p<0.0005
LAZ					
Birth	0.191	0.047	1:282	13.939	p<0.0005
Change 0-6	0.154	0.041	1:224	9.555	p=0.002
Change 6-12	0.412	0.193	1:231	55.193	p<0.0005

All the independent variables were significantly associated with LAZ at 12 months. Lower birth WAZ and LAZ, and smaller change in LAZ between 0-6 and 6-12 months were associated with lower Z scores at 12 months and vice versa. The higher the value of the coefficient B the stronger the predictive relationship. This shows that birth WAZ and change in LAZ from 6-12 months were the strongest predictors of 12 month LAZ with values of the coefficient B of 0.394 and 0.412 respectively.

Table 4-14: The relationship between BAZ at 12 months and birth WAZ and LAZ, and change in WAZ, LAZ and WFLZ

	Beta coefficient	Model summary R square	df	F-statistic	p value
WAZ					
Birth	0.390	0.075	1:285	22.977	p<0.0005
Change 0-6	0.579	0.208	1:231	60.571	p<0.0005
Change 6-12	0.906	0.227	1:231	67.933	p<0.0005
LAZ					
Birth	0.048	0.002	1:282	0.621	p=0.431
Change 0-6	0.063	0.005	1:224	1.071	p=0.302
Change 6-12	-0.064	0.003	1:231	0.689	p=0.407
WFLZ					
Birth	0.149	0.034	1:280	20.674	p=0.002
Change 0-6	0.182	0.061	1:229	32.005	p<0.0005
Change 6-12	0.625	0.241	1:231	73.296	p<0.0005

None of the LAZ variables was significant predictors of BAZ at 12 months while both WAZ and WFLZ were significant predictors. Higher birth WAZ and WFLZ and higher change in WAZ and WFLZ during both time periods were associated with higher BAZ at 12 months. The strongest predictor was change in WAZ from 6 to 12 months, with the highest value of the coefficient B of 0.906.

4.5 Feeding practices

Information on feeding practices was collected at 6 months and 12 months. The findings at both time periods will be presented in this section. This will be followed by an analysis of the association between feeding practices and growth outcomes.

4.5.1 Description of feeding practices at 6 months

MACE clinical staff collected the following feeding information as part of the 6 month follow up questionnaire: type of milk feed the child was receiving; age of introduction of complementary food; and type of complementary food introduced, which is porridge, infant cereal or jarred foods, or a combination.

Results are presented in Table 4-15. In summary, approximately 50% (n=116) of study infants were still receiving some breastmilk at 6 months; either as the sole milk feed or in combination with infant formula. Very early introduction of solids seemed uncommon with only 10% (n=16) of study infants mothers reporting having given complementary foods before 4 months and almost half reporting waiting until the recommended age of 6 months. Use of commercial infant cereals and jarred foods was common.

Table 4-15: Feeding practices at 6 months

	n	%
Milk feeds at 6 months (n= 233)*:		
Breastmilk only	70	29.9
Formula milk only	116	49.9
Breast and formula	46	19.8
Fresh milk	1	0.4
Age of introduction of complementary foods (n=148):**		
<4 months	16	10.7
4-6 months	63	42.6
6 months	63	42.6
Later than 6 months	6	4.1
Type of starchy food introduced (n=233)*:		
Porridge	60	25.9
Infant cereal	146	62.8
No porridge/cereal	26	11.3
Use of commercial jarred foods (n=223)* :**		
Yes	111	49.8
-jarred food only	11	4.9
-jarred food and infant cereal	70	31.4
-jarred food and porridge	30	13.5
No	112	50.2

*57 infants did not attend 6 month visit
** Missing data, 85 infants-original MACE 6 month questionnaire did not include this question *** Missing data for 10 infants

4.5.2 Description of feeding practices at 12 months

Information on type of milk feed was collected at the 12 month visit by MACE clinical staff. The WHO IYCF Indicators questionnaire on complementary feeding practices, adapted to include a question on intake of inappropriate foods, was administered by the researcher to a sub sample of 94 mothers. These were the mothers of infants who came to the clinic for their 12 month follow up visits between March 2016 and August 2017.

4.5.2.1 Milk feeds at 12 months

A little over a third of study infants were still receiving breast milk at 12 months while almost two thirds were receiving infant formula. Only four infants (1.4%) were receiving fresh milk or maas as a milk feed. More detailed information is provided in Table 4-16.

Table 4-16: Milk feeds at 12 months: n=290

12 month milk feeds	n	%
Breastmilk only	63	21.6
Infant formula only	183	63
Breast and formula	40	13.7
Fresh milk or maas	4	1.4

4.5.2.2 Adequacy of complementary feeding practices

Complementary feeding practices were assessed using the WHO IYCF Indicators questionnaire based on a 24-hour dietary recall. The following components were assessed; dietary diversity (a dietary diversity score (DDS) greater than 4, out of a possible 7, is considered adequate) and frequency of feeding (at 12 months at least 3 meals per day for breastfed infants; 4 meals per day, including formula feeds, for formula fed infants). Both the adequate dietary diversity and minimum meal frequency (MMF) indicators must be met in order to achieve minimum adequate diet (MAD) classification. The seven food groups used to assess dietary diversity were: starches, grains and tubers; Vitamin A rich fruit and vegetables; other fruit and vegetables; iron rich foods; eggs; legumes and nuts; and dairy.

Complementary feeding practices were poor with a mean DDS of 3.56 (range 1-6), below the adequate diversity score cut off of 4. Just under half of the infants (n=45; 48%) achieved a score above 4 and only one third (n=31; 33%) achieved the MAD indicator. The discrepancy between the number of children achieving adequate DDS and MAD is due to the fact that formula fed infants require a DDS of 4, excluding milk feeds to achieve the MAD indicator. Only one child did not achieve the MMF score. Iron rich food intake was good with 87.4% (n=82) of infants receiving some form of iron rich food in the previous 24 hours; over half, 59.3% (n=56), in the form of flesh foods. Of those who did not consume flesh foods, a further 28.1% (n=26) consumed iron fortified infant formula or cereals. Processed meat consumption was low with 5.7% (n=5) of infants consuming polony.

The percentage of infants consuming foods from each of the seven food groups, as well as some additional foods of interest, in the previous 24 hours is depicted in Figure 4-2.

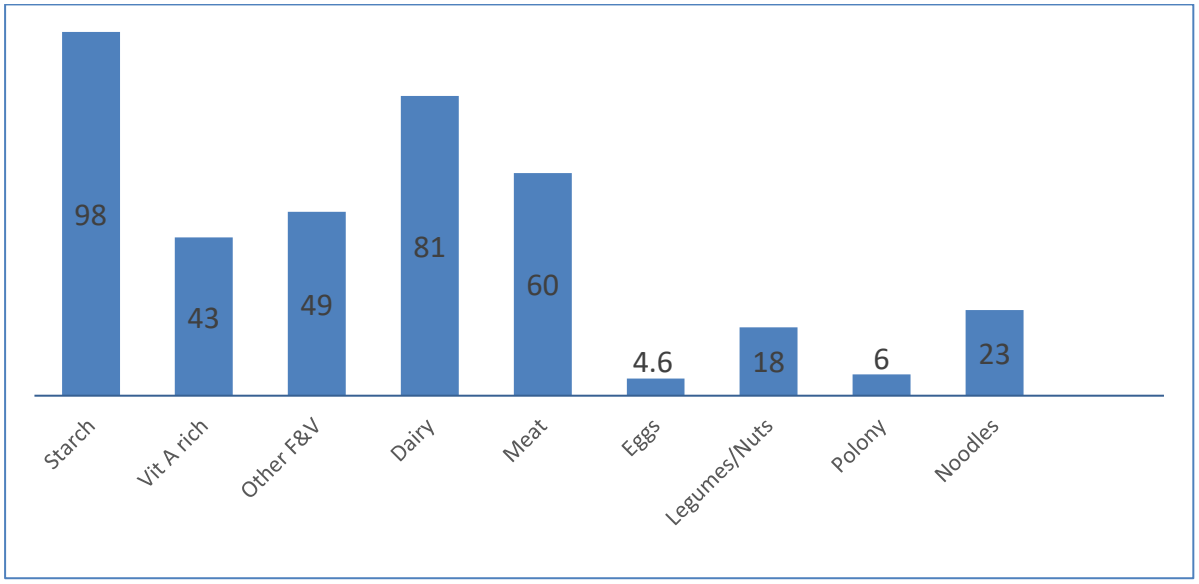

Figure 4-2: Percentage of children receiving foods from the 7 food groups in the previous 24 hours (n=94)

The starch group was the most commonly consumed followed by dairy and meat. Less than half of the infants had consumed fruit or vegetables the previous day. Eggs were the least consumed group with only 4.6% (n=4) of infants having consumed them. Nearly one quarter of infants (23.8%; n=22) had received instant noodles the previous day.

4.5.2.3 Consumption of inappropriate foods and drinks

A question on intake over the previous month of fruit juice, SSBs, sweets and high fat snacks was included in the IYCF Indicators questionnaire. Mean intake is presented in Table 4-17.

Table 4-17: Mean daily intake of inappropriate foods and drinks (n=94)

	Mean daily intake	Minimum	Maximum
Fruit juice	0.4499 (± 3 times a week)	0	3
Sugar score (SSB, juice, sweets)	0.2922 (± twice a week)	0	3
High fat snacks (crisps, vetkoek)	0.5182 (± 4 times a week)	0	3
Total	1.2602	0	4.14

High fat snacks were the most commonly consumed type of food, followed by fruit juice, which is often considered by mothers to be a healthy choice for their infants. Mean total consumption of inappropriate foods was more than once a day. The percentage of infants in the study population consuming each category of inappropriate foods at least once a week was as follows: fruit juice, 51% (n=48); SSBs, 45% (n=42) and HFS, 72% (n=68).

4.5.3 Association of feeding practices with stunting and overweight at 12 months

The association of feeding practices with stunting and overweight in the study infants at 12 months of age was investigated. Chi square tests of independence were performed to test if any significant associations existed between infant feeding practices and the outcome variables (stunting and overweight/obesity) in the infants at 12 months of age. There were no significant associations between any of the infant feeding practices investigated and the outcome variables. The cross tabulation of feeding practices across LFA and BMI for age categories at 12 months is presented in Table 4-18.

Table 4-18: Infant feeding practices across LFA and BMI for age categories at 12 months

	LFA Categories				BMI Categories							
	Stunted		Severely stunted		Wasted		At risk of overweight		Overweight		Obese	
	n	%	n	%	n	%	n	%	n	%	n	%
6 month milk feeds:												
Breast milk only (n=71)	3	4.2	2	2.8	1	1.4	16	22.5	6	8.5	6	8.5
Formula only (n=116)	4	3.3	0	0	1	0.8	27	22.5	23	19.2	10	8.3
Breast & formula (n=46)	2	4.2	0	0	2	4.2	9	18.8	3	6.3	2	4.2
Age of introduction of complementary foods:												
<4 months (n=16)	1	6.3	0	0	0	0	1	6.3	3	18.8	2	12.6
4-6 months (n=63)	3	4.8	0	0	3	4.8	17	27	9	14.3	3	4.8
6 months (n=63)	1	1.6	2	3.2	1	1.6	12	19	12	19	7	11.1
>6 months (n=2)	1	50	0	0	0	0	0	0	0	0	0	0
Type of complementary food (6 months):												
Porridge (n=60)	1	1.7	0	0	2	3.3	13	21.7	11	18.3	6	10
Infant cereal (n=146)	7	4.8	1	0.7	2	1.4	30	20.7	16	11	10	6.9
Jarred food- Yes(n=112)	4	3.6	1	0.9	2	1.8	25	22.3	11	9.8	8	7.1
Jarred food-No (n=110)	3	2.7	1	0.9	2	1.8	21	19.1	18	16.4	10	9.1
12 month milk feed:												
Breast milk only (n=62)	3	4.8	1	1.6	1	1.6	15	24.2	7	11.3	4	6.5
Formula only (n=183)	8	4.4	1	0.5	1	0.5	42	23	30	16.4	14	7.7
Breast & formula (n=40)	3	7.5	0	0	2	5	6	15	4	10	2	5
Fresh milk/maas (n=4)	0	0	0	0	0	0	1	25	1	25	1	25
12 month complementary diet:												
DDS≥4 (n=42)	1	2.4	-	-	-	-	8	19	10	23.8	3	7.1
DDS< 4 (n=45)	3	6.7	-	-	-	-	10	22.2	11	24.4	4	8.9
MAD (n=28)	1	3.6	-	-	-	-	5	17.9	5	17.9	1	3.6
No MAD (n=59)	3	5.1	-	-	-	-	13	22	16	27.1	6	10.2
IRF (n=76)	3	3.9	-	-	-	-	15	19.7	18	23.7	7	9.2
No IRF (n=11)	1	9.1	-	-	-	-	3	27.3	3	27.3	0	0

4.6 Additional findings

An interesting association between maternal HIV status and infant feeding practices was identified and is presented in this section. The prevalence of stunting and overweight/obesity in the study infants at 24 months is also presented together with further analyses of growth patterns in relation to 24 month outcomes.

4.6.1 HIV and infant feeding practices

Prevalence of breastfeeding, formula feeding and mixed breast and formula feeding in HIV positive and HIV negative mothers is shown in Table 4-19. Results from a chi–square tests of independence show that there is a significant relationship between maternal HIV status and formula feeding/breastfeeding practices at 6 months (Fisher's exact=30.330, $p<0.0005$). Mothers who were HIV positive were significantly more likely to be giving only infant formula at 6 months as a milk feed than mothers who were HIV negative. HIV negative mothers were far more likely to be giving both breastmilk and infant formula. There was also a significant relationship between maternal HIV status and initiation of breastfeeding at birth (Fisher's exact=21.431, $p<0.0005$). The IYCF Indicators questionnaire, which was administered to a sub sample of 87 mothers, included a question that asked whether the child had ever been breastfed. HIV positive mothers were significantly less likely to have initiated breastfeeding than HIV negative mothers.

Table 4-19: Maternal HIV status and infant feeding practices

	Mother HIV positive		Mother HIV negative	
	n	**%**	**n**	**%**
6 month milk feeds (n=240) *				
Breast milk only	22	26.8	50	31.6
Infant formula only	58	70.7	62	39.2
Breast and formula milk	2	2.4	46	29.1
Total	82		158	
Ever breastfed (n=87)**				
Yes	16	53.3	54	94.7
No	14	46.7	3	5.3
Total	30		57	

*50 mothers did not attend 6 month visit
** Sub sample of mothers who had completed the IYCF Indicators questionnaire

4.6.2 Anthropometric data at 24 months

Due to time constraints associated with collecting longitudinal data, the original objectives used 12 month overweight and stunting as the outcome variables. However, during the 12 months it took to collect the 12 month IYCF Indicators data, 145 children with 12 month data reached the age of 24 months. It was decided to include their anthropometric data in this report. This section presents these anthropometric data at 24 months and data on rate of growth in the second year of life, followed by analysis of the association of this data with growth outcomes.

4.6.2.1 Anthropometric status

The results are presented in Table 4-20 together with the 12 month data as a comparison. Both the IOTF classification and WHO reference were used to classify overweight and obesity. The Cole classification can only be used in children two years and older.

Table 4-19: Comparison of anthropometric status at 12 and 24 months

	12months(n=291)	24 months (n=144*)
Stunting		
Stunting (LFA Z < -2 ≥ -3)	5.2%	8.3%
Severe stunting (LFA Z < -3)	0.7%	2.1%
Total	5.9%	10.4%
Mean LAZ; SD	- 0.01; 1.24	-0.51; 1.16
Overweight and Obesity		
WHO Classification (n= 291 at 12 months; 144 at 24 months)	14.5%	18.9%
Overweight (BAZ > +2 ≤ +3)	7.2%	7.7%
Obese (BAZ > +3)	21.7%	26.6%
Combined overweight and obesity	0.81; 1.42	0.95; 1.45
Mean BAZ ; SD	0.6; 1.33	0.35; 1.3
Mean WAZ;SD		
IOTF Classification (n=139)**		20.8%
Overweight		10.8%
Obese		31.6%
Combined overweight and obesity		

* One child had missing length data

** Not all the children attending the 24 month visit had reached 24 months of age

While direct comparisons cannot be made, as 24 month data was only available for a subset of the 12 month sample, the prevalence of both stunting and overweight however, increased from 12 to 24 months. Using the IOTF classification resulted in more infants being classified as overweight or obese compared to the WHO reference. The mean LAZ decreased while the mean BMIZ increased.

4.6.2.2 Description of rate of growth from 12 to 24 months

Change in WAZ, LAZ and WFLZ between 12 and 24 months was calculated and classified as "slow" (≤0.67); "normal" (-0.67 to +0.67) and "rapid" (≥+0.67) growth. The prevalence of each category of growth in the study sample is presented in Table 4-21.

Table 4-20: Prevalence of growth categories: 12-24 months

	Slow growth		Normal growth		Rapid growth	
	n	%	n	%	n	%
WAZ (n=145)	46	31.7	82	56.6	17	11.7
LAZ (n=144)*	72	50	49	34	23	16
WFLZ (n=144)*	48	33.3	59	41	37	25.7

*****missing length data for 1 subject

The percentage of children in the "rapid" growth category declined further from the 6-12 month time period while "slow" growth was the most common category for LAZ. The prevalence of each category compared to the 0-6 and 6-12 months data is shown in Figure 4-3.

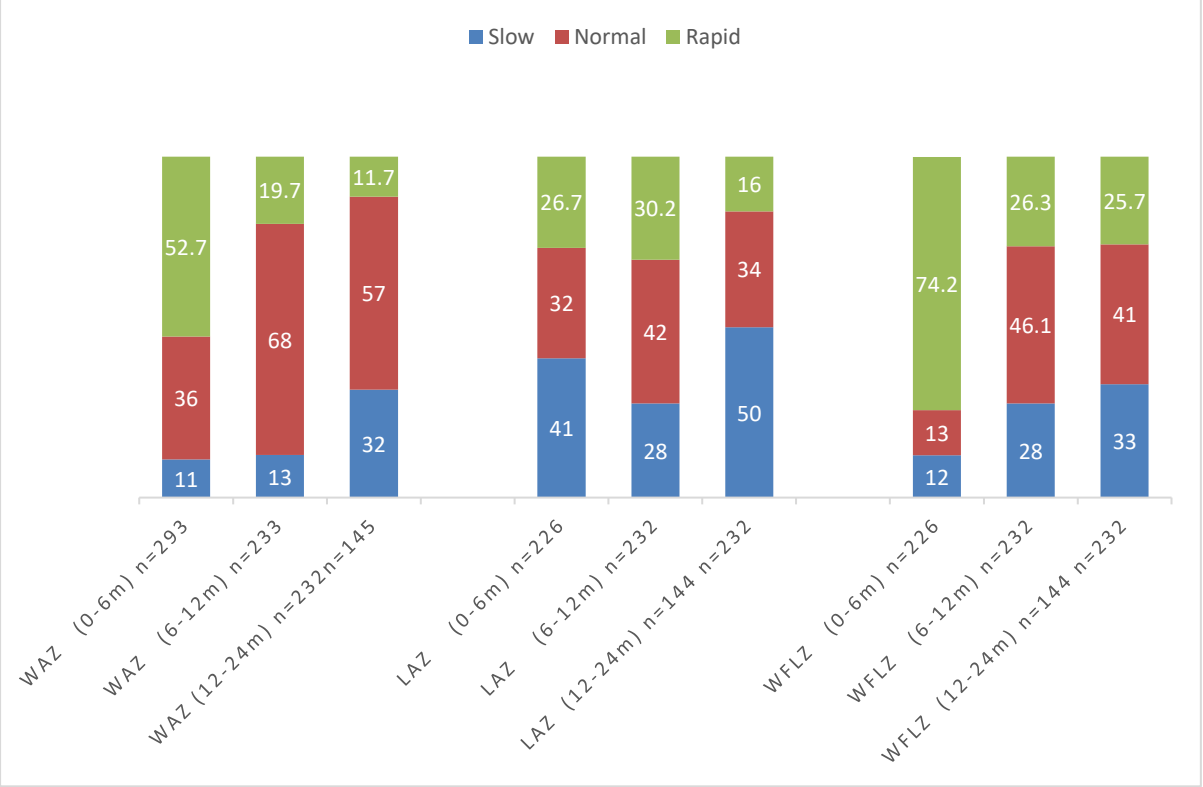

Figure 4-3: Percentage of infants in each growth category: 0 - 6, 6 - 12 and 12 - 24 months

4.6.2.3 The association of birth anthropometric data and growth patterns with growth outcomes at 24 months

Regression analysis was performed to explore the causal relationships between the independent variables birth anthropometric status; rate of growth from 0-6 months; 6 to 12 months; and 12 to 24 months and the dependent variables LAZ and BAZ at 24 months. The results related to LAZ and BAZ are presented in Table 4-22 and 4-23 respectively.

Table 4-21: The relationship between LAZ at 24 months and birth LAZ, and change in LAZ.

	Beta coefficient	Model summary R square	df	F-statistic	p value
LAZ					
Birth	0.178	0.198	1:141	5.726	p=0.018
Change 0-6	0.145	0.216	1:125	6.117	p=0.015
Change 6-12	0.097	0.120	1:127	1.844	p=0.177
Change 12-24	0.344	0.319	1:142	16.100	p<0.0005

Birth LAZ and change in LAZ between birth and 6 months remained significant predictors of LAZ at 24 months. Change in LAZ from 6-12 months was no longer significant. The strongest predictor of 24 month LAZ was change in LAZ from 12 to 24 months with a value of the coefficient B of 0.344.

Table 4-22: The relationship between BAZ at 24 months and birth WAZ, and change in WAZ, and WFLZ.

	Beta coefficient	Model summary R square	df	F-statistic	p value
WAZ					
Birth	0.265	0.033	1:143	4.877	p=0.029
Change 0-6	0.528	0.170	1:128	26.243	p <0.0005
Change 6-12	0.627	0.106	1:128	15.141	p <0.0005
Change 12-24	0.501	0.082	1:143	12.694	p<0.0005
WFLZ					
Birth	0.207	0.255	1:141	9.767	p=0.002
Change 0-6	0.102	0.140	1:126	2.523	p=0.115
Change 6-12	0.325	0.212	1:127	5.976	p=0.016
Change 12-24	0.492	0.400	1:142	27.114	p<0.0005

Change in WAZ from 6-12 months remains the strongest predictor of BAZ at 24 months with a value of the coefficient B of 0.627. The strongest predictor of BAZ at 24 months when looking at change in WFLZ was the 12-24 month period, value of the coefficient B, 0.492.

In order to determine whether being overweight or obese at 12 months was a significant predictor of overweight or obesity at 24 months further analysis was performed. The results of the linear regression analysis are presented in Table 4-24.

Table 4-23: The relationship between BAZ at 24 months and BAZ at 24 months

	Beta coefficient	Model summary R square	df	F-statistic	p value
BAZ 12 months	0.606	0.398	1:142	93.865	p<0.0005

BAZ at 12 months is strongly predictive of 24 month BAZ as can be seen by the high value of the coefficient B of 0.606. The higher the BAZ at 12 months the higher the BAZ at 24 months. This is further demonstrated by the fact that all the infants who were obese at 12 months were either obese or overweight at 24 months.

4.7 Summary of results

The following are the most noteworthy findings:

The sample consisted of 290 infants at 12 months, 54.1% were males and 45.9% females. The prevalence of stunting at 12 months was 5.9% while combined overweight and obesity prevalence was 21.7% with a further 22.1% classified as being at risk of overweight. Anthropometric analysis of 144 of these children at the age of 24 months showed that their nutritional status had deteriorated in the second year of life. Stunting rates had nearly doubled to 10.4%. Combined overweight and obesity prevalence had increased to 26.6%. This prevalence was even higher at 31.6% when using the Cole classification.

Maternal average age was 26.0 and the majority of mothers had completed matric. Maternal HIV prevalence was 35%. Average household size was 6 members and a large proportion of households reported having either no income or less than R2000 per annum. Access to tap water was universal and 98.9 % had electricity, while 8.7% lived in informal housing. Apart from type of housing, there was no association between sociodemographic variables and growth outcomes at 12 months. Infants living in flats were more likely to be stunted than those living in houses. Infants living in informal housing were more likely to be severely stunted, wasted, at risk of overweight and overweight than those living in formal housing.

Analysis of growth patterns revealed a very high proportion of infants with rapid gains in weight and weight for length in the first 6 months that decreased thereafter. The analysis of the

relationship between birth anthropometric status and patterns of growth, and growth outcomes at 12 and 24 months showed:

- Children classified as stunted at 12 months had lower mean birth WAZ and LAZ, while overweight children had higher mean birth WAZ. Linear regression analysis showed a similar pattern using continuous data with both birth LAZ and WAZ significant predictors of LAZ and BAZ at 12 months and 24 months.
- Children obese at 12 months had a pattern of more rapid weight gain than infants with normal BMI at 12 months starting in the first 6 months of life and continuing thereafter. Their higher BAZ at 12 months persisted into the second year with all children who were obese at 12 months being either obese or overweight at 24 months.
- Change in LAZ from 6 to 12 months was most predictive of 12 month LAZ while change in WAZ from 6-12 months was the strongest predictor of both 12 and 24 month BAZ.
- Higher BAZ at 12 months is strongly predictive of high BAZ at 24 months.

Infant feeding practices in the study infants were described. Breastfeeding rates at 6 months were 30% with a further 20% receiving a combination of breastmilk and infant formula. Breastfeeding rates were significantly lower amongst HIV positive mothers compared to HIV negative mothers, who were also more likely to give both infant formula and breastmilk. Half of the mothers fed their infants solid foods before the age of 6 months and only one third of infants met the WHO IYCF MAD indicator. Consumption of instant noodles was common as was intake of inappropriate foods such as SSBs and high fat snacks. There were no significant associations of feeding practices with growth outcomes at 12 months.

The relationship of patterns of growth in the first year of life, in particular the 6 to 12 month period, with BMI outcomes at both 12 and 24 months was the most significant finding of this study. These results will be discussed in the next chapter.

5. DISCUSSION

This study aimed to determine the prevalence of stunting and overweight at 12 months in the MACE study infants; describe potential risk factors identified from the review of the literature; and determine their association with growth outcomes. The results that were presented in the previous chapter will now be discussed.

5.1 Prevalence of stunting and overweight

The prevalence of stunting and overweight in the MACE study infants at 12 and 24 months compared to other South African studies from the last 10 years is presented in Table 5-1. The other studies in Table 5-1 included children in the same age range and used the WHO 2006 growth standard, unless otherwise indicated. The importance of comparing studies with similar age categories and using the same growth reference was highlighted previously. The prevalence of stunting was far lower than other South African surveys of children in the same age range, while that of overweight was higher than most. By 24 months of age both stunting and overweight had increased and this was in line with other studies, however the stunting prevalence remained comparatively low. The low stunting rates could be partly explained by the exclusion of pregnant women with diabetes and hypertension.

Table 5-1: Comparison of the prevalence of stunting and overweight in MACE and other studies

	Stunting			**Combined overweight and obesity**				
					WHO		**IOTF**	
	Age	**n**	**%**	**Age**	**n**	**%**	**n**	**%**
MACE	12 mths 24 mths	290 144	5.9 10.4	12 mths 24 mths	290 144	21.7 26.6	 139	 31.6
Drakenstein Budree *et al* 2017a; 2017b	12 mths	342	13	12 mths	342	8	-	-
Breede Valley du Plessis *et al*., 2016	0-23 mths	218	28.8	0-23 mths	218	21.8	-	-
Agincourt (Kimani-Murage, 2010)	12 mths 24 mths	137 160	32 20	12 mths 24 mths	- -	- -	 160	 10
SANHANES-1 Shisana *et al*., 2013	0-3 yrs	1090	26.5	2-5 yrs	-	-	1291	23.8

Interestingly, combined overweight and stunting was only present in one infant at 12 months in the MACE study and increased to 4 children at 24 months (3%). This is in contrast to the Limpopo study which reported a very high prevalence of combined stunting and overweight of

19% at 36 months (Mamabolo *et al*., 2005), and a number of other studies which found that stunting was a significant risk factor for being overweight (Steyn *et al*., 2005; Popkin *et al*., 1996). Given the increasing prevalence of concurrent stunting and overweight from 12 to 24 months this trend should be monitored in follow up studies in this cohort. A Mexican study reported rates of 5-10% in children aged 2 to 6 years (Fernald & Neufeld, 2007) which is only slightly higher than the 3% at 24 months in MACE study infants.

There were no gender differences in stunting and overweight prevalence in line with other studies in younger children (Kimani-Murage, 2013; Shisana *et al*, 2013). Gender differences only become apparent in older childhood and adolescence when boys have higher rates of stunting while overweight is more prevalent in older girls (Kimani-Murage, 2013; Shisana *et al*, 2013). An exception to this was the MRC/HST study which found higher rates of stunting amongst boys under the age of 5 years (Lesiapeto *et al.*, 2010). The low stunting and high overweight prevalence in the MACE study infants indicates that in this urban population the nutrition transition may be fairly advanced and over nutrition is more of a problem than undernutrition, although the previously discussed issue of exclusion of hypertensive and diabetic mothers must be kept in mind.

5.2 Maternal and household characteristics

Low stunting prevalence can be explained when considering the maternal and household characteristics of the cohort. This was an urban population with almost universal access to tap water and electricity. Lack of water and sanitation is associated with increased episodes of diarrhoea, a significant contributor to stunting (Danaei *et al*., 2016; Black *et al*., 2013). The MRC/HST study, conducted in 2003 in rural areas of KwaZulu-Natal and the Eastern Cape, that had a stunting prevalence of 28%, reported that 75% of the population accessed water from dams and rivers while only 26% had electricity (Lesiapeto *et al*., 2010).

Maternal education levels in the MACE study were relatively high with 70% of mothers having a matric or tertiary qualification and only 1% having primary school education or less. The fact that virtually all the mothers had at least some secondary education probably explains the lack of an association between education and growth outcomes. Stunting has been linked to poor maternal education in both international and local studies. Mothers with low levels of education are more likely to have poor caring practices such as not accessing health care services, for example immunisations; as well as neglect or lack of psychosocial stimulation (Black *et al.*,

2013). Poor complementary feeding practices, particularly lack of dietary diversity were also associated with maternal education in Asian studies (Kabir *et al*., 2012; Senarath *et al*., 2012 ;) and a study in Zambia (Mallard *et al*., 2014). Both the rural Limpopo study and the Agincourt study in South Africa found an association between stunting and young or student mothers (Kimani-Murage, 2013; Mamabolo *et al*., 2005).

Access to water and electricity, and relatively high levels of maternal education may have ameliorated the effect of SES on stunting, considering that reported household income was predominantly very low. In the MACE cohort the only sociodemographic factor that had any association with growth outcomes was the type of housing. Living in a flat, rather than a house, was associated with stunting; while living in informal housing was associated with both severe stunting and overweight, indicating that both these growth outcomes are associated with low SES. This is in line with the 2016 South African DHS which found that stunting and overweight were both more prevalent in the lowest quintile of SES (DoH *et al*., 2017). A number of other South African studies that investigated determinants of stunting and overweight also found an association between stunting and low SES (Budree *et al*., 2017b; Lesiapeto *et al*., 2010; Mamabolo *et al*., 2005) but the relationship with overweight was mixed. The Agincourt study found that higher SES was associated with overweight (Kimani-Murage, 2013) while in the HST/MRC study overweight was associated with lack of a regular source of income (Lesiapeto *et al*., 2010). In HICs, childhood overweight is more prevalent in children of low SES (Skinner et al., 2018; Olds *et al*., 2011) and it would appear that this is becoming the trend in South Africa.

HIV exposure was neither associated with stunting nor overweight at 12 months in the MACE infants. The effect on birth outcomes was not explored, as infants born preterm were excluded from this study. While the negative impact of untreated HIV infection on infant growth is well documented (Venkatesh *et al.*, 2010), there has been mixed evidence on the effects of HIV exposure and/or maternal ARV therapy (ART) on growth outcomes in HIV exposed but uninfected (HEU) children. A systematic review on the topic found that some trials investigating birth outcomes reported an increased prevalence of preterm birth while others found no effect (Heidari *et al*., 2011). The recently published results from the 2012-2013 South African PMTCT evaluation showed that HEU infants had a greater risk of preterm birth, SGA and LBW; and of being underweight for age at 6 weeks compared to HIV unexposed infants.

Preconception ART also increased the odds of preterm delivery compared to post conception ART initiation (Ramokolo *et al.*, 2017).

The systematic review on the effect of maternal ART on infant outcomes also reviewed studies investigating later growth outcomes. Some studies found lower LAZ but they appeared to be transient (Heidari *et al*., 2011). Infants in an American study exposed to Tenofovir (TDF) had slightly lower mean head circumference and LAZ at 12 months than infants on a non TDF regimen (Siberry, *et al*., 2012). The authors highlighted that while the difference was small, <0.5 cm on average, and of uncertain significance, further monitoring of longer term growth outcomes in HEU infants was recommended (Siberry *et al*., 2012). The MACE study finding of no difference in growth outcomes at 12 months between term HIV exposed and unexposed infants, is particularly welcome in view of the high antenatal prevalence of HIV in South Africa and in KZN in particular. The sample size is, however, relatively small so the findings should be interpreted with caution.

It must be noted that the fact that the MACE study excluded pregnant women with hypertension and diabetes could have contributed to the low stunting prevalence. These women would have been more likely to have experienced the double burden of malnutrition themselves, placing their offspring at greater risk for LBW and subsequent stunting.

It is interesting to compare the MACE study stunting prevalence to another current longitudinal birth cohort study, the Drakenstein study in the Western Cape. Stunting prevalence in the Drakenstein cohort at 12 months, although lower than other national and regional surveys at 13%, was a little over double that of the MACE cohort at 5.9% (Budree *et al*., 2017b). Stunting in the Drakenstein cohort was associated with low birthweight, which was linked to very high rates of antenatal smoking at 54%, and alcohol intake of 26%, in mothers of mixed race ethnicity. Birthweight and linear growth were both lower in mixed race infants. The MACE cohort mothers in contrast, had rates of antenatal smoking and alcohol intake below 5% and they were not associated with either birthweight or 12 month growth outcomes. The small numbers in each group however mean that these results should be interpreted with caution. The MACE cohort has a similar ethnic mix to the Drakenstein cohort, comprised mainly of Black African and mixed race subjects. Although data on ethnicity was not collected in the MACE study, home language was used as a proxy for ethnicity and there were no differences in growth

outcomes between the two groups. This suggests that lifestyle factors, rather than ethnicity, are responsible for the disparities in growth outcomes between ethnic groups in the Drakenstein study. Clear messaging regarding the harmful effects of alcohol and smoking on the developing fetus and community initiatives aimed at supporting mothers to make healthy choices are some potential interventions although a long term solution requires addressing the underlying poor socioeconomic conditions.

5.3 The relationship between birth weight and length and growth patterns; and growth outcomes at 12 and 24 months

There was congruence between the findings using both categorical outcomes (ANOVA) and continuous data (linear regression analysis). Both analytical methods found links between birth anthropometrics and 12 month growth outcomes, and additionally identified the 6-12 month period as being of greatest significance. They also found no link between birth LAZ and linear growth, and BMI categories or BAZ at 12 months.

5.3.1 Birth weight and length

In the MACE study, both birthweight and length of the infants were associated with stunting and LAZ at 12 month and 24 months. Overweight at 12 months and BAZ at 12 and 24 months were also associated with birthweight, but not birth length. This emphasises the importance of the maternal aspect of the first 1000 days. As was discussed previously, 20-25% of childhood stunting has its roots in poor foetal growth (Danaei *et al*., 2016; Black *et al*., 2013) while childhood overweight is also thought to start in utero (Black *et al*., 2013).

The association of low birthweight with increased risk of stunting was reported in the Drakenstein and Agincourt studies (Budree *et al.*, 2017b; Kimani-Murage, 2013). A link between high birthweight and risk of obesity at 9-11 years of age was reported by the International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE), which included South African schoolchildren (Qiao *et al*., 2015). Of interest was the finding that infants from LMICs were at increased risk of obesity at lower birthweights, >3500 g, than infants in HICs, whose risk only increased above 4000 g. The authors suggested that this could be partly attributed to differences in maternal nutritional status between LMICs and HICs (Qiao *et al*., 2015). This is demonstrated in the MACE study where infants classified as overweight

at 12 months had a mean birthweight of 3396 g, which is 300 g heavier than infants of normal BMI at 12 months, although not exceptionally high.

This finding of a positive relationship between birthweight and later BAZ is similar to results from a number of international birth cohort studies, however they used growth outcomes in older children, typically aged 5 to 7 years (Weng *et al*., 2012). The Millennium Cohort study and the Avon Longitudinal study both reported increasing risk of childhood overweight with increasing birth weight (Brophy *et al*., 2009; Reilly *et al*., 2005). Higher birthweight remained a predictor of higher BMI at 24 months in the MACE study infants, but follow up at the age of 5 years is recommended to determine if this association remains significant in later childhood. Considering the relationship of lower birthweight with stunting, optimal nutrition and health care during pregnancy is important to ensure a healthy birthweight that protects against both stunting and overweight.

5.3.2 Patterns of growth

There were four main findings from the analysis of growth patterns:

- The first 6 months was characterised by rapid weight gain in more than 50% of study infants.
- Growth in the 6-12 month period was most strongly predictive of both growth outcomes at 12 months
- Obese infants at 12 months had significantly greater gains in weight starting from the first 6 months and continued to be either overweight or obese at 24 months.
- There was disproportionate weight gain in relation to linear growth in the cohort.

These findings will now be discussed in more detail.

There was a marked difference in rate of growth between the first and second 6 months of life in the MACE study infants. In the first 6 months, over half the study infants had gains in WAZ ≥+0.67, placing them in the "rapid" growth category. The mean change in WAZ was +0.777 for the study sample, similar to the +0.7 change reported in the Drakenstein study (Budree *et al*., 2017b). This was not accompanied by gains in LAZ, resulting in 75% of the infants falling into the "rapid" growth category for WFL. Growth patterns changed in the second 6 months with most of the study infants falling into the "normal" growth category for WFA and LFA. A similar pattern of rapid weight gain in the first 6 months, followed by slowing in the 6-12 month period was reported in the Drakenstein study (Budree *et al*., 2017b). Rapid weight gain in the

first 1 to 2 years of life has been associated with risk of obesity in later childhood in a number of studies (Druet *et al*., 2011; Ong & Loos, 2006), including one using data from the South African Birth to 20 cohort (Cameron *et al*., 2003).

The birth to 6 month period has been identified in some studies as being particularly significant for later obesity risk (Chandler-Laney *et al*., 2013; Taveras *et al*., 2009) whereas in the MACE study the change in LAZ and WAZ in the 6-12 month period was the most predictive of 12 month LAZ and BAZ respectively. Change in LAZ in the second 6 months of life was most strongly predictive of 12 month LAZ, while change in both WAZ and WFLZ during that time period was most strongly predictive of BAZ at 12 months. ANOVA results confirmed the importance of the 6-12 month period: Infants classified as obese, overweight and at risk of overweight at 12 months had significantly greater mean change in WAZ and WFLZ than those classified as having normal BMI.

Change in LAZ from 6-12 months was no longer significant when using 24 month LAZ as the outcome while 12 -24 month change in LAZ was the most predictive. Change in WAZ, however, during the 6-12 month period, remained most predictive of BAZ at 24 months. The consistent association of early rapid weight gain with later childhood obesity may be due either to nutrition, or the environment, or may be a genetic marker for future weight gain trajectory (Druet *et al.*, 2011). Follow up of the study infants at 4-5 years is recommended to determine whether rapid weight gain in the 6-12 month period remains the most significant predictor of later overweight and obesity.

The finding that infants obese at 12 months of age had already started on a rapid weight gain trajectory in their first 6 months, and that their increased BMI persisted into the second year of life, has been demonstrated in other studies. An American study found that overweight and obese 5 year olds had consistently higher BMIs than normal weight children starting from 2-4 months of age (Ludington-Hoe *et al*., 2013), similar to the findings of a Chilean study (Corvalan *et al*., 2009). The "growth acceleration hypothesis" proposes that early and rapid growth, particularly in the first 6 months of life, programs the infant metabolic profile to be susceptible to obesity in later childhood and adulthood (Young *et al.*, 2012; Singhal, 2007). The Isle of Wight study discussed previously had identified four different growth trajectories in children from birth to 18 years, two of which are relevant to the MACE findings. The first one was early, transient overweight where infants were overweight at 1 year, however BMI

decreased thereafter. The second one was early, persistent obesity where infants were obese by 4 years and BMI never decreased (Ziyab *et al*., 2014). In the MACE study, all of the infants who were obese at 12 months and who had 24 month data, remained either obese or overweight at 24 months; indicating that they were probably on the persistent obesity trajectory. Additionally, their mean change in WAZ from birth to 12 months was +2.97. This puts them at an extremely high risk for later obesity when one considers the results of the meta-analysis by Druet *et al* (2011) which showed that infants with a change in WAZ >+1.33 in the first year had an almost fourfold risk of later childhood obesity.

The necessity in LMICs of balancing prevention of overweight with promotion of healthy growth, in particular linear growth, was discussed at length in the literature review. The general consensus was that, unlike in HICs, rapid weight gain in the first 24 months, particularly if accompanied by gain in length, may be desirable in terms of human capital outcomes and does not appear to pose a long term risk for later obesity or cardio metabolic outcomes. In contrast, rapid weight gain after this time, has no positive effects and does increase the risk of later obesity and NCDS (Adair *et al*., 2013). The finding therefore of a disproportionate gain in weight in relation to length in the MACE cohort from birth to 24 months, is a cause for concern. Mean birth WAZ of -0.23 was below the WHO reference and had increased to +0.35 by 24 months while LAZ decreased from +0.34 to -0.51. This is very similar to the Drakenstein study findings at 12 months where mean WAZ increased from -0.7 to +.01, while LAZ decreased from +0.1 to -0.5 (Budree *et al*., 2017).

5.4 Infant feeding practices and growth outcomes

Breastfeeding practices, age of introduction of solid foods and the quality of the complementary feeding diet were assessed. Despite poor feeding practices, there were no associations with growth outcomes at 12 months, echoing the findings of the Drakenstein study (Budree *et al*., 2017a). The hypotheses of the study, namely that poor breastfeeding practices, and lack of dietary diversity were associated with both stunting and overweight, are therefore rejected.

5.4.1 Breastfeeding practices

Information on initiation of breastfeeding was only collected from the sub sample of mothers who completed the IYCF Indicators questionnaire. The findings were similar to other South

African studies, with high initiation rates of 95% in HIV unexposed infants, comparable to the rural KZN MRC study with 96% (Faber & Benade, 2007). Initiation rates were significantly lower, just over 50%, in HIV exposed infants. The Drakenstein study reported initiation rates of 86% in the cohort but less than 50% in HIV exposed infants (Budree *et al*., 2017a) while in another Western Cape study, in Worcester, none of the HIV positive mothers initiated breastfeeding (Goosen *et al*., 2014). The Worcester study was conducted in 2011, while the Drakenstein study collected data from 2012-2015 and the findings probably reflected prior Department of Health policy that provided free infant formula to HIV positive mothers (DoH, 2007). Following the Tshwane Declaration (Tshwane Declaration, 2011), the Department of Health IYCF Policy changed in 2013 and free formula was discontinued, HIV positive mothers were advised to exclusively breastfeed for 6 months then introduce complementary foods and continue breastfeeding to 12 months (DoH, 2013b). In KZN the free formula supply to HIV positive mothers was discontinued two years earlier, from January 2011 (KZN DoH, 2010). Therefore, it is surprising that breastfeeding initiation rates were not higher in HIV exposed infants in the MACE study.

The difference in feeding practices between HIV positive and HIV negative mothers was also apparent at 6 months and 12 months. At 6 months 73% of HIV positive mothers were giving formula milk as the only milk feed and this increased to 87% of mothers by 12 months. The corresponding figures in HIV negative mothers were 39% and 51%. Mixed feeding of both breastmilk and formula at 6 months was significantly more common in HIV negative mothers at 29.5% compared to HIV positive mothers at only 2.5%. In the Drakenstein study, exclusive breastfeeding rates at 6 months in those HIV positive mothers who had chosen to breastfeed were 26%, double those of the HIV negative mothers, at 13% (Budree *et al.*, 2017a). This indicates that the message regarding the importance of exclusive breastfeeding in the first 6 months has perhaps been misinterpreted to apply to HIV positive mothers only. It could also reflect the fact that HIV positive mothers may receive more detailed breastfeeding counselling from nursing staff and lay counsellors.

By the age of 6 months, half of the MACE mothers had discontinued breastfeeding completely, while only 35% of infants were still receiving breastmilk at 12 months. These rates are far lower than the Drakenstein study, which reported breastfeeding rates of 58% at 12 months (Budree *et al*. 2017a), but similar to those in the Breede valley study, with rates of 32.5% in

infants aged 12-15 months (du Plessis *et al*., 2016). Breastfeeding rates at 12-17 months in the urban mothers in the Faber *et al*. (2016) KZN study were also fairly low at 44%. All of these figures reflect poor adherence to the South African IYCF Policy which recommends breastfeeding up until two years and beyond, including for HIV positive mothers (DoH, 2017a). The low rates in both the MACE and the Faber *et al* (2016) studies, which were both conducted in KZN, are unexpected as the province has been singled out for its commitment to, and support of, breastfeeding promotion (DoH *et al*., 2014). The effect of maternal HIV status on feeding choice, as discussed previously, may be a contributory factor when considering that KZN is the province with the highest antenatal HIV prevalence, at 44% (DoH, 2017b).

The lack of an association between breastfeeding and growth outcomes is not unexpected. Breastfeeding is vital for child survival in LMICs (Jones *et al*., 2003) but it has not been shown to decrease stunting (Black *et al*., 2013). Continued breastfeeding at 12 months was in fact associated with lower LAZ in the Drakenstein study as well as in a review of DHS data from 9 countries (Budree *et al*., 2017a; Jones *et al*., 2014). Breastfeeding has been shown to be moderately protective against childhood overweight in a number of birth cohort studies in HICs (Weng *et al*., 2012) but this association may be confounded by maternal education or SES as a RCT in Belarus failed to find a protective effect (Kramer *et al*., 2007). Results from a birth cohort study conducted in Brazil, which is a LMIC, found no association between duration of breastfeeding and obesity at 11 years of age (Neutzling *et al*., 2009). A strength of the Brazilian study was that there was very little risk of confounding by socio economic factors because breastfeeding duration was not associated with socioeconomic status or maternal education. The Promise EBF study in contrast, found that infants who were not breastfed at 12 weeks were at greater risk of rapid weight gain between 12 and 24 weeks and of being overweight or obese at 24 months (Ramokolo *et al*., 2015). The potential mechanisms for a protective effect of breastfeeding include nutritional composition factors, particularly lower protein content (Poskitt & Breda, 2012); bioactive factors, such as leptin or ghrelin that may elicit an endocrine effect (Weng *et al*., 2012; Young *et al*., 2012); and behavioural factors, namely enhanced ability to self-regulate intake, leading to lower energy intake (Yang & Huffman, 2013; Young *et al*., 2012).

5.4.2 Age of introduction of complementary foods

There appears to be a positive trend towards introducing complementary foods at a later age, when looking at the findings from MACE and other recent studies, compared to studies conducted 10-15 years ago. In the MACE study the mean age of introduction of complementary foods was 5.1 months (SD 1.14) with only 10% of infants receiving solids before 4 months of age. The Drakenstein study reported that 19% of infants had received solid food before 14 weeks of age, of which 8% had received it by 6-10 weeks (Budree *at al*., 2017a). These figures are considerably lower than those of studies conducted in the early 2000s. In the Limpopo birth cohort, 80% of infants had received solid foods by the age of 3 months, 44% of them by 1 month (Mamabolo *et al*., 2004), while in a rural KZN study 61% of infants had received solids by 4 months of age (Faber & Benade, 2007). The Promise-EBF trial which collected data between 2006 and 2008, found that almost two thirds of infants had received infant cereal by the age of 3 months, 28% of them by 3 weeks of age (Ramokolo *et al*., 2015). Previous advice given to mothers was to introduce complementary foods between 4-6 months. The South African IYCF Policy of 2007 (DoH, 2007) adopted the 2001 WHO guideline of exclusive breastfeeding until 6 months of age, followed by the introduction of complementary food. There appears to be greater awareness amongst mothers of this recommendation in recent years. Both the MACE and Drakenstein study found no association between the age of introduction of solid foods and either stunting or overweight. There has been conflicting evidence from international studies with a systematic review concluding that while there was no clear evidence, very early introduction, before 4 months of age, may increase the risk (Pearce *et al*., 2013). Potential mechanisms include either increased energy intake, resulting in early rapid weight gain (Thompson & Bentley, 2013); or a hormonal effect, for example, by increasing ghrelin levels (Pearce *et al*., 2013).

5.4.3 Complementary feeding practices

5.4.3.1 Feeding practices at 6 months

The limited data that was available on feeding practices at 6 months in the MACE study infants indicates that commercial infant foods, particularly infant cereals, were the predominant choice. Nearly two thirds of the sample received infant cereal, with only a quarter receiving porridge while half the sample received commercial jarred foods. Approximately one third of the sample received both infant cereal and jarred foods. The Drakenstein cohort had an even

higher daily consumption of infant cereal at 6 months of almost 90% (Budree *et al*. 2017a). The Faber & Benade (2007) study reported a greater consumption of porridge than infant cereal with 52% of infants receiving infant cereal on most days while 88% of infants received porridge. This probably reflects the fact that this was a rural population. A later study conducted in both a rural and an urban setting in KZN found that the consumption of infant cereal was more common in the urban setting (Faber *et al*. 2016).

Infant cereal, although unaffordable for many, has been found to make an important contribution to iron intake, with the rural KZN study reporting 51% of total iron intake coming from infant cereals (Faber, 2005). A beneficial effect of iron fortified commercial infant foods, including cereal, on LAZ was reported in an Indonesian study (Diana *et al*. 2017). In South Africa there is mandatory fortification of maize meal and bread flour with a range of micronutrients, including iron and zinc, but the amounts consumed by infants are too small to have a significant impact on micronutrient intake (Faber *et al*. 2016). South African complementary feeding guidelines, while recognising that infant cereals can improve micronutrient intake and are convenient, also emphasise the importance of improving dietary diversity and specifically intake of animal protein foods (du Plessis *et al*., 2013). These are also expensive but are required in only small amounts and cheaper sources such as chicken livers can provide high levels of micronutrients for far less money than commercial infant foods. As an example, three tablespoons of chicken livers (45 ml) provides 4.5 mg of iron and 2.3 mg of zinc (FoodFinder®), compared to the 6 mg of iron and 2 mg of zinc in 150 ml of Nestlé Cerelac® infant cereal, for less than half the price[3].

While infant cereals are high in micronutrients, commercial jarred foods make little contribution to micronutrient intake and are far costlier (Faber, 2005). A review of the nutritional value of commercial compared with home prepared meals, highlighted the conflicting findings on the topic but advised that, provided caregivers followed infant feeding guidelines, home prepared foods had a number of advantages including: the ability to vary texture; greater control over ingredients, and cheaper cost (Lockyer, 2016). Lobstein *et al* (2015) in the 2015 Lancet Obesity series raised the issue of the rapidly increasing processed infant foods market which may be preventing the transition from breastmilk to family foods.

[3] June 2018 prices: chicken livers R10.99/250g=R2.43 per 45ml serving; Cerelac® R32.99/ 250g= R6.60 per 50g serving (150ml)

The concern is that they accustom infants to highly processed foods from an early age and these taste preferences then continue into later childhood and beyond.

5.4.3.2 Feeding practices at 12 months

Complementary feeding practices were assessed using the WHO IYCF Indicators for infants from 6-23 months, with some adaptions made for local conditions and the inclusion of a section on the intake of inappropriate foods and beverages. The questionnaire, administered by the researcher to a sub set of 94 caregivers, revealed poor dietary diversity with a mean DDS of 3.56, below the cut off for an adequate DDS of 4. This is similar to the mean DDS of 3.26 for children 6-13 months old in the Breede Valley study (du Plessis *et al*. 2016). The association of dietary diversity with micronutrient adequacy was demonstrated by Faber et al. (2016), where a complementary diet with a DDS ≥4 provided significantly greater amounts of protein and a number of micronutrients, including calcium, iron and zinc than a diet with a DDS <4. Less than half the sub set of MACE infants achieved MDD while only one third achieved the MAD indicator. These results are comparable to other South African studies that have reported on complementary feeding practices using the IYCF Indicators; two of the studies reported that less than half of infants achieved MDD (du Plessis *et al*., 2016; Faber *et al*., 2016). The Drakenstein study reported much higher rates with 75% of infants achieving MDD, but the MAD indicator could not be assessed as information on frequency of feeding was not collected (Budree *et al*. 2017a). The MAD Indicator was assessed in the Breede Valley study and the 2016 DHS; both reporting less than 50% of infants achieving MAD, with the DHS particularly low at only 21% (DoH *et al*., 2017; du Plessis *et al*., 2016). To put the findings into a global context, a recent analysis using UNICEF data from 101 countries found that, in infants aged 12-17 months, only 31.5% achieved the MDD indicator and this figure decreased to 18% for the MAD indicator (White *et al*., 2017).

Iron rich food consumption was fairly high at 87%, including infant cereal and formula; very similar to that reported in the Breede Valley study at 89%. The intake of flesh foods was also similar, 60% in MACE compared to 55.6% in Breede Valley (du Plessis *et al.* 2016). Consumption of animal protein foods is recommended as they are good sources of iron and zinc which are typically problem nutrients in the complementary diet and, together with calcium, are associated with linear growth (Krasevec *et al*., 2017). There was a high meat consumption in the Drakenstein study with 77% of infants consuming animal protein but 55%

was in the form of polony (Budree *et al*., 2017a), while only 5% of infants in the MACE study had consumed polony in the previous 24 hours. Polony and other processed meat consumption is not recommended because of the high sodium and saturated fat content. Of concern in the MACE study was the fact that almost a quarter of infants had consumed "two minute" noodles, which are also very high in sodium. Consumption of high levels of sodium in infancy has been linked to higher blood pressure in later life (Huffman *et al*., 2014). Additionally, noodles are purely a source of refined carbohydrate with very little nutritional value and replace more nutrient dense foods in the diet.

The high levels of consumption of inappropriate infant foods such as SSBs and HFS, for example crisps, in the MACE study have also been reported in the Drakenstein study (Budree *et al*., 2017a) and in two KZN studies (Faber *et al*., 2016; Faber& Benade, 2007). There seems to be an increasing trend towards the consumption of crisps in more recent times. In the earlier rural KZN study, crisps were consumed once a week by 35% of 6-12 month old infants (Faber & Benade, 2007); this figure had nearly doubled in the more recent KZN study (Faber *et al*., 2016) and was even higher, at 72%, in the MACE sub set. This trend is not limited to South Africa; increasing consumption in LMICS of inappropriate high fat and high sugar snacks and beverages has been reported with implications for both stunting and overweight and longer term NCD risk (Contreras *et al*., 2016; Huffman *et al*., 2014). This is due to their combination of high energy- but low nutrient-density and high contents of sugar, salt and fat, particularly trans fats. In a LMIC context setting these foods also displace healthier, more nutrient dense options in the food budget (Huffman *et al*., 2014).

Fruit juice is often seen as a healthy option by mothers but the American Academy of Pediatrics does not recommended this for infants below 12 months (Heyman & Abrams, 2017) as it is thought to act as a gateway drink to SSBs in later childhood (Sonneville *et al*., 2015). It is therefore concerning to note that in South Africa there is a fruit juice specifically marketed for infants from 6 months. Fruit juice was consumed at least once a week by 51% of 12 month old infants in the MACE subset and daily by 83% in the Drakenstein cohort (Budree *et al*., 2017a).

Despite the poor complementary feeding practices in MACE study infants, such as low dietary diversity and the frequent consumption of inappropriate foods and beverages, there was no association with either stunting or overweight. This could be due to the small sample size of

87 infants. These findings are however very similar to those of the Drakenstein study which also used the WHO IYCF Indicators but had a larger sample size and collected data at different time points. Poor feeding practices were also identified in the Drakenstein study but there was no association with growth outcomes except that longer duration of exclusive breastfeeding was associated with lower LAZ at 12 months (Budree *et al*., 2017a).

The WHO Indicators are a useful tool to assess feeding practices and make comparisons across studies, both nationally and internationally, but their association with growth outcomes has been mixed. A pooled analysis of data from 14 countries found lower risk of both stunting and underweight if the indicators for timely introduction of solid foods, MAD, intake IRF and MDD were met (Marriott *et al.*, 2012). A later review used data from 9 countries but did not pool the data. It found lack of consistency across countries with some countries showing a positive relationship between MDD indicators and MAD and HAZ, but others showing no association (Jones *et al.*, 2014). The authors suggested that this may be due to the fact that at a cut off of four food groups the Indicators lack specificity, meaning they will often misclassify an adequate diet as inadequate. However, a recent analysis of DHS data from 39 countries found that increasing dietary diversity and animal source food consumption, was associated with lower risk of stunting. The authors recommended that MDD be set at five rather than four food groups (Krasevec *et al*., 2017). While they were developed for use in large scale surveys such as DHS, it was envisaged that the Indicators could prove useful in smaller surveys (WHO, 2008a). A small 2017 Canadian longitudinal study used the Indicators to investigate the association of dietary diversity with overweight and found that infants fed homemade complementary foods had greater dietary diversity and reduced adiposity at 12 months (measured by DEXA) compared to those who received commercial infant foods (Mok *et al.*, 2017).

It must be noted that a number of birth cohorts conducted in HICs, using different dietary assessment tools, have also failed to make conclusive findings on the association between feeding practices and overweight (Smithers *et al*., 2011; Brophy *et al*., 2009; Reilly *et al*., 2005). A systematic review on the types of food introduced and risk of childhood overweight concluded that high energy and protein intake, particularly dairy protein, appear to be associated with higher BMI and body fat mass in later childhood; although the evidence was not definitive. No specific foods or food groups were linked to childhood BMI (Pearce &

Langley-Evans, 2013). Given the association in some studies between energy and protein intake and overweight, follow up studies in MACE children should include a more detailed dietary assessment that allows for the determination of energy, macronutrient and micronutrient intake.

5.5 Summary

The prevalence of stunting in MACE study infants at both 12 and 24 months was considerably lower than other South African studies in infants of the same age range while levels of overweight and obesity were generally higher. The low levels of stunting can be explained by the urban/peri-urban nature of the cohort with virtually all households having access to tap water and electricity. Another socioeconomic contributing factor is the relatively high levels of maternal education, as lack of maternal education has been linked to stunting mainly through its impact on caring and feeding practices. The exclusion of mothers with hypertension and diabetes from the MACE study was another potential contributing factor to the low stunting prevalence. The association of stunting with low SES has been fairly well documented but the MACE study adds to evidence from the most recent South African DHS that both stunting and overweight are linked to low SES.

The finding of an association between lower birthweight and length, and stunting; and higher birthweight and overweight; emphasises the need for optimal nutrition and care during pregnancy to ensure a healthy birthweight. Early identification of infants gaining weight exceptionally fast from as early as the first 6 months is important as this was strongly predictive of being overweight or obese at 24 months in the MACE study and evidence from other studies suggests that they are at markedly increased risk of obesity in later childhood. The 6 to 12 months period appears to be particularly important as rapid weight gain during this time was the most strongly predictive of higher BAZ at both 12 and 24 months. This is in contrast to other studies that found the first 6 months to be more significant.

Infant feeding practices were very similar to those reported in other South African surveys with high levels of breastfeeding initiation, which were not sustained by 6 months; and generally poor complementary feeding practices, characterised by low dietary diversity and high intake of inappropriate foods. Breastfeeding practices of HIV positive mothers were also similar to those reported in other surveys with lower rates of breastfeeding initiation and mixed feeding

compared to HIV negative mothers and higher rates of formula feeding. There is poor compliance with the South African IYCF policy. Despite poor infant feeding practices they were not associated with growth outcomes at 12 months, in line with the findings of the Drakenstein study.

6. CONCLUSION

South Africa, as with other LMICs, has a double burden of malnutrition, with high rates of stunting and overweight in young children. A number of sociodemographic factors, together with sub optimal nutrition during the first 1000 days from conception to two years of age, have been implicated in the development of poor growth outcomes. This study used longitudinal data collected between 2013 and 2017 from 290 mothers and infants enrolled in the MACE birth cohort study, Durban, to investigate early life risk factors for stunting and overweight in a LMIC setting.

This study had the following objectives:

- To describe the prevalence of stunting and overweight at 12 months of age in infants enrolled in the MACE study.
- To describe the maternal and household characteristics of the study infants and to determine their association with the growth outcomes of stunting and overweight at 12 months.
- To describe birth anthropometrics, and patterns of growth from 0-6, and 6-12 months, and to determine their associations with growth outcomes at 12 months.
- To describe infant feeding practices at 6 and 12 months and to determine their association with growth outcomes at 12 months.

This chapter concludes the findings of the study in relation to the objectives described above. It discusses the limitations of the study and makes recommendations for future research.

6.1 Conclusions of the study findings

6.1.1 The prevalence of stunting and overweight in the MACE study infants

The study found that at 12 months of age MACE study infants had a lower prevalence of stunting and a higher prevalence of overweight compared to other South African surveys. Data was also available for half the study infants at 24 months of age and both stunting and overweight prevalence increased in the second year of life although stunting prevalence remained relatively low. The findings indicate that, in this urban setting, over nutrition is now a greater problem than undernutrition.

6.1.2 Maternal and household characteristics and their association with growth outcomes

Households were predominantly low income but with relatively high levels of maternal education and universal access to tap water and electricity. Antenatal HIV prevalence was 35.3% and HIV exposure had no effect on growth outcomes at 12 months of age. The only household factor associated with growth outcomes was type of housing; both stunting and overweight were associated with children living in informal housing or flats as opposed to formal houses. This indicates that low SES is linked not only to stunting, which has been well documented, but also to overweight. This is a less well established association in LMICs. The low stunting rates documented in the survey can be explained by the access to basic services such as water and electricity as well as the good level of education in the MACE survey mothers. This emphasises the importance of nutrition sensitive interventions that address the underlying social determinants of stunting.

6.1.3 Growth patterns and their association with growth outcomes

Stunting and overweight start in utero with lower birthweight associated with lower HAZ at 12 and 24 months while higher birthweight is associated with higher BAZ. This emphasises the need for optimal nutrition and care during pregnancy to ensure a healthy birth weight. When looking at patterns of growth, the 6-12 month period is the most significant for prediction of later overweight; rapid weight gain during that time should be detected early through routine growth monitoring to allow for intervention. Furthermore, infants obese at 12 months were already gaining weight significantly faster from birth than infants of normal BMI at 12 months. They remained overweight or obese at 24 months, indicating that very rapid gains in weight in the first 6 months of life were the beginning of a growth trajectory that placed the infant at high risk for later obesity and should not be regarded as a transient stage that the child will grow out of. The 12-24 month period seems to be most important for stunting prevention as it was most predictive of 24 month LAZ and stunting prevalence doubled in the second year of life. The disproportionate increase in weight in relation to linear growth in the study sample is of concern and if the trend continues it could lead to even higher prevalence of overweight at a later age.

6.1.4 Infant feeding practices and their association with growth outcomes

Feeding practices were similar to those reported in other South African surveys with low levels of breastfeeding by 6 months; introduction of solid foods before 6 months in half of the sample; and a poor quality complementary feeding diet that lacked dietary diversity. Furthermore, the consumption of inappropriate foods and beverages was high. Despite this there was no association between poor feeding practices and growth outcomes. This finding was perhaps not surprising in relation to the breastfeeding practices as many other studies have shown no association with stunting and found mixed results with overweight. The fact that the 6-12 month period was most significant in predicting overweight indicates that complementary feeding practices are implicated however, the small sample size of infants with 12 month IYCF Indicators data probably prevented the detection of any significant associations. The finding that HIV positive mothers were significantly less likely to breastfeed than HIV negative mothers, shows that there was a lack of awareness of DoH guidelines and that misperceptions regarding the risk of transmission may persist.

6.2 Study limitations

Although the MACE study had collected data on household income and maternal education, in a country like South Africa, with a large informal economy, it is recommended that assessment of SES includes a greater number of variables. The South African Stress and Health Study (SASH) used a composite score of four aspects: maternal education; employment status; household income, including social grants; and a household asset and market access score. (Myer *et al*., 2008). Some of this information was not collected in the MACE study and, as the study is still ongoing, should be incorporated into follow up questionnaires to allow for accurate determination of SES. In particular, income from grants and more detailed information on the type of housing, including RDP housing should be included The association of informal housing with overweight and stunting indicates that there is a link that was not identified with the income data currently collected.

Pregnant women with hypertension and diabetes were excluded from the MACE study which aims to determine the association of environmental pollution with respiratory outcomes at 5 years. The exclusion of these women, who are more likely to have experienced the double burden of malnutrition themselves and therefore more likely to deliver LBW infants at risk for

stunting, is a limitation that could have contributed to the lower than expected stunting prevalence.

The sample size of 290 infants with longitudinal anthropometric data from birth to 12 months and 144 infants with data up to 24 months was comparable with other regional South African studies. Limited information on milk feeds, age of introduction of solid foods and types of foods given at 6 months had been collected by MACE clinical staff for these infants. There was very little detail collected on feeding practises and the MACE questionnaires should be adapted to collect more accurate data.

It was only possible to collect more detailed WHO Indicators data from 87 infants as this was the number of infants who attended their 12 month follow up visits in the year allocated to dietary data collection. An additional 7 infants had WHO Indicators data but no anthropometric data so their Indicators information was used for descriptive purposes only. Therefore, while the data was accurate as all the caregiver interviews were conducted by the researcher, the small sample size limited the ability to detect any associations with growth outcomes. The WHO Indicators provided only qualitative information regarding the complementary diet, and a more detailed questionnaire allowing for quantitative assessment of nutrient intake could have perhaps detected an association between feeding practices and growth outcomes.

6.3 Recommendations

6.3.1 Recommendations for dietetic practise

It is important that dietitians ensure that training of staff conducting routine growth monitoring and promotion activities has an explicit focus on identifying early those infants on a rapid growth trajectory, particularly in the 6-12 month period, as they are at increased risk of later overweight. The emphasis in the past has been on identifying undernutrition and staff need to be aware of the increasing problem of childhood overweight. This is in line with the Strategy for Prevention and Management of Overweight and Obesity. The new Road to Health booklets include a section on detecting overweight in infancy while the KZN DoH has new Standard Operating Procedures (SOPs) for Malnutrition which includes SOPs on management of overweight and obesity in infancy, later childhood and adolescence.

Infant feeding messages should focus on ensuring optimal linear growth while avoiding excessive weight gain. This includes complementary feeding advice aimed at improving dietary diversity, particularly intake of micronutrient rich foods, and limiting intake of inappropriate energy dense, but nutrient poor foods and beverages. The draft Paediatric Food Based Dietary Guidelines, which have recently been field tested, emphasise all of these points and should be finalised and disseminated without unnecessary delay. Additional platforms for dissemination of optimal infant feeding messages, such as the Road to Health booklet, radio shows and social media, form part of the DOH Side by Side campaign, launched in 2018, and it is hoped that improvements in IYCF practices will result.

In line with the WHO Guidelines and KZN SOPs, routine supplementation of stunted children with energy and protein supplements is not advised in view of the longer term risks of overweight and NCDs.

6.3.2 Recommendations for future research

This study has made some important preliminary findings and therefore the need for follow up of these study infants at the age of 5 years is strongly recommended. This is important to determine whether birth anthropometric data and patterns of growth, particularly in the 6-12 month period, remain significant predictors of BAZ in later childhood. The finding that they are associated with high BMI at 24 months indicates that there may be a longer term risk. The question of the significance of overweight or obesity at 12 or 24 months in predicting overweight in later childhood is also important to investigate, as is the association of slow linear growth in the second year of life with later stunting outcomes.

The significant association of rapid weight gain in the 6-12 month period with higher BAZ at both 12 and 24 months indicates that complementary feeding practices are implicated in the development of overweight but the small sample size and lack of quantitative data meant that associations with growth outcomes were not identified in this study. It is recommended that the MACE fieldworkers continue to use the WHO Indicators questionnaire to collect data which will increase the sample size. The information is also useful to provide qualitative data regarding infant feeding practices. In addition future research should focus on collecting more detailed quantitative information on the complementary feeding diet to enable the calculation of macro and micronutrient intake which could be used to investigate associations with growth

outcomes. The role of responsive feeding practices in the prevention of overweight is another aspect of complementary feeding that should be further investigated. Further qualitative research is also recommended that explores the attitudes and beliefs of mothers regarding infant feeding practises, including HIV and infant feeding, and identifies reasons/barriers for poor adherence to the IYCF Policy.

The MACE study has collected information on maternal diet and maternal BMI. Although beyond the scope of this study, future research should investigate the association between maternal nutritional status and diet, and growth outcomes in childhood. The fact that birth weight and length were associated with stunting and overweight at 12 and 24 months of age is indicative of the importance of the gestational period of the first 1000 days in later growth outcomes.

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APPENDIX A: MACE STUDY PROPOSAL

THE MOTHER AND CHILD IN THE ENVIRONMENT (THE MACE STUDY)

THE ADVERSE EFFECTS OF AMBIENT AND INDOOR POLLUTION ON CHILDHOOD RESPIRATORY DEVELOPMENT

A proposal for a birth cohort study in the eThekwini Municipality

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CONTENTS

INTRODUCTION

The prevalence of various chronic health outcomes in childhood, such as endocrine, respiratory and neuropsychological disorders have been increasing worldwide (Newacheck and Taylor, 1992) In developing countries, where acute infectious diseases remains the largest threat to the health of children under the age of five, the prevalence of these chronic non-communicable disorders are similarly increasing (WHO, 1998; Deen et al., 1999). The reasons for this are still unclear, however, a variety of factors have been postulated, including, environmental factors, such as ambient and indoor pollution and diet; genetic factors and biological risk factors (ISAAC, 1998). It is likely that the impact of environmental factors commence early in conception (Yeatts et al., 2006). In order to understand how these various risk factors are associated with health outcomes, cohort studies commencing in utero, and following children until the manifestation of these outcomes, probably provide the best epidemiological approach.

Pregnancy and birth cohort studies provide one of the most powerful research methods for medical and social research. Data collected about the growth and development of children, from *in utero* through to early childhood can be used to determine which biological and environmental factors can be associated with the health and optimal development of the foetus, infant and child.

Non-communicable respiratory disorders among children are of particular concern. Not only does this result in substantial infant mortality, but among those infants surviving early years, are likely to experience adverse respiratory health in later childhood or adult life (Sram et al., 2005). The global increasing prevalence of childhood asthma in recent decades is seen as the most important non- communicable respiratory disease of childhood. The “hygiene hypothesis” has been postulated for this increase in prevalence. However, there is growing evidence in the scientific literature that shows that children from lower socio-economic backgrounds have a higher prevalence of disease, and also greater severity (Joseph et al., 2010; Akinbami et al., 2002). The pathophysiological mechanisms for these disorders are complex, and act through multiple pathways (Figure 1)

Understanding the reasons behind the profile of this and other non-communicable childhood respiratory disorders allows for the development of appropriate interventions. A birth cohort with well defined health outcomes, measuring a multiple of factors at different stages of development, including biochemical, genetic, epigenetic and environmental factors can provide such an understanding. We have completed an pilot study, which investigated genetic and biochemical inflammatory markers among pregnant women (n=100), while assessing their exposure to key pollutants. This proof of concept study, although lacking power to address associations, suggested that those mothers exposed to industrial pollution have differential levels of biochemical inflammatory markers compared to those without such exposure.

The industrial basin in south Durban provides an opportunity to investigate these associations. The Durban south industrial basin (DSIB) is a complex mix of heavy industry and residential communities with high levels of community organisation, there has been a substantial focus by researchers, academics and the media on the prevalence of poor health outcomes as well as levels of ambient air pollution (Matooane et al, 2002). These have included, both published and unpublished studies, a large number of media reports, community organisation reports and commissioned reports (governmental, corporate and non-governmental). Studies have been conducted looking at exposure-health outcomes relationships, as well as documenting the environmental pollution patterns in the Basin. Studies among the communities in this area suggest that children of school-going age are at a higher risk for asthma and other respiratory outcomes, when compared to children of similar age and socio-economic status from less polluted northern communities within the city (eThekwini Health, 2007; Kistnasamy and Knapp, 1992; Nriagu et al, 1999; Kistnasamy et al., 2008; Naidoo et al., 2012)

This area consists of approximately 200 000 people living in 25 designated "suburbs". The area has been reported to have a high unemployment rate, with approximately 52% of the population not economically active. The DSIB is subject to significant levels of ambient air pollution because of its geographic relationship with certain stationary sources of air pollutants. Specifically, two major petroleum refineries are within the community, together with a pulp and paper manufacturer, Mondi. Available reports indicate that each of these refineries has emitted, on average, in the range of 35 000 to 40 000 kg of SO_2 per day. Through rigorous monitoring and enforcement, the total industrial SO_2 emissions were reduced from 107 tons per day in 2000, to 61 tons per day in 2005 (eThekwini, 2007). Owing to a combination of its geographical relationship to the refineries, land contours, prevailing meteorological conditions, the use of a relatively short emissions stacks at these facilities (50 – 100 meters), the lack of or relative ineffectiveness of emission control devices on refinery stacks, the many sources of so-called fugitive air emissions at refineries, emissions from industrial and passenger vehicles, as well as the proximity of other industries and until recently, the Durban International Airport, the community is believed to be at risk for intermittent substantial exposure to ambient air pollutants.

The recognition of this pollution risk is reflected in the various attempts to monitor levels of pollution in the Basin. This has included an industry-funded South Durban Sulphur Dioxide Management Systems Committee (SDSDMSC) which has been continuously monitoring one pollutant of concern, sulfur dioxide, at the Settlers' Primary School since June 2000. More recently, the establishment of the “Multipoint Plan” (MPP) of the local government (eThekwini Municipality) has resulted in a comprehensive continuous Air Quality Monitoring

System (AQMS). Available data from these and other monitoring systems suggest that although in the past pollutant measures, particularly sulphur dioxide, have frequently exceeded World Health organisation (WHO), the South African Department of Environmental Affairs (DEAT), since the implementation of the MPP, there has been a gradual decline in the ambient pollutant levels. (eThekwini, 2007)

This longitudinal birth cohort study aims to examine the effects of environmental influences on the health and development of a cohort of women attending ante-natal clinics in the South Durban Basin, compared to a cohort of women from the less industrially polluted community north of the city. Priority outcomes will be indicators of respiratory development, as well as adverse respiratory outcomes. Risk factors to be investigated will include ambient and indoor pollution, dietary impacts, particularly reduced intake of antioxidants, genetic polymorphisms, epigenetic changes and adverse birth outcomes. Additionally, the proposed investigation will address the hypothesis that asthma risk may be influenced by environmentally induced epigenetic changes. This is cutting edge research which is gaining momentum in the USA and UK, but still in its infancy in Africa. We will determine if factors, such as environmental pollution, nutrition and genetic polymorphisms may interact to result in adverse outcomes such as low birth weight, intra-uterine growth retardation and premature births, in a population of pregnant females from communities with high levels of industrial environmental pollution, compared to pregnant females without such exposures, but with similar socio-economic status. Children will be followed up from before birth to six years of age. The Mother and Child in the Environment (MACE) study has the advantage of repeated measures of specific environmental exposures, collection of biological specimens over time, and comprehensive clinical outcomes assessment. This will contribute to a wealth of information on specific environmental triggers and critical time windows of susceptibility. Additionally, an African population will, for the first time, be evaluated in a study of environmental epigenomics.

HYPOTHESIS, OVERALL AIM AND SPECIFIC OBJECTIVES

Research Hypotheses

- Childhood organ development is adversely and permanently affected by ambient pollution resulting in permanent deficits in organ function, including the respiratory system. These impacts occur in utero.
- Ambient pollution may result in adverse birth outcomes such as low birth weight and intra- uterine growth retardation, which in turn may be risk factors for the development of respiratory diseases.
- Additional exposures and risk factors such as indoor air pollution, inadequate dietary intake, environmental tobacco smoke, genetic polymorphisms and epigenetic changes and other unmeasured socio-economic factors contribute to these adverse outcomes
- Subsets of children can be identified who are more susceptible to the effects of air pollution. This may be through oxidative stress mechanisms and genetic predisposition.
- Specific biomarkers and genetic profiles can predict the likelihood of adverse outcomes
- Human immunodeficiency viral infections contribute to these adverse outcomes, and may modify the effects of the other exposures.
- Among those who are susceptible and those predicted to develop adverse outcomes, appropriate and early interventions will alter the course of the disease or outcome.

Overall Aim

To determine the effect of ambient and environmental pollution exposure on childhood respiratory development, and the modification of effect by birth factors, genetic predisposition, epigenetic changes and oxidative stress, and to test the effectiveness of dietary intervention in reducing the risk for adverse respiratory outcomes.

Specific Objectives

- To characterise environmental exposure patterns among a cohort of pregnant women and subsequently, their offspring among communities with documented high levels of industrial exposure and a comparison communities without such exposures;
- To describe perinatal risk factors including exposure to ambient and indoor pollutants, maternal smoking, or maternal exposure to passive smoking, allergen exposure (ingestion of peanuts, egg protein etc) and atopy status, diet generally and specifically dietary intake of antioxidants, HIV and anti-retroviral treatment status and other maternal stressors likely to impact on the foetus;
- To describe neonatal, infant and early childhood risk factors, such as adverse birth outcomes (low birth weight, intra-uterine growth retardation and preterm deliveries), dietary factors (breastfeeding or formula feeding; intake of antioxidants etc), exposure to allergens (aerosol or dietary) and atopy status, exposure to environmental pollutants, ambient and indoor;
- To assess genetic status of these children with respect to a set of polymorphisms in specific genes associated with response to oxidative stress and respiratory biomarkers, cellular changes from environmental pollutants, and to response to therapeutic interventions (dietary supplementation with vitamin C and vitamin E.) These genes include GSTM1, GSTP1, and TNF .
- To investigate the genome wide DNA methylation status of the neonate using umbilical cord blood;
- To investigate the relationship between genome wide DNA methylation status and prenatal environmental exposures;
- To investigate the relationship between genome wide DNA methylation status and respiratory outcomes in children.

- To assess baseline level of airway inflammation through measurement of FeNO, sputum ECP, metalloproteinase, neutrophil and eosinophil count; fibrinogen, C-reactive protein (CRP); as well as more general markers of asthma severity (spirometry, peak expiratory flow, airway hyperresponsiveness, symptoms, emergency ward visits and hospitalizations, and perhaps short acting beta agonist rescue use, corticosteroid use) at various stages of childhood within the cohort
- Among the older children, to assess associations of short term fluctuations in ambient air pollutants with fluctuations in pulmonary function, symptom reports, and other markers of inflammation using generalized estimating equations and/or generalized linear mixed models. To assess effect modification of these associations by asthma severity, asthma type, medication use, allergen exposures, serum specific IgE/IgG4 ratios to common inhalant allergens, specific polymorphisms and combinations of polymorphisms.
- To assess the long term effect of ambient pollution on lung development generally and on adverse respiratory outcomes specifically, adjusting for perinatal risk factors, genetic polymorphisms, adverse birth outcomes, oxidative stress and indoor environmental pollution, through the development of appropriate multivariable regression modelling;
- Evaluate the effectiveness of a dietary intervention (supplementation with vitamin C and vitamin E), in a randomized control trial, to reduce symptoms, improve pulmonary function and decrease markers of underlying inflammation among children with asthma. This objective assesses the hypothesis that in those children with evidence of suboptimal baseline antioxidant intake (evaluated through a combination of serum measurements of carotenoids, vitamin E, and food diaries), dietary supplementation will significantly improve all of these markers.
- To investigate effect modification of this intervention effect by polymorphisms related to oxidative stress. This objective will test the hypothesis that children at high-risk for

adverse effects of exposure to ambient pollutants on the basis of their genotypes (e.g., GSTM1 null, etc.), will show a greater effect of dietary supplementation.

BACKGROUND AND SIGNIFICANCE

Introduction

The scientific literature has consistently documented the association between various ambient and indoor pollutants and adverse health outcomes among children. These outcomes extend from birth outcomes such as low birth weight, prematurity and birth defects through to later childhood including respiratory outcomes such as asthma and neurological outcomes such as low IQ or learning disabilities (Glinianaia et al, 2004). These outcomes have been documented with a variety of pollutants, although some outcomes are pollutant-specific. Responses to pollutant insults vary within populations. These could be due to the nature and dose of particular exposures, genetic predisposition or inadequate defense mechanisms to respond to specific stressors, such as oxidative stress.

Over recent times research into the potential impact of air pollution on the health of the developing foetus has grown rapidly. Foetuses may be considered a further sub group of a vulnerable population, which are known to be highly susceptible to a variety of toxins, because of their exposure pattern and physiologic immaturity (Sram et al, 2005; Perera et al, 1998). Studies conducted in the United States, Brazil, China, Mexico and the Czech Republic have all related adverse birth outcomes such as low birth weight, intrauterine growth retardation, preterm birth and foetal mortality to ambient air pollution (Xu et al, 1995; Pereira et al, 1998; Bobak et al, 2000; Perera et al, 1998; Loomis et al, 1999; Woodruff et al, 1997). Neonatal susceptibility to environmental pollutants may be caused by either direct or indirect hits on several cell types to influence cell differentiation, proliferation and/or maturation. Air pollutants may also alter the normal developmental pattern for metabolic, immune and neurological functions that are constantly changing during *in utero* and postnatal growth. The sensitivity of neonatal cells to environmental toxins is likely to be completely different from these same cell types found in the adult. Delivery of an environmental toxicant to the respiratory system is also dramatically different during the foetal compared with the postnatal period (Pinkerton and Joad, 2006). There is accumulating evidence that there is a need for longitudinal studies in the developing world (International Interest Group, 2003).

The period in which the developing foetus is most susceptible to organ maldevelopment is the period of organ formation during weeks 3-8 following fertilisation. Birth weight reflects intrauterine growth and wellbeing and is recognised globally as an indicator of perinatal and infant health. A low birth weight (less than 2500g), from either premature birth or intrauterine growth retardation (IUGR) is strongly associated with increased infant mortality and morbidity. These neonates are more likely to have hypertension and coronary heart disease (Sram et al, 2005). As well as being a marker of early child health, low birth weight is associated with increased risk of developing various diseases in later life including heart disease and type 2 diabetes (Spinillo et al, 1995; Osmond and Barker, 2000).

Lung and airway development begins about 24 days after fertilization, with the pre-acinar airway pattern being finalised by 17 weeks of gestation. Respiratory bronchioles and acini continue to grow until about 36 weeks of gestation. The airways are almost fully developed by birth, with postnatal development in length and diameter of already developed tissue. Thus insults in early foetal life and early childhood have consequences later in childhood and adulthood (Devereaux, 2007). A number of factors influence the growth and maturation of the developing lung. These include transcription factors and molecular factors directing the development of epithelial and cellular proliferation. In addition, the enzyme systems necessary for the development of the respiratory system occurs late in the gestational period, and continues into early childhood. This includes the glutathione-S-tranferases, the cytochrome P450 mono-oxygenase systems and the other anti-oxidant enzymes (Pinkerton

and Joad, 2006). Environmental tobacco smoke, bioactivated compounds and oxidant gases have shown to adversely impact on the developing lung (Pinkerton and Joad, 2006).

Although numerous studies have shown that exposure to outdoor air pollution exacerbates asthma, the effect on lung development has been less clear (American Academy of Pediatrics, 2004) Children are more vulnerable to the effects of air pollution than adults; they have increased exposure levels due to higher minute ventilation and higher levels of physical activity. Eighty percent of the alveoli are formed in the post natal period and changes in the lung continue until adolescence. This means that during the early post neonatal period the developing lung is highly susceptible to damage after exposure to environmental toxins. (American Academy of Pediatrics, 2004) To understand the health outcomes of exposure to a variety of environmental factors in the respiratory system of children requires careful consideration that lung development is a multistep process and cannot be based on studies in adults. Most babies and preschool children spend a very high fraction of their time indoors – if this is a critical window of exposure to allergens and the subsequent development of asthma, greater emphasis on the investigation of the indoor environment is necessary.

Genetic and Molecular Changes in utero

The complications observed during pregnancy and birth outcomes are primarily as a result of foetal development which is dependent on the state of maternal health. Pregnancy has been accepted as an inflammatory state in the maternal system accompanied with elevated oxidative stress, hence susceptibility to damage of biomolecules (Furness et al., 2011). A myriad of contributing factors are implicated in pregnancy complications which range from diet and lifestyle to genetic variation between individuals. Monitoring DNA damage is most frequently used to assess exposure to environmental pollutants. The measurement of DNA damage is of importance in pregnancy, both at the genomic and mitochondrial level, as the human foetus is exposed to a variety of environmental agents and drugs that cross the placenta (Furness et al., 2011). DNA damage has been well studied and several classes of products identified such as chromosome loss, single and double strand DNA breaks, DNA fragmentation, and base oxidation. Mitochondrial DNA, due to its close proximity to the electron transport change, is susceptible to damage due to pollutant induced oxidative stress (Figure 2). The significance of changes in the genome in foetal development is well described and understood (Furness et al., 2011).

Epigenetic changes such as DNA methylation, chromatin and histone modification and microRNA expression, however, are stable and heritable changes which does not in itself affect the DNA sequence (Li et al., 2010). MicroRNA expression have been associated with embryonic development (Prieto et al., 2011), cell cycle and more importantly plays a crucial role in coordinating the inflammatory response by directly affecting the transcriptome and thus heavily influences gene expression patterns (Asirvatham et al., 2009).

There is emerging evidence for modulation of the transcriptome by environmental factors (Jardim, 2011). MicroRNA expression profiling and its functional significance during pregnancy have been only recently documented (Prieto et al., 2011; Kotlabova et al., 2011) The effects of air pollution on these microRNA species during pregnancy are unknown. We hypothesise that industrialisation and ambient pollution exacerbates the inflammatory state during pregnancy and contributes to birth defects and pregnancy complications by influencing the level of microRNA expression and mitochondrial function.

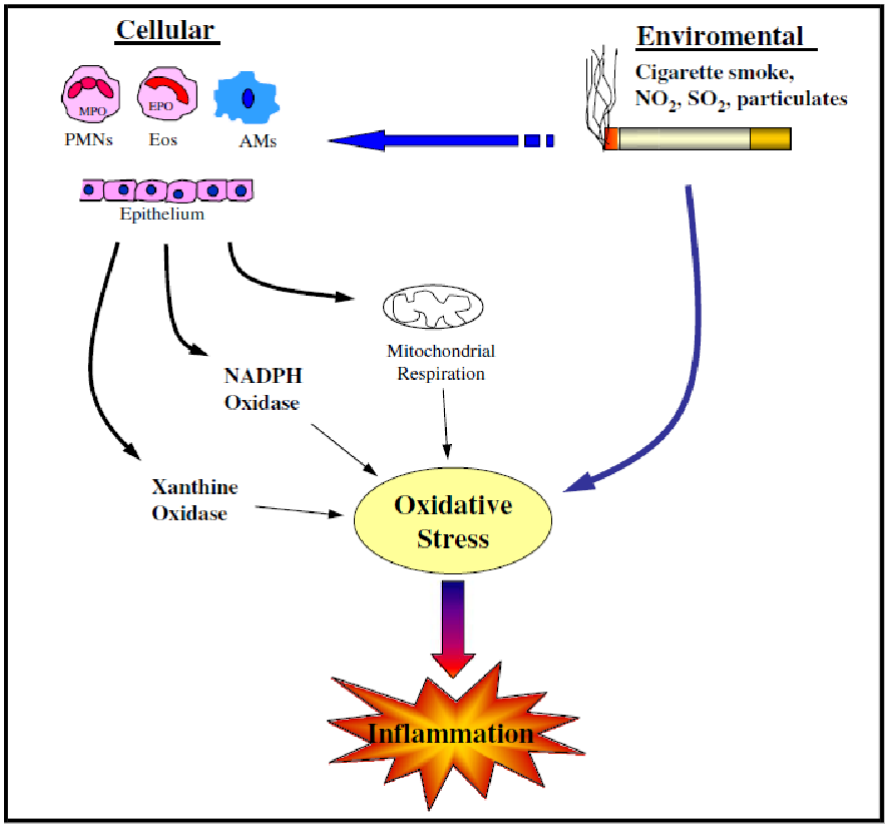

Figure 2: Sources of oxidative stress. Oxidative stress derived from environmental pollutants results in inflammation. Cellular-derived oxidative stress from mitochondrial respiration, the NADPH oxidase system or xanthine oxidase system. Inflammation may have a feed forward effect triggering inflammatory cells to contribute to oxidative stress, exacerbating and intensifying the inflammatory response. PMNs, polymorphonucleocytes; Eos, eosinophils; AMs, alveolar macrophages. (Adapted from Kirkham, 2006)

Studies have shown that asthma has a strong genetic component and research has shown that the genetic and environmental factors may interact to exacerbate the disease. Findings of studies evaluating gene environment interactions clearly indicate the importance of these interactions in respiratory disease. Large cohort studies on childrens' respiratory health and genetics have been done or currently underway in several countries in the developed world but to date there has been no similar work done on the African continent. While there are other birth cohort studies from Africa, this is the first to include both genetics and environmental exposures as risk factors for the development of respiratory disease. However, there are few examples from the literature of specific gene-environment interactions in relation to asthma. The clearest examples of genetic interactions for inhaled pollutants exist for ozone, environmental tobacco smoke and endotoxin (London, 2007).

The genetic and environmental factors that mediate asthma risk and expression are intricately linked. Environmental and genetic factors interact pre- and post-natally to determine phenotype in the child as shown in Figure 2(Carroll et al., 2005; Miller and Ho, 2008).

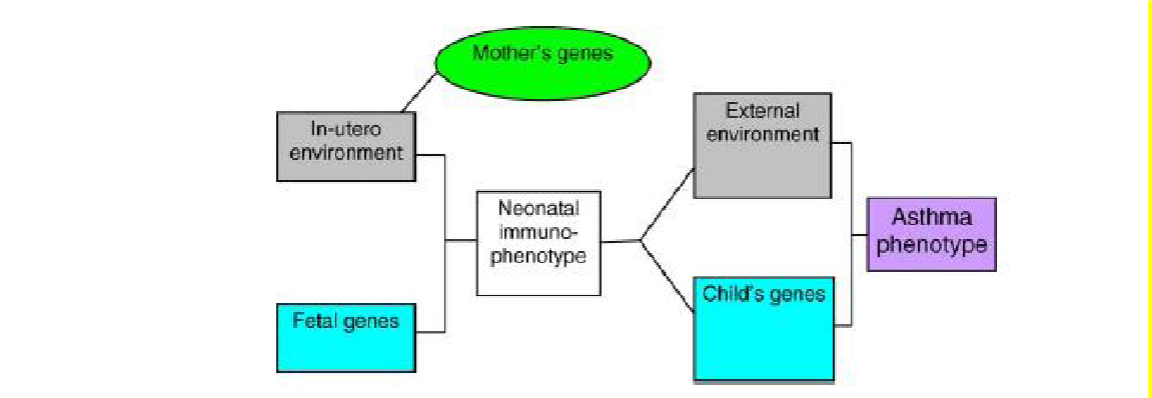

Figure 3 Proposed model of gene–environment interactions in determining asthma phenotype (Carroll et al., 2005).

Developments in laboratory sciences over the recent past have begun to identify a variety of markers of inflammation and inflammatory responses related to oxidative stress, including within the respiratory system. Some of these could be predictors of adverse respiratory outcomes in later life. The lung in the newborn is subject to a variety of oxidative stressors, including the large increase in oxygen concentration, and exposures to various environmental pollutants and irritants. This effect is exacerbated in the premature infant (Kelly, 2003). We have chosen to investigate GSTM1, GSTP1 and TNF-308 genes since these polymorphic genotypes have common functional variants (SNPs) which affect susceptibility to disease and have been implicated in possible gene- environment interactions in the context of respiratory diseases. GSTM1 and GSTP1 have been linked to oxidative stress responses, while TNF-308 has been associated with inflammatory responses. Research has mainly focused on a handful of common polymorphisms with well described functional effects in genes thought to be involved in oxidative stress responses (London and Romieu, 2009). The single most commonly examined is a highly prevalent deletion polymorphism of the glutathione S-transferase M1 gene (*GSTM1*). Deletion of both copies of the *GSTM1* gene, referred to as homozygous deletion or the null genotype, abolishes *GSTM1* activity (Strange et al., 2001) The high frequency of the *GSTM1* null genotype, ranging from 25 to 60% depending on the ethnic group, enables examination of this polymorphism in studies that were not originally powered to study interactions. The second most commonly studied gene in relation to either ETS or ambient air pollution is glutathione S-transferase P1 (*GSTP1)*. A functional polymorphism (Ile105Val; AA→AG/GG) occurs at relatively high frequency (Strange et al., 2001). The majority of published studies looking at interactions between air pollutant exposures and either or both *GSTM1* and *GSTP1* polymorphisms show positive findings, but not always in the same direction for *GSTP1* (London and Romieu, 2009). TNF alpha is a 157 amino acid cytokine protein manufactured by white blood cells to stimulate and activate the immune system in response to cancer, infection, exposure to endotoxin, or other products of bacterial, viral, parasitic or inflammatory origin. Overproduction of this compound can lead to disease where the immune system acts against healthy tissues. (TNF)-alpha is a pro-inflammatory cytokine that has been implicated in many aspects of the airway pathology in asthma (Li et al., 2006).

Epigenetics plays an important role in the regulation of a wide variety of genes, which includes the genes involved in the inflammatory immune response (Bousquet et al., 2004). These epigenetic modifications may help to explain the patterns of inheritance seen in asthma and explain how they interact with environmental factors (Miller and Ho, 2008). Aberrant DNA methylation, altered histone modifications, specific microRNA expression, and other chromatin alterations orchestrate a complex early-life reprogramming of immune T-cell response, dendritic cell function, macrophage activation, and a breach of airway epithelial barrier that dictates asthma risk and severity in later life. (Ho, 2010). This may be particularly valid when accounting for the effects of environmental stressor such as cigarette smoke and air pollution on enhancing the risk of asthma in children exposed in utero. Epigenetic changes do not alter the underlying genetic code of the person but affect a cell's

transcriptional programme in response to environmental challenge and, importantly, are reversible throughout a person's life (Adcock et al., 2006). Complex diseases such as asthma are ‘‘programmable’’ by specific early-life environmental exposures. The prenatal period (during growth of the airways and development of the immune system) is a critical window of programming. In this regard maternal exposure to ETS, traffic related pollutants, viral infection, dust mites, and certain nutritional factors during pregnancy have been shown to increase the risk of asthma in offspring (Miller and Ho, 2008).

The first epigenetic mechanism recognised was DNA methylation, which is a reversible modification of DNA structure, adding a methyl group to the 5 position of a cytosine residue often as part of a CpG island or cluster which generally results in gene silencing (Ho, 2010). Epidemiologic studies have investigated the association between demographic, environmental and behavioral risk factors with both white blood cells (WBC) global and gene-specific DNA methylation (Terry et al., 2008). Pollutants such as ozone have been linked to epigenetic changes in the lung. For example, as with smoking, air pollution can result in oxidative stress, leading to DNA lesions and hypomethylation. Furthermore, pollution decreases methylation across the genome which was associated with oxidative stress (Miller and Ho, 2008). There are numerous gene-specific DNA methylation studies. Methylation of the ACSL3 59-CGI gene was found to be significantly associated with maternal airborne PAH exposure exceeding 2.41 ng/m3 (OR = 13.8; p =0.001) and with a parental report of asthma symptoms in children prior to age 5 (OR = 3.9; p,0.05). Thus, if validated, methylated ACSL3 59CGI in umbilical cord white blood cells DNA may be a surrogate endpoint for transplacental PAH exposure and/or a potential biomarker for environmentally-related asthma (Perera et al., 2009). DNA methylation might also be an epigenetic mechanism that can explain the lifelong effect of exposure to tobacco smoke in utero on asthma risk. Breton et al (2009) recently examined DNA methylation status in buccal cells from a cohort of children born to mothers who did or did not smoke during pregnancy. Children exposed to maternal smoking had lower methylation of the AluYb8 repeat element, indicating global DNA hypomethylation.

A large body of research has implicated specific time periods when individuals seem to be more susceptible to the effects of environmental exposures and other asthma triggers. These include prenatal development, early childhood, and adolescence (Ho, 2010). Experimental animal models also have shown that intrauterine exposure to airborne pollutants may increase the risk for respiratory disease in offspring. Differential DNA methylation of promoter regions of reprogrammable genes may be an important mechanism in establishing the imprint. Therefore a mother’s exposure to pollution during pregnancy may predispose her child to asthma, these alterations could occur, not only in target organs/ tissues such as fetal lung or the immune system, but also in peripheral tissues such as fetal circulating white blood cells and placental tissues, thus providing opportunities to discover and develop highly sensitive and specific, minimally invasive biomarkers for exposure assessments or clinical indications. There are limited published data that have examined the effects of prenatal exposure to ambient air pollutants, nutrition and other environmental exposures on DNA methylation patterns and potential association with asthma phenotypes in the offspring. This is an important gap in our understanding of asthma pathogenesis. (Miller and Ho, 2008). The dynamic nature of epigenetic regulation in contrast to the static nature of the gene sequence provides a mechanism for reprogramming gene function in response to changes in lifestyle trajectories. Thus, epigenetics could provide an explanation for well documented gene–environment interactions. An important implication of the possible involvement of epigenetics is the potential for therapeutic intervention. Moreover, once we understand the rules through which different environmental exposures modify the epigenetic processes, we might be able to design behavioural and therapeutic strategies to prevent and revert deleterious environmentally driven epigenetic alterations (Swanson et al., 2009)

(a) Markers of Inflammation, Oxidative Stress and interventions

Developments in laboratory sciences over the recent past have begun to identify a variety of markers of inflammation and inflammatory responses related to oxidative stress, including within the respiratory system. Some of these could be predictors of adverse respiratory outcomes in later life. The matrix metalloproteinases (MMP) 9 and 2 are type IV

collagenases released from inflammatory cells have been shown to have a role in respiratory basement membrane inflammation (Schock et al, 2001; Sweet et al, 2004). The latter studies showed that the ratio of MMP to its tissue inhibitor (tissue inhibitor of metalloproteinase-1 (TIMP-1)) was related to chronic lung disease in the first weeks of life.

The lung is subject to a variety of oxidative stressors in the newborn, including the large increase in oxygen concentration, and exposures to various environmental pollutants and irritants. This effect is exacerbated in the premature infant (Kelly, 2003). Oxidative stress adversely impacts on the regulation of the T-helper cytokines. Additionally, pregnancy stimulates the T-helper2 (Th2) pathway with a consequent an up-regulation of interleukin (IL)-4 and IL-10 production and concurrent down-regulation of IL-2 and interferon-g (IFN-g) (Vance and Halloway, 2002). Sensitisation occurs in the early in pregnancy, with priming of T-helper precursors by 18-22 weeks of gestation. IgE and Th2 cytokines are present in the amniotic fluid, and ingested and aspirated by the foetus, exposing the respiratory tract to these immunoglobulins and cytokines. This creates the environment for the development of allergic sensitisation.

Among pregnant women with asthma, T-lymphocyte activity results in increased production of IL-4, and IFN-gamma, which was shown to have a negative correlation with maternal peak flow and birth weight of infants, suggesting that both maternal respiratory systems and fetal development are affected (Tamasi et al, 2005). Inflammatory markers such as IL-1beta and TNF-alpha in amniotic fluid, blood and tracheal aspirates have been associated with adverse foetal respiratory outcomes (Viscardi et al, 2004). Neonates with evidence of respiratory impairment at birth were commoner among mothers with higher levels of circulating interleukins.

Studies investigating the relationship between markers of oxidative stress (such as plasma thiobarbituric acid-reactive substances (TBARS), glutathione, glutathione peroxidase, and 6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and impacts on lung function among adults have shown a positive relationship with some markers, and inverse relationship with others on lung function, but only in men (Ochs-Balcom et al, 2005). Similarly, intake of antioxidant vitamins have been positively associated with lung function parameters (Schunemann et al, 2002). Zinc plays major role as an ant-oxidant and as an anti inflammatory marker. These functions of zinc highlight the importance of this trace element‘s role in various pathology, including asthma (Truong-Tran et al, 2001; Zalewski et al,2005). An association has been noted between selenium deficiency & chronic asthma, with low clinical improvement post selenium replacement.(Allam et al., 2004)

Several intervention studies have examined the impact of introducing vitamin supplementation to counter the effects of oxidative stress on lung development. It is argued that substances such as Vitamin A, C and E may have a protective effect (Romieu and Trenga, 2001; Sempertegui, 2001; Gilliland et al. 2003). Individuals with the lowest intakes of vitamin C (97.3mg/day) were associated with a >5-fold increased risk of bronchial reactivity compared to those with higher intakes (104.5mg/day) (Soutar et al, 1997). It has also been argued that introduction of Vitamin E during pregnancy may reduce wheeze and eczema in the second year of life (Martindale et al, 2005). Studies have shown that reduced maternal intake of vitamin D and E results in increasing symptoms of wheeze and asthma in children up to five years of age (Devereaux 2007). Preterm infants have been shown to have reduced levels of antioxidant vitamins, placing them at greater risk for oxidative stress (Baydas, 2002)

Antioxidants reduce phagocytotic NADPH oxidase activities and also impact on apoptotic pathways necessary for the clearance of airway phagocytes (Kelly F, 2003). Supplementation of diets with these antioxidants, such as Vitamins E and C have shown inconsistent response to protecting against asthma symptoms, however some studies have shown a response for specific air pollutants such as ozone (Romieu, 2002). Children who did not have a daily intake of fruit had almost a 79ml lower FEV_1 compared to children eating at least one fruit a day (Cook et al, 1997). These respiratory outcomes-vitamin intake effects have been replicated in other studies (Forasteire et al, 2000)

Exposure to ambient and indoor pollutants, particularly cigarette smoking, oxone and particulates are postulated to impact on oxidative pathways, increasing oxidative stress. Studies have examined whether the impact of such exposures could be modified by antioxidant supplementation (Romieu 2002). The latter was able to show that there were protective effects against ozone associated with vitamin C and E supplementation amongst asthmatic children.

METHODOLOGY

Overview

Cross sectional studies are limited in their ability to investigate associations between environmental pollution and multiple health outcomes, or predictors that may vary over time. The south Durban communities have been subjected to decades of environmental pollution from the adjacent industries, and have expressed concerns about a variety of outcomes, including respiratory, haematological, immunological and malignancies. In addition, cross sectional studies cannot assess the effectiveness of interventions. The methodology proposed for this study utilizes a longitudinal cohort design, with a primary focus on respiratory outcomes, however, additional outcomes will be investigated using the broad framework and structure of this project.

Pregnant women will be selected from the public sector ante-natal clinics in participating communities in south Durban. A comparison sample of women with similar socio-economic background, but different exposure profiles will be selected from north Durban. In each of these communities, located in close proximity to these clinics (south and north), are environmental monitoring stations managed by the eThekwini municipality. These pregnant women will be followed up during their pregnancy, through to labour and delivery. The neonate will subsequently be followed up through infancy and on regular intervals until the age of 6 years.

A randomised control trial study design will be developed to introduce dietary supplementation for the study participants (children), and evaluate its effectiveness in reducing adverse respiratory outcomes among the sample.

Selection of Communities, study population and study sample

The methodology to address the hypothesis, together with the overall objective and specific aims will be through a detailed assessment of a cohort of pregnant women attending the public sector ante-natal clinics in the Merebank, Bluff, Wentworth and Austerville areas, in south Durban, with a comparison sample of pregnant mothers of similar socio-economic status living in a less heavily industrial north of the city. These communities were deliberately selected for various reasons, including their proximity to the heavy industry outlined in the Introduction (in the south) or the absence of such (in the north), the presence of environmental monitoring stations, measuring a variety of ambient pollutants, as indicated; the presence of strong community based organisations and the experience of the research team in working with these communities, as reported in the background. In addition, all women with uneventful pregnancies and anticipated normal deliveries, attending these public sector ante-natal clinics, present at three hospitals in the south (Wentworth Hospital, Prince Mshiyeni Hospital and King Edward VIII Hospital) and at three hospitals in the north (Addington and Mahathma Gandhi, and King George V Hospitals).

All pregnant women that meet our inclusion criteria, presenting to these ante-natal clinics over a recruitment period of two years, will be invited into this study. The children born to these women from the pregnancy of interest will subsequently form the child participants of the study. The following are the criteria for pregnant women entering into this longitudinal study:

PREVIOUS STUDIES IN THE SOUTH DURBAN BASIN

Given the historical context of the communities located in the Durban South and the high levels of community organisation, there has been a substantial focus by researchers, academics and the media on the prevalence of poor health outcomes as well and levels of ambient air pollution.

These have included, both published and unpublished studies, a large number of media reports, community organisation reports and commissioned reports (governmental, corporate and non- governmental). Studies have been conducted looking at exposure-health outcomes relationships, as well as documenting the environmental pollution patterns in the Basin. This review attempts to provide an overview of some of these findings.

Health and Exposure related epidemiologic studies

In an unpublished study conducted in the early 1990s, Kistnasamy and Knapp found that learners at a primary school located between the two major refineries in the DSIB (Settlers' Primary School) were significantly more likely to report lower respiratory symptoms than learners from a school of similar socioeconomic/ethnic make-up in Chatsworth (prevalence ratios of 3.63, 2.71, 3.09, and
3.01 for cough, chest congestion, wheeze, and visiting a doctor for chest illness, respectively) (Kistnasamy and Knapp, 1992). Other community based studies have documented prevalence rates for doctor diagnosed outcomes such as asthma in children (10%) and among adults (12%), higher than that documented for other populations in the scientific literature (Nriagu et al, 1999).

In a study among students and teachers at the Settlers' Primary School, Kistnasamy et al have reported unusually high prevalence rates for asthma, with ranges of any type of non or probable asthma (symptoms assessed) from 53.5% to moderate to severe persistent asthma of 16.8%. In addition, approximately 20% of the study sample had marked airways hyperresponsiveness as diagnosed by methacholine challenge testing, a prevalence higher than any other population based reports in the scientific literature. This study found statistically significant associations between prior day and prior 48 hour PM10, SO_2, and NO_2 levels (continuously measured at the school) and increased respiratory symptoms and diminished pulmonary function measures (measured by digital recording peak flow meters) among students with persistent asthma. These effects were observed during a time period when all ambient pollutant measures were well within national and international standards. (Kistnasamy et al, 2008).

The same research team as for the above study conducted the “South Durban Health Study”, (Naidoo et al, 2007) among seven communities in the highly industrialised south compared with the north Durban. In each community, one primary school was selected according to specific criteria. All students from within one or two randomly selected grade four classrooms were invited to participate in the study. Standardised interviews of child participants and caregivers were conducted, together with spirometry, non-specific bronchial hyperresponsiveness (BHR) testing and skin prick testing (SPT). At each of the schools particulate matter, sulphur dioxide, oxides of nitrogen and carbon monoxide were monitored for a year. Generalized estimating equations (GEE) were used to examine associations between daily mean levels of ambient air pollutants (NO2, NO, SO2, and PM10) and daily measures of pulmonary function (intraday variability and daily nadir values for FEV1 and peak expiratory flow [PEF]) across 4 seasons among 423 primary schoolchildren in models adjusted for age, gender, race/ethnicity, school, caregiver smoking, caregiver education, household income, and season. Asthma severity was considered as an effect modifier. Possible lag effects (1 to 5 days), as well as 5-day averages, were modelled.

Mean daily NO2 concentrations varied from a low of 11 ppb in non-industrial areas to 19 - 24 ppb in the city centre and industrial areas. Average SO2 concentrations varied from 1 - 3 ppb in non- industrial to 12 - 20 ppb in industrial areas. Mean daily filter-based PM10 concentrations ranged from 41 – 57 µg m-3.

The prevalence of reported symptoms consistent with persistent asthma was 32%. Adjusted predicted prevalence of symptom-defined persistent asthma was higher among schools in the south as compared to schools in the north (12.2% vs. 9.6%). A similar geographic difference was seen for marked BHR (8.0% vs. 2.8%). Living in south Durban presented a significantly higher risk for the presence of persistent asthma (OR=1.8) and BHR (OR=2.6). Statistically significant lagged decrements in pulmonary function associated with higher ambient concentrations were present for a substantial proportion of the regression models evaluated for each of the four pollutants, with associations stronger and much more frequent among those children with persistent asthma, as compared to those with mild intermittent or no asthma.

Inclusion Criteria

- The recruited participants will have to be resident in the geographical area within which the clinic and monitoring station is located. They will have to be in this area for the full duration of the pregnancy, and for the follow-up period of 5-6 years. The children (from the pregnancy of interest) must also be resident in the communities for the duration of follow- up. It will be preferable for the mothers to be living within a 5km radius of the monitoring station, but this will not be essential. Based on our previous studies among these communities, extended residency, sometimes over a couple of generations, is the norm.
- Preferably such females should be at gestational age less than 20 weeks. However, females presenting above 20 weeks will not be excluded.
- Women who test positive for human immunodeficiency virus (HIV) during their routine ante- natal testing will be included in the study.

- Participating females with any complications of pregnancy such as hypertension, diabetes, placenta previa or genital tract infections, or other complications which result in adverse growth effects on the foetus will be included.

The following will not exclude participation:

- Females with multiple pregnancies will be excluded

Periods of Follow-up

Assessments conducted during each trimester of pregnancy, shortly after delivery, and at regular intervals in the neonatal and infancy period: The cohort children will be followed up at the age of 1, 3, 6, 12, and 18 months, and from then on at yearly intervals within 3 months of the child's birthday up to the age of 5 years. Further follow-up and assessment will depend on the availability of funding.

Collection of questionnaire data

(a) Carefully selected interviewers drawn from the communities and will be trained and supervised to conduct baseline interviews with participants. Training will include techniques and practice in conducting interviews in a consistent and neutral fashion. The instrument that was used in the pilot study will be revised for use in the cohort study. The latter instrument consists of standardised questions extracted from past and current European birth cohorts, as well as environmental health studies conducted by the research team previously. Components of this questionnaire will include demographic information; antenatal history; place of birth and residential history and potential confounding factors such as maternal; smoking, exposure to cigarette smoke, occupational and environmental exposures, dietary history, pre-existing medical conditions, past medical and obstetric history. Other questions will include residential and occupational history, general quality of life; use of biomass fuels, smoking and neighbourhood matters (Appendix 1). The questionnaire will be administered to the pregnant females on enrolment into the study. Data will be captured at the time of the interview, using a mobile telephone system, automatically uploading data onto the study database using wireless technology called Mobile Researcher.

(b) Follow-up interviews will be conducted with the pregnant women in each trimester subsequent to enrolment, and just prior to delivery to evaluate any change in status, particularly in exposures, dietary changes, pregnancy complications.

(c) Maternal interviews about the health of the child, including respiratory diseases in the neonatal period, especially the need for oxygen therapy, feeding regimes (for the one year olds and above, information on dietary intake using a validated food frequency questionnaire will be included (Rockett et al, 1997a and 1997b) and vaccination history will be conducted within a week of delivery, and repeated at 6 weeks, 3, 6, 12, 18, 24 and 36 months (Appendix 2). At the ages of 4 and 5 years, interviews about respiratory health and symptoms, will be conducted with the child themselves, with verification of the information provided by the mother (Appendix 3).

(c) Questionnaire language: All questionnaires will be available in English and isiZulu. The isiZulu versions will be translated from English and then backtranslated by a second translator to assure that the instruments are truly equivalent.

Clinical Assessments

Abdominal ultrasounds are not available at the participating clinics. In instances where this assessment is performed, the data will be obtained. The basic clinical data of all the participating children will be obtained from the birth records, including intra-labour history such as fetal distress, normal, assisted or caesarean deliveries; birth weight and other anthropometric data, APGAR scores etc. Each child will be assessed at each assessment interval. These assessments will include growth and developmental milestones, as well as detailed clinical respiratory evaluation. Lung function assessments will be conducted, and these are detailed below.

Biochemical Assessments

(a) Maternal Blood Assessments

These biochemical asssessment plan is based on the assessments we conducted within the Birth Outcomes Pilot.

Third trimester maternal blood samples and cord blood at birth will be taken for laboratory analysis. Samples will be analysed for markers of oxidative stress (lipid peroxidation), allergic potential and atopy. These include the interleukins IL-4, IL-5 and IL-13, cytokines tumour necrosis factor (TNF) and interferon (INF), salivary cortisol, IgE, IgA and vitamins C, D and E and beta-carotene. The role of the mitochondria in the development of oxidative stress will be determined by analysing protein levels and mRNA expression of sirtuins, uncoupling proteins and mitochondrial DNA damage of PBMCs.Sample preparation for mitochondrial analysis will include the isolation of mitochondria based on MACS Technology. Briefly, the density and viability of isolated PBMCs (Histopaque 1077) will be determined by Trypan Blue exclusion and resuspended in cold PBS (10^7 cells/ml) and pelleted (300 xg; 10 min; 4^oC). Pellets will be resuspended in Cell Lysis Buffer (1ml) and homogenised using a 29G needle (20x) prior to the addition of and incubation with separation buffer (9ml) and 50µl of AntiTOM22 microbeads (4^oC, 1 hour with gentle shaking). Samples are then transferred to prepare LS Columns in a magnetic field of a MACS separator. Following 3 washes (3ml wash buffer), columns will be transferred to collection tubes and collected using 1x Separation buffer for further analysis. Mitochondrial yields will be split for RNA isolation, Protein isolation and DNA isolation.

The mitochondrial DNA damage assay that will be used is a quantitative PCR assay which determines the level of mitochondrial damage by quantifying the presence in circulation. Briefly, a pre-mixed SYBR®Green Supermix, primers and DNA template (10ng/µl) will be

made up to a volume of 25µl per reaction, as well as a corresponding housekeeping gene and amplified using a BioRad Chromo4 Thermocycler.

MicroRNA analysis will be conducted using the TaqMan® MicroRNA 384-well Array cards. Briefly, total RNA will be isolated using an in-house triazol-phenol extraction after which, microRNA will be isolated and cDNA synthesised using the TaqMan® miRNA Reverse Transcriptase Kit. Total mature microRNAs will then be determined by Quantitative PCR methods on the Applied Biosystems Viia7 instrument.

The JC-1 Mitoscreen assay (BD Biosciences) will be used to assess mitochondrial membrane polarity. Many toxic agents tend to affect mitochondrial ATP production. Several known pollutants (e.g. lead) tend to interfere with haem synthesis and compromise the electron transport chain. A rapid assessment of mitochondrial integrity is the measurement its polarity. Depolarized mitochondria are good indicators of toxicity and apoptosis. Peripheral lymphocytes (approximately 10^5) will be transferred into 5ml polystyrene cytometry tubes. The JC-1 dye (150ul) will be added to lymphocytes and allowed to incubate at 37^0C for 10 minutes. Thereafter, lymphocytes are washed twice in JC-1 wash buffer and re-suspended in 200ul flow cytometry sheath fluid. Labelled lymphocytes are enumerated by flow cytometry using a 4-colour FACS Calibur flow cytometer. Data is recorded for green and red fluorescent channels from 50,000 events for each sample. Analysis is performed with FlowJo 7.1 (Tree Star Inc.) software.

The COMET assay measures DNA damage in PBMCs resulting from exposure to a broad spectrum of genotoxic and cytotoxic compounds *in vivo*. Under both neutral and alkaline conditions, DNA damage is visualized by the characteristic comet shape formed as a result of the migration of genetic material from the nucleus towards the anode. The length of the DNA tail is measured using SCION image.

Lipid peroxidation occurs when polyunsaturated lipid come into contact with reactive oxygen species. Lipid hydroperoxides can degrade into, among other things, malondialdehyde, which forms a 1:2 adduct with thiobarbituric acid (TBA). This assay is a good measure of oxidative stress.

Correlations between lipid peroxidation and mitochondrial depolarization will be done. The result is a coloured product with a maximum absorbance at 532 nm.. Finally, spectrophotometric measurements are made at both 532 nm (maximum absorbance for TBARS) and 600 nm. The absorbance at 600 nm is due to the solution and is subtracted from the absorbance at 532 nm. Absorbances from the blank reaction are also measured to account for substances that may also result in a signal at 532 nm.

The GSH-Glo™ Glutathione Assay is a luminescence-based assay for the detection and quantification of glutathione (GSH). The assay is based on the conversion of a luciferin derivative into luciferin in the presence of glutathione, catalyzed by glutathione S-transferase (GST). The signal generated in a coupled reaction with firefly luciferase is proportional to the amount of glutathione present in the sample. This assay is a measure of intracellular antioxidant concentration.

The Human Inflammatory Cytokines & Receptors RT² Profiler™ PCR Array profiles the expression of 84 key genes involved in the inflammatory response. This array contains genes involved in mediating immune cascade reactions during inflammation. The chemokines, cytokines, and interleukins involved in the inflammatory response are represented as well as their receptors. Using real-time PCR, it can easily and reliably analyze expression of a focused panel of genes related to inflammation with this array. The PCR array is a set of optimized real-time PCR primer assays on 96-well plate for pathway or disease focused genes as well as appropriate RNA quality controls. The PCR array

performs gene expression analysis with RT-PCR sensitivity and the multi- gene profiling capability of a microarray. The cDNA template is mixed with the appropriate ready- to-use PCR master mix, aliquoted in equal volumes to each well of the same plate, and then run on a real-time PCR cycling program.

Analysis of samples for trace elements zinc ,copper & selenium as well analyses of vitamin A, C and E and FeNO, sputum ECP, metalloproteinase, neutrophil and eosinophil count; fibrinogen, C- reactive protein (CRP) will be carried out by the chemical analytic laboratories at the National Institute for Occupational Health.

As a substantial focus of this study is on basic sciences, **laboratory quality assurance and validation** of tests form an important aspect of this project. This takes several forms. Firstly, many of these tests have been conducted previously by the Departments of Medical Biochemistry, using, in the main, internationally standardised kits. These analyses have been conducted in scientific projects, which have been published in peer-reviewed journals – for example, Prof Chutturgoon (Medical Biochemistry) has done research on oxidative stress and rheumatoid arthritis as well as inflammatory markers and cardiac disease, and other aspects of oxidative stress, as well as micronutrients and various diseases. Analytic methods used are all based on internationally standardised techniques (as stated above) and reported in the peer-reviewed literature. The laboratories are accredited by the accreditation body (SANAS) in South Africa. As additional measures, for all new tests conducted or new methods used, split samples will be sent to either nationally accredited or international laboratories using extenal standardisation processes, such as the Chemical Analytic laboratories at the National Institute for Occupational Health.

(b) Genotyping

These tests were performed during the conduct of the pilot.

Collection of blood from the mother will be done by venipuncture during the third trimester. Genomic DNA will be extracted using a PUREGENE DNA isolation kit (GENTRA, Minneapolis, MN). DNA samples will be banked and stored at -20C. Umbilical cord white blood cells (UCWBC) will be collected for genomic DNA isolation for the neonate and for DNA methylation analysis. UCWBC provide a reasonable surrogate for our target organ/tissue (lung/immune cells) because they contain stem cells that can populate the lungs in later life and also provide a rich source of T cells (Miller and Ho, 2008) which are important producers of cytokines and other asthma mediators. The Illumina DNA Methylation Analysis kit (450K) will be used to determine genome wide DNA methylation.

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Single nucleotide polymorphisms (SNPs) in GSTM1 (null vs positive); GSTP1 (Ile-Val) and TNFα-(308) will be determined using standard PCR procedures, including in some instances, restriction fragment length polymorphism analysis (RFLPs). DNA sequencing will be used to verify PCR products and appropriate quality control procedures will be followed **(**Appendix 4**).**

(c) Cord Blood Assessments

Cord blood for methylated DNA testing, IgE levels as well as metals, such as lead and manganese and vitamins A, C, E and beta-carotene will be done.

(d) Neonatal and Infant Assessments

The neonate will be subjected to biochemical markers of oxidative stress, allergy, atopy and genetic polymorphisms. These assessments will include: salivary cortisol, interleukins IL-4, IL5 and IL-13, cytokines IFN and TNF, IgE and IgA, Vitamins C, D and E and beta-carotene. Inflammatory markers, such as Eosinophilic cationic protein (ECP); neutrophil and eosinophil count; fibrinogen, C-reactive protein (CRP).

(e) Childhood assessments

All children participating in the study will be requested to provide a blood sample collected using standard techniques for assessment of blood lead, mercury and manganese, as well as the markers of oxidative stress, inflammation, allergy and atopy. All of these assessments, will be done annually from the age of 3 years until the end of follow-up. In order to reduce the degree of contamination during the blood sampling, the following washing procedure for the area to be punctured will be used. The skin will be carefully scrubbed with soap and water, followed by an alcohol swab. The cleaned surface will be rinsed with distilled water and dried with metal-free tissue paper before puncture. Only trained nurses or phlebotomists will be employed in the collection of blood samples.

Immediately after the cleaning, the puncture will be made using a Minilancet or a similar device. About 2.5 mL of the blood will be collected in a lead-free plastic vacutainers containing EDTA powder. The vacuutainers will be capped and stored for analysis in temperature controlled storage boxes. The personnel involved in the blood sampling will be instructed on how to minimize sample contamination, by wearing acid-washed polyethylene gloves and avoiding contact between sampling material and any unclean material prior to fingerstick.

Lung Function assessments

Minute ventilation, mean tidal inspiratory and expiratory flow measures (newborns and infants) ; forced expiratory manouevres and peak flow measures (3-4), spirometry (4-5 years old) and methacholine challenge testing (6 year olds) will be done over the course of the study.

Funding dependent, testing will be conducted in unsedated newborns and infants during natural sleep, using an infant mask using the European Respiratory Society (ERS)/American Thoracic Society (ATS) standards of infant lung function testing, with an ultrasonic flowmeter (Bates et al, 2000).

All American Thoracic Society (ATS) guidelines for conducting spirometry will be followed among the older children (American Thoracic Society, 1995). Spirometers will be calibrated at least twice a day with a three-liter syringe. Technologists who have undergone training in standard technique will conduct spirometry. Spirometry will be performed in a sitting position with nose clips. The lung function indices of primary interest will include forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Special instructions will be given to participants to ensure that tested individuals do not take any anti-asthmatic inhalers (12 hours before) or oral asthma medications (48 hours before) prior to the test. Participants with an obstructive pattern at baseline (FEV1/FVC < 0.75) will be administered an inhaled bronchodilator and have testing repeated.

All children will be assessed at the assessment centre. Transport will be arranged for these participants.

(b) Intensive Phase Assessments

To address Specific Objective 6 (airway response to short term fluctuations in pollutant measures), intensive phases of data collection (health measures and pollutant monitoring) will be conducted. All children will be asked to participate, however, only children above the ages of 4 will undergo the intensive lung function assessments. These intensive phases will take place for a full week (including the weekends) at the midpoint of each of the four seasons for each of the years of follow-up. These will be in end January (summer), end April (autumn), end July (winter), and end October (spring). This data will be used to determine whether there is an association between daily fluctuations in ambient air pollution levels and fluctuations in health status. The ability of the research investigators to collect such data successfully was demonstrated during the South Durban Health Study.

Based on this previous study, this population of children appeared suitably sensitive to potential acute health effects associated with fluctuations in ambient air pollution.

(a) Daily Diaries

Each child-participant, with the assistance of a parent or other adult caretaker, will be asked to keep a daily diary for the full week of these intensive phases, completed before going to bed on each day. In these diaries will be recorded symptoms, activities, medication use, encounters with the medical system, time spent indoors/outdoors, and child and parent quality of life measures. These diaries will be used to create single day summary severity, medication, and quality of life scores for use in statistical analyses examining the relationship between daily and seasonal fluctuations in exposure measures and fluctuations in these outcome measures. An example of a similar diary used in the South Durban Health Study can be found in (Appendix 5).

(b) Tri-daily measures of pulmonary function during the intensive phases

The AirWatch® (Imetrikus.com, Mountain View, California, USA) airway monitoring equipment to monitor fluctuations in peak expiratory flow (PEF) and forced expiratory volume at one second (FEV_1) of each participant, will be used. This portable, hand-held device has a number of distinct advantages over methods used previously to obtain repeated measures of a forced expiratory manoeuver in field studies. First, unlike the case with traditional peak flow meters, the FEV_1 is also obtainable. FEV_1 has inherently greater reproducibility than PEF and is a more clinically relevant measure. Second, results of up to 500 expiratory maneuvers are digitally stored in each Air Watch. A unique patient identifier and the time and date of each expiratory maneuver can be manually downloaded into a data base. Each participant will receive his/her own peak flow device, which will be kept at the participant's home, and will be clearly labeled with the participant's full name to ensure that devices are not interchanged during retrieval, data download and returning to participant. At the end of each intensive phase, the data will be manually downloaded.

The quality of such peak flow and FEV1 measures collected in the field tends to be quite variable, but is responsive to focused training of participants in good technique with frequent reinforcement. We will conduct training sessions with the participants in the proper performance of peak flow manoeuvers. As part of this training we will individually coach and observe each participant to ensure his or her ability to perform valid and reproducible expiratory manoeuvers. In addition, trained technicians will randomly visit the homes of the participants and observe expiratory manoeuvers to reinforce proper technique. We will re-train the participants at the beginning of each of the subsequent one-week intensive data collection periods. As additional quality control, FEV_1 obtained by means of the Airwatch will be compared to those obtained by the trained technician performing the full spirometry. Readings from the airwatch that is less than 80% or more than 120% of the technician obtained recording will be rejected.

On each of the days during the week of intensive monitoring, participants will be asked to perform a session of three consecutive manoeuvers once in the morning before 07h00, again on return at 15h00 and again before going to bed. The highest PEF and highest FEV_1 from each session, even if from different manoeuvers, will be used in data analyses.

We have implemented the above serial peak flow recordings among children, using similar handheld devices previously.

(c) Skin Prick tests

This will be done on all the children at 4 years of life. All children will be requested to participate in skin prick testing. Antigens to be tested will include mixed cockroach, mixed dust mite, mould mix (*Aspergillus*, *Cladosporium* and *Penicillium*), cat, dog, mouse, rat, mixed grasses, plus histamine as a positive control and saline (or glycerol) as a negative control. Participants will be informed to stop any antihistamines and any other reactive

medication (H_2 antagonists, tricyclic antidepressants, corticosteroids etc) at least 24 – 72 hours pre-test. The test will be applied to the volar surface of the forearm, and will be read approximately 15 – 20 minutes later. The wheal and erythema will be read and measured according to a standardised method, and an outline of the wheal and erythema will be recorded on see through tape for a permanent record. A greater than 2 mm difference in mean diameter between allergen and control wheal will be considered positive. A medical doctor will be on site to clear each participant to receive skin testing and will have proper medications and resuscitation equipment in the exceedingly unlikely event that individual has a severe reaction to a skin test. Collection of this data will allow for the assessment of whether skin test positivity is associated with increased susceptibility to the effect of ambient air pollutants.

(d) Measurements of Ambient Air Pollutants and Exposure Assessment Modelling

(a) Continuous monitoring – site locations

The location of the communities under study (Austerville, Merebank and Bluff in the south, and Newlands East in the north) were deliberately selected because of the location of continuous monitoring stations in these communities. These stations are part of the Air Quality Management System (AQMS) of the eThekwini metropolitan government. The number of sites in the different geographical areas also vary, with a single site (Ferndale) in the less industrialised north (Newlands East), and six sites (Nizam, Wentworth, Southern Works, Settlers, Ganges and Grosvenor) in the south.

(b) Continuous monitoring – parameters

Currently the pollutants measured at these sites vary, and the details are provided in Appendix 6. Additional monitoring will be set up at stations where key pollutants of interest are not being monitored. All stations should monitor particulate matter less than 10 microns in diameter (PM10), particulate matter less than 2.5 microns in size (PM2.5), oxides of nitrogen (NO_x), sulphur dioxide (SO_2), oxone (O_3), carbon monoxide (CO), temperature and relative humidity (RH). These measurements will be compared to TEOM measurements. Additional parameters will include wind direction and speed. A strict quality assurance plan will be adhered to during the monitoring (See the Quality Assurance Project Plan in Appendix 7.)

(c) Analysis of collected samples

Samples will be analyzed, to the extent possible, using the available technology and laboratory services in the country. Each laboratory will participate in a quality assurance program (see below) to ensure that data quality objectives are achieved.

(d) Exposure Modelling

All pregnant women selected into the study will have to be resident within a reasonable radius of these monitoring stations for the entire duration of their pregnancy and birth, and for as much of the follow-up period as possible. Children will only contribute data to the study for the period in which they are resident in proximity to these stations.

No personal sampling for any pollutants will be conducted – all data will be obtained from the above mentioned area monitors. However, based on the geographic, spatial and temporal variablity of data from previous studies conducted by the researchers in Durban and historical data from the Air Quality Monitoring System in the city and on time activity surveys of each of the participants (pregnant female up to time of delivery, and thereafter participating child) conducted at various times during the study, personal exposure metrics will be calculated. This will allow factors such as pregnant females working outside or spending vacations of the monitoring station range to be factored into the exposure metric. It is recognised that the absence of personal monitoring is a weakness, but the cost of either continually monitoring or phase monitoring of each individual participating in the study will be exorbitant. The development of land use regression techniques has been employed in our pilot, and a similar strategy will be employed here, for the key pollutants of interest.

A land use regression model (LUR) regresses pollutants of interest against site-specific geographic predictors (independent variables) such as traffic, land use, topography and

meteorology in a multivariate regression model. The parameter estimates derived from this regression equation are then used to estimate pollutant levels at unmonitored locations such as participant homes. The use of site-specific variables in this method, captures small-scale variability more effectively than other well known methods of interpolation. The development of a LUR model comprises two key steps, i.e. determining pollutant concentrations either from an existing monitoring network or though passive sampling efforts and subsequently deriving geographic predictor variables within varying buffer distances around each sampling site. This data is translated into a regression equation, which is applied within a GIS framework, thus rendering a pollutant concentration map which provides information on exposure at unmonitored locations such as the participant homes.

(e) Sampling details

The sampling procedures for all of the following pollutants was successfully employed by the research team during the conduct of the South Durban Health Study, and we therefore have full confidence in our ability to implement this strategy, as well as ensure the requisite quality assurance.

PM2.5: TEOM samplers will be used. TEOM samplers can be used to measure PM2.5 although it is recognized that this is not a US EPA Federal Reference Method (FRM) at present, due to some artifact issues. However, correlation between TEOM and gravimetric measurements is expected to be high. Previous collocation studies conducted by the researchers, using the TEOMs and gravimetric samplers showed this to be true (Naidoo et al, 2007). With appropriate filter media (see below), the same samplers will be used to collect filter samples for metals and other analyses.

Filter samples will use 24-hour samples. Sampling periods from 8:00 am to 8:00pm samples, rather than calendar day, are acceptable and more easily achieved. Further, this averaging period is preferable for the health study since it better corresponds to the exposure period prior to lung function and other health measures in the children and adults being measured.

Trace metals. Selected PM10 and PM2.5 samples, collected on quartz fiber filters, will be analyzed for trace metals, including chromium, copper, manganese, and lead, all key toxic pollutants. The best analytical performance is obtained using Inductively Coupled Argon Plasma Mass Spectroscopy (ICP-MS). Individual filters will be analyzed; no composite samples will be used although this is a lower cost option. We proposed to develop estimates of annual average levels for trace metals and the spatial gradient across South Durban, including levels at the population sites, the traffic impacted sites, and the North Durban sites. At these sites, we propose 24-hour sampling every 12^{th} day, giving an 1 year total of 31 observations, which should be sufficient to provide a robust annual average. There will be 10 percent trip and field blanks included in the analysis. The determination of ambient trace metal concentrations is a critical input in the health risk assessment including the prediction of cancer and systemic disease.

Pollen/Moulds. Daily measurements are required during the intensive period. Outdoor pollen counts will be performed using sterilized filter cassette (AirCheck) or equivalent methods; we will also consider the use of final stage Anderson impactors and appropriate culture media. Because these are short-term samples, each sampling event will utilize at least two replicates.

(e) Assessment of Indoor Environment

A random sample of 25% participating households will be selected for detailed indoor environmental assessments. These assessments will be done at particular points during the follow- up. An assessment will be conducted during the pregnancy phase, preferably in the first trimester, as this may be a critical period for the development of allergies in the child, and repeated at least twice during follow-up – once in the first year of the life of the participating child, and again before the end of follow-up. The data from this random sample will be used to develop a model, which will be used to predict that pollution found in homes not similarly assessed. The methods described below have been utilised by the research team previously in the South Durban Health Study.

(f) Walkthrough indoor assessment

The indoor environment of the household of each participant will be assessed by means of a walkthrough evaluation, using a standardised instrument (this instrument will be modified from one successfully used by the investigators in the South Durban Health Study -- (Appendix 8). The instrument includes room by room observation together with some questions put to an adult respondent. This assessment will include building age, type, condition and temperature, dust and lead control behaviour; moisture problems (sources, indications, ventilation, heating); indoor air quality (combustion products -- particularly use of biomass fuels for cooking and heating, formaldehyde, radon, asbestos, home chemicals); and nearby environment, including proximity to pollution sources.

(g) Household dust

This will be collected for allergen assessments: specifically dustmite, cockroach, and total moulds. Dust samples will be additionally assessed for lead and other metals. These will be analysed at the University of KwaZulu-Natal and externally validated at the University of Michigan. Vacuum samples will be collected from each household: one from the room the child sleeps in will be directly measured, the second a composite sample of other rooms in the house. The protocol to be followed will be based on one successfully used previously in the above-mentioned study (Appendix 9).

(h) (c) Indoor monitoring

Selected indoor airborne contaminants monitoring will be conducted in selected households. Monitoring for O_3, SO_2, NO_x and VOCs is feasible using passive samplers with sampling periods of approximately 1 to 3 weeks. PM10 or PM2.5 and airborne spores require the use of active samplers. These samples will be repeated and simultaneously conducted outdoors. Outdoor pollen counts and indoor airborne endotoxins will also be sampled and analysed using electrostatic dust fall collector (EDC) (Noss et al, 2008). The number of households selected for indoor monitoring will be sufficient to constitute a validation subset, very useful to establish, for example, the indoor/outdoor ratio of major pollutants, the range of variation from the community air pollution monitor, and other purposes.

(i) Antioxidant Intervention – Randomised Control Trial Design

Based on our previous studies, we expect a prevalence of persistent asthma (based on symptoms reported by mother/caregiver) of approximately 20%, across the city. From a total study sample of 1100 children, an expected 220 are likely to exhibit asthma-like symptoms. Using a double blind randomised control study design, 50% of this subset of the study sample will be given antioxidant supplementation and the remainder a placebo. In order to increase the number of particpants, additional known asthmatics from within the communities under study, recruited from local family practitioner practices in these communities will be included into the trial. Children will be recruited at the age of 3 years and followed up as per the study plan described previously. The specific observations between treatment and placebo groups that will be considered will be symptoms, based on reports from caregivers, lung function estimates from intensive phases and technician conducted spirometry, markers of inflammation, oxidative stress and allergy, responses to pollutant fluctuations, as measured in the intensive phases.

Health Risk Appraisal Exercises

An exercise attempting to characterise health risk in the basin concluded that the SO_2 associated risk for the community was low to acceptable (Matooane and Diab, 2001). The study, using the US Environmental Protection Agency (US EPA) health risk assessment model, made assumptions about similarities in exposed populations in the US and in a developing country as represented by the Durban South community. These assumptions included the "averaging time", "exposure scenarios" and "inhalation rates" - unless these assumptions are adequately tested in the field, the conclusions of this study can be considered untested, as acknowledged in part by the authors. Moreover, it would appear

that the presented methodology may not take sufficient cognizance of the increased sensitivity of asthmatics to adverse respiratory effects of air pollutants.

More recently, as part of the South Durban Health Study, health risk assessments in the south Durban found that a substantial health risk exists for both acute and chronic diseases (Naidoo et al, 2007). Long term exposures to major air toxic pollutants, including trace metals, polycyclic aromatic hydrocarbons (PAHs), persistent chlorinated pesticides, polyhalogenated dioxin/furans (D/F), polychlorinated biphenyls (PCBs), and volatile organic compounds (VOCs), were estimated using periodic measurements over 2004-5 at three sites: two sites in the Durban South Industrial Basin, and a comparison site in north Durban. Additionally, lead and manganese were monitored in blood drawn from 358 children. Using current estimates of chronic cancer and non-cancer toxicities, individual lifetime risks and hazard indices were calculated to identify risk drivers. The differences between the sites were evaluated, and results to risks estimated for other cities was compared. A small number of pollutants contribute most of the total estimated cancer risks (1.8–2.5 x 10^{-4}), and the following classes pollutants warrant attention due to the presence of elevated ambient concentrations: (1) VOCs, specifically benzene, which was high (average of 9 µg/m^3) at multiple sites in the DSIB and had a strong spatial gradient, showing the importance of local sources. (2) Selected D/F congeners and PAHs (e.g, naphthalene), which showed a diffuse spatial pattern and suggested the importance of both local and regional sources. This also indicates that such exposures are widespread among the population in Durban. (3) Selected metals, including chromium, nickel, lead, and manganese. Non-cancer risks fell just below a hazard index of one, the usual criterion. Based on both ambient and biomonitoring, elevated exposures of toxics are found in portions of Durban (Naidoo et al, 2007).

Environmental studies and available Environmental Data for DSIB

The peculiarities of geography and land development strategies for the Durban South region have been documented in numerous reports since the late sixties, strongly implying a lack of appropriate town planning on the part of the local government. The DSIB is bounded on the southeast by the Bluff ridge (70 - 100m high) and in the northeast by the Berea ridge (110m high) (Diab, 1995). This allows for the drainage of cold night air along the Umhlatuzana and Umbilo rivers in the Durban south, damming against the Bluff ridge, and diverted northwards to the Berea ridge, and subsequently toward the sea. Through land warming during the day, sea currents direct the air back inland, causing continuous re-circulation (Matooane, 2001). These lesion patterns are accompanied by temperature inversions from late autumn to early spring, limiting the vertical diffusion of pollutants (Diab, 1995).

The Durban south industrial basin (DSIB) is a complex mix of heavy industry and residential communities. Owing to a combination of its geographical relationship to the refineries, land contours, prevailing meteorological conditions, the use of a relatively short emissions stacks at these facilities (50 – 100 meters), the lack of or relative ineffectiveness of emission control devices on refinery stacks, the many sources of so-called fugitive air emissions at refineries, emissions from industrial and passenger vehicles, as well as the proximity of other industries, the community is believed to be at risk for intermittent substantial exposure to ambient air pollutants.

The key pollutants that are monitored in the DSIB are SO_2, NO_x, PM and to a lesser extent, CO. Lead, O_3 and volatile organic compounds (VOCs) are not monitored intensively. Most of the monitoring in DSIB is done by private consultants contracted to the Durban Metro for management of the Durban Metro Air Quality and Emission Survey, which has been responsible for the Durban South Sulphur Dioxide Monitoring System.

The ambient pollutant findings from the recent South Durban Health study revealed NO_2 concentrations lowest in the north (mean of 11 ppb), highest in the city centre and industrial areas (19 - 24 ppb), and lower at sites in the south (12 - 14 ppb). Average SO_2 concentrations varied widely; with low concentrations (1 - 3 ppb) at sites in the north; medium to high concentrations (6 -

10 ppb) at central and south-central sites and (12 - 20 ppb) in the south. The SO_2 spatial distribution reflects the location of emitting industries in the South Basin. Annual average PM_{10} concentrations was in a narrow range of 38 – 57 μm m^{-3}, across north and south sites. Maximum 24-hr average concentrations approached or exceeded 150 μg m^{-3} at most sites. The highest concentrations were observed at one south site, and one north site, two widely separated monitors. $PM_{2.5}$ concentrations measured at three sites were nearly identical, 20 – 21 μm m^{-3}, with maximum 24-hr concentrations ranging from 79 to 131 μg m^{-3}. PM_{10} and $PM_{2.5}$ concentrations across the region were high relative to international norms and standards. (Naidoo et al, 2007)

Although a steady decline has been observed for annual SO_2 levels since 1989, at some monitoring stations the international WHO standard has been exceed since 1995. One hour and 24 hour exceedances of national Department of Environmental Affairs and Tourism (DEAT) standards are reasonably common occurrences, with approximately 176 one hour exceedance episodes in 1999 and 17 24 hour exceedances between 1997 - 1999 have been reported. Similar patterns of exceedances have not been shown for NO_x (Matooane, 2001).

Lead exposures have been poorly documented in the Durban South area. Reported airborne lead concentrations range from 0.44ug/m^3 - 6.42 ug/m^3, which exceed national and international standards several fold (Nriagu et al., 1996). Dust lead loadings among primary schools in the basin ranged from 9 - 264 ug/m^3, much higher than recorded in other studies elsewhere in the world (Liggans et al., 1998). This contrasts to the findings of the South Durban Health Study of 8.7ug/m^3 in the north and 6.3ug/m^3 in the south (Naidoo et al, 2007).

Studying Birth Outcomes in Durban

In preparation for a longitudinal birth cohort, the researchers embarked on a pilot study, intending to test the proposed study's ability to recruit participants, work with the healthcare facilities, test instruments and in-field procedures (phlebotomy and transport of samples to laboratories); basic science assessments and follow-up of patients until delivery. The pilot also focused on the development of exposure assessment strategies for the cohort study.

In this pilot of 100 women (50 from north and south Durban respectively), there were only meaningful differences between education and HIV status. Average years of education was lower and HIV positive prevalence was higher in the south compared to the north. Although a substantial percentage of participants were exposed to environmental tobacco smoke (ETS), neither this or other risk factors varied geographically.

There were three stillbirths and 8.7% low birthweight (<2.5kg) newborns. The mean gestational age (weeks), (38.2, south; 38.4, north), birthweights (kg) (3.1, south; 3.2, north) and apgar scores and 1 and 5 minutes did not differ significantly between north and south. No differences were seen for adverse birth outcomes, although these were consistently higher in the south. The biochemical assays (Table 1) only varied between the two regions for apoptosis and GSH.

(j) Table 1: Biochemical markers for participants from the south and north

	South (mean (SD))	North (mean (SD))
Malondialdehyde (MDA)	0.07 (0.07)	0.07 (0.05)
Comet tail length*	0.56 (0.13)	0.47 (0.10)

Mitochondrial depolarisation	44.4 (18.7)	43.32 (20.6)
Apoptosis*	15.2 (7.46)	20.24 (11.8)
Caspase 3/7	20.86 (22.82)	15.30 (25.3)
Caspase 8	14.8 (16.55)	85.89 (431.6)
Caspase 9	36.7 (90.3)	52.7 (131.6)
GSH* 5.3 (0.9) 6.9 (1.8)		

Biochemical evidence of oxidative stress did not vary between north and south. These markers were not associated with low birthweight. HIV status was not associated with low birthweight. Living in the south resulted in a non-statistically significant elevated risk (odds ratio=1.3, 95% CI: 0.4-3.6) for adverse birth outcomes, adjusted for ethnicity, education, income, HIV status and cigarette smoke (personal or passive). Geographical area was also a significant predictor of the GSH, Comet Tail Length and apoptosis biomarkers.

(k) Summary

We have successfully completed a pilot birth cohort study investigating air pollution and risk factors for adverse birth outcomes in 2011. It served as preparation and proof of concept for a full scale longitudinal birth cohort study which will examine the effects of environmental influences on the health and development of a cohort of 1200 women attending ante-natal clinics and children will be followed up from before birth to six years of age to evaluate respiratory health. Participants will be recruited from south and north Durban in South Africa, areas of high and low ambient pollution respectively. This is a unique opportunity investigate the epigenetic contribution of environmental and dietary contributors to respiratory health. The proposed study has the potential to address key questions, such as those concerning the influence of timing of exposure, dose of exposure, diet, and ethnicity on susceptibility to asthma development. The study will further investigate the argument that prenatal environmental exposures may induce epigenetic modification and subsequently influence the risk for asthma.

The proposed project has three distinct features:

- Research in epigenetics is ongoing in Europe, the USA and Asia, however thus far; Africa has not entered this arena of research in terms of the link between epigenomics and epidemiology. This is the first opportunity to study an African ethnic group in this context.

- While studies have been published on gene-specific DNA methylation, there is less data on genome wide DNA methylation and association with environmental exposures and asthma.

- Despite progress made in experimental and cross sectional studies, fundamental questions related to asthma risk can only be addressed by cohort studies using specimens linked to well- annotated clinical and environmental exposure data which will be collected during the MACE study.

DATA MANAGEMENT AND ANALYSIS

Data Management

A data manager will be employed to track outgoing and incoming instruments, validate interviewer collected responses, ensure accuracy of data and clarify any obvious omitted data or errors. Data capturers will capture data as this becomes available, using pre-designed databases. All questionnaire, lung function, peak flow and exposure data will be double entered, by different capturers, blinded to the previous entries, with subsequent

checking for errors. All data will be maintained under tight security to ensure no breach of confidentiality.

Data Analytic Strategies, including Sample Size Calculations

Our study proposes the evaluation of several independent variables and health outcomes over the duration of follow-up in the cohort. Attempting to establish an appropriate sample size is a challenge. For the purposes of ensuring an adequate sample size for addressing our primary concerns (ambient pollution and childhood asthma), we have considered data generated from our previous studies in these communities. However, because of our interest in understanding the relationship of environmental exposures to birth outcomes, as contributory factors to asthma outcomes, we additionally considered these variables in the sample size calculations.

Our previous studies of asthma, atopy and airway hyperresponsiveness in these communities, showed the prevalence of persistent asthma in the pollution affected communities of 16.5 and 9.1% in the non-polluted communities, prevalence of marked and probable bronchial hyperresponsiveness of 18.8% and 12.6% respectively; prevalence of atopy of 35%, and covariate adjusted risk estimates of asthma between polluted and non-polluted communities of 1.3. The prevalence of adverse birth outcomes generally and low birth weight specifically in an unexposed population in South Africa is about 15% (Van der Merwe, 2011). In our pilot study, our low birth weight findings were 13.7% and 5.9% in the high vs low pollution exposed participants respectively, while overall adverse birth outcomes were 25.5% and 21.6% respectively. Based on these findings, a sample size of 1200 children should be adequate to detect effects between pollutant exposure and respiratory outcomes, while adjusting for the various covariates indicated below, with a 95% level of confidence and power of 80%.

These children will be recruited in utero, over a period of two years from the above-mentioned antenatal clinics in the communities under study. For all analyses a 5% alpha level will indicate significance. Analyses will be performed using STATA version 12.

Our pilot study indicated that at least 30-40 pregnant mothers present in each geographical location (north and south) per week. The non-random arm of the study will recruit additional mothers with histories of atopy, as well as mothers who are HIV positive

This study includes a variety of exposure, outcome, intermediate, and potential confounding variables of interest. Key exposure (independent) variables include: ambient air pollution (specific pollutants, including sulphur dioxide, particulate matter and oxides of nitrogen and metals); indoor air pollutants, including particulate matter and volatile organic compounds, cigarette smoking and use of biomass fuels; indoor dust lead and allergen levels. The primary outcome (dependent) variables of interest are respiratory related and birth outcomes which may impact on respiratory health during development of the lungs. The respiratory outcomes include peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) measured at repeated intervals during the course of the study; bronchial hyperresponsiveness (BHR) measured at the age of 6 years; specific respiratory symptoms, especially cough, wheeze, shortness of breath and chest tightness; any asthma and persistent asthma. The non-respiratory dependent and/or intermediate variables quantitatively assessed in the study will be skin test allergic status, and markers of inflammation, including eosinophilic cationic protein (ECP); neutrophil and eosinophil count; fibrinogen, salivary cortisol and C-reactive protein (CRP). Markers of oxidative stress, allergy and atopy will also be additional covariates assessed (these include: interleukins IL-4, IL5 and IL-13, cytokines IFN and TNF, IgE and IgA, Vitamins C, D and E, beta-carotene and the matrix metalloproteinases). Birth outcomes of particular interest include birthweight, stillbirth and preterm deliveries. Other outcomes of interest assessed through interviews will include reported medical history or symptoms consistent with respiratory infections. Maternal and child dietary intakes, particularly with regard to the intake of antioxidants will also be intermediate variables of interest.

Birth outcomes will be defined as follows: low birthweight (LBW), birthweight less than 2500 g; very low birthweight (VLBW), birthweight less than 1500 g; intrauterine growth

retardation (IUGR), birthweight at a given gestational age and sex less than 10th percentile based on national standards for live births; preterm birth, birth at gestational age less than 37 completed weeks; and stillbirth, fetal loss at gestational age 28 weeks and more, birthweight 1000 g or more, or fetal length 35 cm or more. LBW will be adjusted for gestational age, while IUGR is restricted growth at any gestation. For lung function outcomes, because of the lack of any useful validated external prediction equations, internal predictors will be developed by adjusting for age, age^2, height, height^2 and sex.

Before conducting formal statistical analysis, preliminary analysis will be performed. Frequency tables and descriptive statistics such as means and variances of the variables of interest will be calculated.

Statistical analyses will proceed from univariate analyses, which are used to describe the characteristics of the study population and to examine the crude associations between variables of interests. Such analyses will provide us a rough picture of the data and would be useful in the subsequent stratified and multivariate analyses. For categorical dependent variables, odds ratios will be calculated. For continuous dependent variables, correlation coefficients will be calculated. Stratified and multivariate analyses using multiple regression models for cross-sectional data and longitudinal data will then be performed to study the effects of ambient air contaminant levels on symptoms and pulmonary function status while adjusting for potential confounding variables. Descriptive analysis will be followed by chi-square tests to investigate possible differences in DNA methylation between participants from the 2 areas and between categories of environmental exposures and asthma outcome. Logistic regression will be performed to evaluate the association between parental report of asthma and methylation status; and between different environmental exposures and methylation status.

Advantages of a longitudinal study include the ability of studying the change of an outcome over time and increased power of analysis. However, analysis of longitudinal data is often complicated, since the observations obtained from the same subject over time are likely to be correlated and conventional regression techniques, such as linear and logistic regression, which assume independence among observations, lead to invalid results. Statistical methods specifically designed for analyzing longitudinal data will be used to account for within-subject correlation.

For continuous outcome variables, such as FEV1, PEF linear mixed models will be used. Linear mixed models account for within-subject autocorrelation over time using subject-specific random effects. Distributions of these continuous outcome variables will be examined and appropriate transformations will be taken to achieve normality. Logistic regression models will be developed for discrete outcome variables, such as frequency of specific lower respiratory symptoms (cough, wheeze, shortness of breath, chest tightness). We will adjust for relevant covariates, such as smoking status and demographic variables. Effect modifications will be examined by including interaction terms in the models. The baseline disease status of participants will receive particular attention as an effect modifier.

Bivariate analysis will be conducted for the exposed and comparison communities separately, looking for statistically significant differences between the populations, using chi square tests (categorical outcomes) and differences in means (continuous outcomes). Multivariable analysis will also be conducted by stratifying on comparison status and then combining all data into single regression models, while controlling for levels of exposure and other variables of interest.

HUMAN SUBJECTS

Institutional Ethical Clearance

The research proposal will be submitted to the Ethics Committee of the University of Natal Medical School. The latter committee consists of academics from the Medical School, representatives of communities and of the private sector, headed by a specialist in Biomedical Ethics.

Individual Informed Consent

Individual informed consent will be necessary for all participants. In the case of children, this will be obtained from their parent or guardian. In this instance each participant will be given a comprehensive explanation in the language of their choice. The content of this discussion will include the aims of the research, the purpose of the interview, the tests that will be conducted on them, use of their data and the confidentiality of all results. It will be emphasised that participation is voluntary and withdrawal at any time is permitted. Each participant will be asked to sign a consent form. No financial incentives will be provided for participation in the study.

Participants' Confidentiality

All participants will be individually given a copy of their results, together with interpretations of the data and if indicated, referral to an appropriate centre of their choice for further medical management. Individual results will be strictly confidential and will only be accessible to the research team. These results will be released to any clinician/guardian/agency should this be desired by the individual participant.

Publication of Results and Reports

In the publication of research results and reports, all data will be treated as grouped, thus no individual will be identified from such documentation. Semi-annual or more frequent reports will be submitted to all stakeholders, as required by contract. A draft final report will be also submitted for comment. All comments will receive careful consideration. As per the required guidelines of the University of Natal, the final content of the articles submitted to peer review scientific journals will be the responsibility of the researchers.

Ethical Issues

Individual informed consent will be necessary for all participants. In this instance each participant will be given a comprehensive explanation in the language of their choice. The content of this discussion will include the aims of the research, the purpose of the interview, the tests that will be conducted on them, use of their data and the confidentiality of all results. It will be emphasised that participation is purely voluntary and withdrawal at any time is permitted. Each participant will be asked to sign a consent form. A separate consent form will be administered documenting the taking of biological samples for genetic analysis and storage. Women may still enter into the study is they refuse consent to storage of biological samples and genetic analysis. No financial incentives will be provided for participation in the study; however as this is a longitudinal cohort study some form of incentive will be used to improve retention rates. These will take the form of gift vouchers and small gifts in the form of baby blankets and food.

PUBLIC HEALTH SIGNIFICANCE

The findings of this project will provide a better understanding of the interactions between environmental factors, biochemical and genetic factors that result in adverse respiratory health. These findings will allow for appropriate interventions at all levels, including

improved control of environmental pollution and improved diets during pregnancy and for the newborn.

Potential applications of this research include primary prevention (such as aiding at understanding the biology of diseases, potentially leading to the development of strategies based on mass intervention or target risk intervention approaches, this could facilitate the setting up of a genetic risk profile for the development of asthma and would enable us, for the first time, to take preventative action early in life for children with an increased genetic risk increased genetic risk to allergic diseases), secondary prevention (such as the development of primary tests for population wide screening) and therapy (such as choosing among alternative interventions or assist in the design of new drugs which are more specific, effective and safe (Bierbaum 2007, Bierbaum and Heinzmann, 2007; Castro-Giner, 2006).

APPENDIX B: MACE ENROLMENT QUESTIONNAIRE

Mother and Child in the Environment (MACE)
Enrollment Questionnaire

1. Date:____/____/____ **2. Mother Identification No.** ☐☐☐ - ☐☐☐ - ☐☐☐
Day Month Year

Gestational Week _____

1. Name of respondent:	_____ First _____ Middle _____ Surname
2. Phone numbers:	**home:** _____ **work:** _____ **cell:** _____ **other:** _____
3. What is your physical address?	_____ House No. _____ Road/Street _____ City _____ Postal Code
4. How old are you?	_____years
5. What is your date of birth?	_____/_____/_____ day month year ☐9 Refused
6. What is your marital status?	☐1 Married ☐2 Living together ☐3 Single ☐4 Divorced ☐5 Separated ☐6 Widow ☐7 Other _____
7. What is the highest grade or year of school you completed? **[READ CHOICES – select only one]**	☐1 Never attended school or only pre-school ☐2 Class 1 – Std 5 (Grades 1 through 7) ☐3 Std 6 – Std 9 (Grades 9 through 11- Some high school) ☐4 Std 10 / Matric (Grade 12 - High school graduate) ☐5 Non-degree training ☐6 College / technikon / university (1 year to 3 years) ☐7 Refused to answer

8. What is the highest grade or year of school your baby's father completed? [READ CHOICES]	☐$_1$ Never attended school or only pre-school ☐$_2$ Class 1 – Std 5 (Grades 1 through 7) ☐$_3$ Std 6 – Std 9 (Grades 9 through 11- Some high school) ☐$_4$ Std 10 / Matric (Grade 12 - High school graduate) ☐$_5$ Non-degree training ☐$_6$ College / technikon / university (1 year to 3 years) ☐$_7$ Refused to answer

[INTRODUCTION: INTERVIEWER READS TO RESPONDENT]

The purpose of this questionnaire is to collect information about your pregnancy and reproductive health . If there is a question you do not want to answer, please let me know and we can skip it. All of your responses are confidential and will not shown to anyone outside the study team without your written consent. If you wish to stop the interview at anytime, please advise me. We can continue at a later time at your convenience

A. HOUSEHOLD CONDITIONS	
9. With whom do you live? *(Fill in one or several boxes.)*	☐$_1$ Spouse ☐$_2$ Partner ☐$_3$ Parents ☐$_4$ Parents-in-law ☐$_5$ Children ☐$_6$ No one ☐$_{88}$ Others, describe _____
10. How many people including you live in your home?	☐$_1$ Number of people over 18 years ☐$_2$ Number of people between 12 and 18 years ☐$_3$ Number of people between 6 and 11 years ☐$_4$ Number of people under 6 years
11. Usual language spoken at home:	☐$_1$ English ☐$_2$ Zulu ☐$_3$ Xhosa ☐$_4$ Afrikaans ☐$_{88}$ Other (Specify:_____)
12. How many of your children are at nursery school?	_____ no. of children
13. What is your yearly gross income? (*Include child support, unemployment benefits and other allowances.)*	☐$_0$ No income ☐$_1$ Less than R2 000 ☐$_2$ R10 001–30 000 ☐$_3$ R30 001–75 000 ☐$_4$ R75 001–150 000 ☐$_5$ R150 001 and above ☐$_6$ Refused to answer

14. What is the baby's father's yearly gross income? (*Include child support, unemployment benefits and other allowances.)*	☐$_0$ No income ☐$_1$ Less than R2 000 ☐$_2$ R10 001–30 000 ☐$_3$ R30 001–75 000 ☐$_4$ R75 001–150 000 ☐$_5$ R150 001 and above ☐$_6$ Refused to answer
15. What type of housing do you live in?	☐$_1$ Detached house, Semidetached ☐$_2$ Farm ☐$_3$ Flat, Terraced flat, Apartment building ☐$_4$ Refused to answer ☐$_{88}$ Other _____
16. Has there been water damage, visible signs of fungus/mildew or a smell of mildew in your home in the past 3 months? *(Fill in one or several boxes.)*	☐$_1$ No ☐$_2$ Yes, water damage ☐$_3$ Yes, signs of fungus and mould ☐$_4$ Yes, a smell of mildew
17. What year was this house/structure originally built?	_____ year ☐$_{99}$ don't know
IF RESPONDENT IS UNSURE ASK: 18. Would you say it was built:	☐$_1$ before 1970 ☐$_2$ between 1970 and 1985 ☐$_3$ after 1985
19. How many rooms are there in your home? (counting the kitchen, but not the bathroom or toilet)	_____rooms
20 How long have you lived at this address?	_____ years ☐$_1$ less than 1 year
21. Is your home drinking water from the tap or from a river or dam?	☐$_1$ tap ☐$_2$ river or dam ☐$_3$ well ☐$_{88}$ other, please specify: _____
22. Do any pets live in this home?	☐$_1$ Yes ☐$_2$ No **[If no, skip to Q24]**
23. What kind of pets live here?	
(a). a dog?	☐$_1$ Yes ☐$_2$ No
(b). a cat?	☐$_1$ Yes ☐$_2$ No
c). a bird?	☐$_1$ Yes ☐$_2$ No
(d). any other pet(s)	☐$_1$ Yes ☐$_2$ No ☐$_{88}$ please specify: _____
24. During the past 12 months was a room heater used to heat one or more rooms in this house?	☐$_1$ Yes ☐$_2$ No **[If no, skip to Q25]** ☐$_{99}$ don't know **[If don't know, skip to Q25]**
(a). Was this heater fueled by	☐$_0$ not applicable – no furnace ☐$_1$ paraffin

	☐2 gas ☐3 electricity ☐4 wood ☐5 coal ☐6 Gel ☐88 other, please specify: _____ ☐88 don't know
25. During the past 12 months was one or more wood stoves used in this house?	☐1 Yes ☐2 No ☐99 Don't know
26. During the past 12 months was a fireplace used to heat the rooms in this house?	☐1 Yes ☐2 No ☐99 Don't know
27. During the past 12 months was the stove or oven ever used to heat this house?	☐1 Yes ☐2 No ☐99 Don't know
28. Is a stove or oven used for cooking in this house?	☐1 Yes ☐2 No ☐99 Don't know
(a). What is the primary source of heat for this stove or oven?	☐1 paraffin ☐2 gas ☐3 electricity ☐4 wood ☐5 coal ☐6 Gel ☐88 other, please specify: _____ ☐99 don't know

APPENDIX C: MACE 3RD TRIMESTER QUESTIONNAIRE PART 2

Mother and Child in the Environment (MACE)
3RD TRIMESTER + PART 2

1. Date:____/____/____ **2. Mother Identification No.** ☐☐☐ - ☐☐☐ - ☐☐☐
Day Month Year

Gestational Week _____

Work and leisure	
145. What was your work situation when you became pregnant? (*Fill on one or several boxes for each.)*	☐1 Student [skip to Q162] ☐2 At home [skip to Q162] ☐3 Intern/apprentice ☐4 Military service ☐5 Unemployed/laid off ☐6 Rehabilitation/disabled [skip to Q162] ☐7 Employed in public sector ☐8 Self-employed ☐9 Family member without steady income in family company ☐10 manufacturing ☐11 chemical ☐12 mining ☐13 commercial and retail ☐14 agricultural and farming ☐88 Other _____
146. What kind of work do/did you do?	☐1 general assistant ☐2 clerical/administrative ☐3 machine operator ☐4 farm assistant ☐5 supervisor/manager ☐6 engineering, designer or planning ☐88 other, please specify: _____
147. Did you have an extra job (with or without salary) when you became pregnant? *(For example, accountant, hair dresser, singer in a dance band, club leader)*	☐1 Yes ☐2 No Describe: _____
148. Have you been absent from work more than two weeks during this pregnancy?	☐1 Yes ☐2 No
149. Are you absent from your work at the present time?	☐1 Yes ☐2 No
150. If yes, what is the reason for your absence? *(Fill in one or several boxes.)*	☐1 Medical leave ☐2 Leave of absence ☐3 Sick child ☐88 Other: _____
151 The usual number of paid working hours a week before you became pregnant and at present.	Before the pregnancy hours_____ During the pregnancy hours _____

HABITS	
202. Did your mother smoke when she was pregnant with you?	☐1 Yes ☐2 No Don't know
2034. Are you exposed to passive smoking at home?	☐1 Yes ☐2 No
2045. If yes, how many hours a day are you exposed to passive smoking?	_____ hours a day
2056. Are you exposed to passive smoking at work?	☐1 Yes ☐2 No
2067. If yes, how many hours a day are you exposed to passive smoking?	_____hours a day
2078. Did the baby's father smoke before you became pregnant?	☐1 Yes ☐2 No
2089. Does he smoke now?	☐1 Yes ☐2 No
209. Have you ever smoked?	☐1 Yes ☐2 No (proceed question 104)
2109. Do you smoke now (after you became pregnant)?	☐1 No ☐2 Sometimes ☐3 Daily
(a). If yes, how many	_____ cigarettes per week _____ cigarettes per day
211. Did you smoke during the last 3 months before you became pregnant this time?	☐1 No ☐2 Sometimes ☐3 Daily
(a). If yes, how many	_____ cigarettes per week _____ cigarettes per day
212. How old were you when you started to smoke on a daily basis?	_____ years
213. Have you stopped smoking completely?	☐1 Yes ☐2 No
214. If yes, how old were you when you stopped smoking?	_____ years
215. If you stopped smoking after you became pregnant, in which week of pregnancy did you stop?	_____ week of pregnancy

APPENDIX D: MACE CLINICAL DATA 3RD TRIMESTER QUESTIONNAIRE

Mother and Child in the Environment (MACE)
Clinical data @ 3rd Trimester

1. Date:____/____/____ (Day Month Year) **2. Mother Identification No.** | | | | | | **3. Gestational week** _____

1. Weight	_____kg
2. Height	_____m
3. Body Mass index	
4. Blood pressure	SYS_____ DYS_____
5. Urine analaysis	☐1 Sg ☐2 pH ☐3 Glucose ☐4 Protein ☐5 Blood ☐6 Bilirubin ☐7 Ketones ☐8 Nitrites ☐9 Urobilinogen ☐10 Leucocytes ☐11 None of the above
6. Heamglobin (Hb)	
7. Iron (Fe)	
8. WR	
9. RPR	
10. Pap smear	
11. Do you know your HIV status	☐1 Yes ☐2 No **[If No, skip to Q 19]** ☐3 Refused to answer
12. What is your status?	☐1 Positive ☐2 Negative **[If negative, skip to Q 19]** ☐3 Refused to answer
13. Do you know your CD4 count?	☐1 Yes ☐2 No **[If No, skip to Q15]** ☐3 Refused to answer
14. What is your CD4 count?	_____
15. Do you know your Viral load?	☐1 Yes ☐2 No **[If negative, skip to Q17]** ☐3 Refused to answer
16. What is your viral load?	_____
17. Are you currently on HIV treatment?	☐1 Yes ☐2 No **[If No, skip to Q 19]** ☐3 Refused to answer
18. Please list all HIV Medication	_____ _____ _____ _____ _____ _____

19. Have you had an ultrasound done during this pregnancy?	☐$_1$ Yes ☐$_2$ No **[If No, skip to Q22]** ☐$_3$ Refused to answer
20. What were the results?	☐$_1$ Normal **[If Normal, skip to Q22]** ☐$_2$ Abnormal ☐$_3$ Refused to answer **[Skip to Q22]**
21. If abnormal, explain the ultrasound results	Explain:
22. What is your expected delivery date?	Date:
23. What type of delivery are you expecting?	☐$_1$ Normal vaginal delivery ☐$_2$ Elective Ceaser ☐$_3$ Refused to answer

APPENDIX E: MACE LABOUR/POSTNATAL QUESTIONNAIRE

Mother and Child in the Environment (MACE) **Labour/Postnatal data of Baby** **Date:____/____/____** **Mother Identification No.** ☐☐☐☐☐☐ **Day Month Year**	
Child Identification No. ☐☐☐☐☐☐	
1. Date of delivery:	_____/_____/_____ day month year ☐9 Unknown
2. Time of delivery:	Time: _ _:_ _ am/pm ☐9 Unknown
3. Gestational age:	Weeks_____ ☐9 Unknown
4. What type of delivery occurred?	☐1 NVD ☐2 Caesarian section
5. What is the babies gender?	☐1 Male ☐2 Female
6. What is the babies birth weight?	_____grams ☐9 Unknown
7. What is the babies birth length?	_____cm ☐9 Unknown
8. What is the babies head circumference?	_____cm ☐9 Unknown
9. What is the APGAR score?	_____ 1minute _____ 5 minute
10. Complications of baby	☐1 Jaundice ☐2 Fever ☐3 Sneezing ☐4 Respiratory distress ☐5 Pneumonia ☐6 Sepsis ☐7 TB ☐8 None ☐88 Other _____

	☐9 Unknown
11. Was the mother on any medication during labour?	☐1 Yes ☐2 No **(if no skip to Q12)** ☐9 Unknown
(a) If yes, please list the medication	Medication 1: _____ Medication 2: _____ Medication 3: _____. Medication 4: _____ Medication 5: _____
12. Did the mother receive any of the ffg: drugs after labour?	☐1 Yes ☐2 No **(if no skip to Q13)** ☐9 Unknown
(a). HAART	☐1 Yes ☐2 No ☐9 Unknown
(b). Vit A	☐1 Yes ☐2 No ☐9 Unknown
(c). PMTCT	☐1 Yes ☐2 No ☐9 Unknown
13. Did the mother have any complications during labour?	☐1 Yes ☐2 No **(if no skip to Q14)** ☐9 Unknown
(a) If yes, please list any complications	_____ _____
14. Did the baby receive any medication after delivery?	☐1 Yes ☐2 No **(if no skip to Q15)** ☐9 Unknown
(a) If yes, please list the medication.	_____ _____ _____
15. Did your baby stay in nursery?	☐1 Yes ☐2 No **(if no skip to Q19)** ☐9 Unknown
(a) If yes, how long did the baby stay in nursery?	
(b) Did the baby receive oxygen in nursery?	☐1 Yes ☐2 No ☐9 Unknown
16. Why was the baby admitted to nursery?	

18. When did he/she get discharged?	
19. What are you feeding the baby?	☐$_1$ Exclusive breastfeeding ☐$_2$ Exclusive formulae feeding ☐$_3$ Mixed feeding

APPENDIX F: MACE 6 MONTH FOLLOW UP QUESTIONNAIRE

Mother and Child in the Environment (MACE) **6 month infant follow-up** **Date:____/____/____** **Mother Identification No.** ☐☐☐☐☐☐ **Day Month Year**	
Child Identification No. ☐☐☐☐☐☐	
1. Date of Birth:	_____/_____/_____ day month year
2. Chronological age	_____months
3. Corrected age	_____months
4. GA at birth	_____weeks
5. Baby's birth weight	_____grams
6. Baby's birth Length	_____cm ☐9 Unknown
7. Baby's birth OFC	_____cm ☐9 Unknown
8. Baby's MUAC	_____cm ☐9 Unknown
9. Baby's current weight	_____grams ☐9 Unknown
10. Baby's current length(cm)	_____cm ☐9 Unknown
11. Baby's current OFC (cm)	_____cm ☐9 Unknown
12. Is the baby's Immunization up to date?	☐1 Yes ☐2 No
a) If No, why was your child not	☐1 Child unwell

immunised?	☐$_2$ Mother missed visit ☐$_3$ Clinic had no stock ☐$_4$ Other_____
13. What feeds is your baby receiving currently?	☐$_1$ Breastmilk ☐$_2$ Formulae ☐$_3$ Mixed feeding (breastmilk and formulae) ☐$_4$ Porridge
a) What formulae you are feeding?	☐$_1$ Nan ☐$_2$ Nan HA ☐$_3$ Infarcare ☐$_4$ S26 ☐$_5$ Pelagon ☐$_5$ Isomil ☐$_5$ Lactogen ☐$_7$ Other
b) What porridge are you feeding?	☐$_1$ Nestum ☐$_2$ Ceralac ☐$_3$ Maize meal ☐$_4$ Purity ☐$_5$ Other _____ _____
c) Which month did you start supplementary food?	_____

APPENDIX G: MACE 12 MONTH FOLLOW UP QUESTIONNAIRE

Mother and Child in the Environment (MACE) **1 YEAR infant follow-up** **[To be completed by Doctor attending to patient]**	
Date: **:**____**/**____**/**____ **MID:** _____ **CID:** _____ **Day Month Year**	
1. Date of Birth:	_____/_____/_____ day month year
2. Chronological age	_____months
3. Corrected age	_____months
4. Baby's birth weight	_____grams
5. Baby's birth Length	_____cm ☐9 Unknown
6. Baby's birth Head circumference	_____cm ☐9 Unknown
7. Baby's current weight	_____**grams**
8. Baby's current length	_____**cm**
9. Baby's current OFC	_____**cm**
10. BABY'S CURRENT MUAC	_____**cm** **☐9 Unknown**
A) Classify MUAC	**☐1 Normal** **☐2 Moderate wasting** **☐3 Severe wasting**

11. Is the baby's Immunization up to date? [INTERVIEWER TO CONFIRM WITH RTHC]	☐$_1$ Yes **(If Yes,Skip to Q 13)** ☐$_2$ No
12. If No, why was your child not immunised?	☐$_1$ Child unwell ☐$_2$ Mother missed visit ☐$_3$ Clinic had no stock ☐$_4$ Other_____
13. Did your baby receive de-worming?	☐$_1$ Yes **(If Yes,Skip to Q 15)** ☐$_2$ No
14. If No, why was your child not de-wormed?	_____ _____ _____
15. Did your baby receive Vitamin A?	☐$_1$ Yes **(If Yes,Skip to Q 17)** ☐$_2$ No
16. If No, why has your child not received Vit A?	_____ _____ _____
17. What feeds is your baby receiving currently?	☐$_1$ Breastmilk ☐$_2$ Formulae ☐$_3$ Mixed feeding (breastmilk and formulae) ☐$_4$ Porridge
18. What formulae you are feeding?	☐$_1$ Nan ☐$_2$ Nan HA ☐$_3$ Infarcare ☐$_4$ S26 ☐$_5$ Pelagon ☐$_5$ Isomil ☐$_5$ Lactogen ☐$_7$ Other
19. What supplementary feeds are you feeding?	☐$_1$ Veg only ☐$_2$ Meat only ☐$_3$ All foods ☐$_4$ Other _____ _____ _____

APPENDIX H: 12 MONTH NUTRITION QUESTIONNAIRE

Date:_____/_____/_____ Day month year	MID:_____	CID:_____ Gender: M/F (Circle)

1. Has the baby ever been breastfed	☐1 Yes ☐2 No	
2. Was the baby breastfed yesterday during the day or at night	☐1 Yes ☐2 No	
3. Is the baby receiving any other liquids	☐1 Yes ☐2 No	
4. What other liquids did the baby have yesterday during the day or at night. 5. If YES for B,C or D, ask how many times the previous day or night		
A. Plain water	☐1 Yes ☐2 No	
B. Infant formula eg Infacare/ Nan	☐1 Yes ☐2 No	5. B. ☐☐ TIMES
C. Fresh milk or powdered milk eg Nespray, Klim, Growing Up milks. NOT Cremora or Ellis Brown	☐1 Yes ☐2 No	C. ☐☐ TIMES
D. Maas or yoghurt eg Danone. NOT Yogisip	☐1 Yes ☐2 No	D. ☐☐ TIMES
E. Rooibos or other tea	☐1 Yes ☐2 No	

6. During the past month how often has the baby had the following foods or drinks:

Food or Drink	Never /once a month	Twice a month	Once a week	2-4/ week	Once a day	2-4/day
A. 100% fruit juice						
B. Mix juice eg Fusion, Jumbo, Daly's, Oros, Halls						
C. Fizzy drinks eg Coke, Cooee, Busta						
D. Sweets						
E. Crisps eg Snax, NikNaks, Bhamfoqo or slap (fried) chips, vetkoek/amagwinya						

7. Please describe everything the baby ate yesterday during the day or night, whether at home or outside the home.
   a) Think about when the baby first woke up yesterday. Did he/she eat anything at that time? If YES: Please tell me everything he/she ate at that time. PROBE: Anything else? UNTIL CAREGIVER SAYS NOTHING ELSE. IF NO, CONTINUE TO QUESTION b).
   b) What did the baby do after that? Did she/he eat anything at that time? If YES: Please tell me everything she/he ate at that time. PROBE: Anything else? UNTIL CAREGIVER SAYS NOTHING ELSE.

   REPEAT QUESTION b) UNTIL CAREGIVER SAYS CHILD WENT TO SLEEP UNTIL THE NEXT DAY.
   IF CAREGIVER MENTIONS MIXED DISHES LIKE SAUCE OR STEW/CURRY, PROBE: What ingredients were in that mixed dish? PROBE: Anything else?
   AS THE CAREGIVER RECALLS FOODS, UNDERLINE THE FOOD IN THE TABLE BELOW AND CIRCLE 1 IN THE COLUMN NEXT TO THE FOOD GROUP. IF THE FOOD IS NOT LISTED IN ANY OF THE FOOD GROUPS BELOW, WRITE THE FOOD IN THE 'OTHER FOODS' BOX.

   ONCE THE CAREGIVER FINISHES RECALLING FOODS EATEN, READ EACH FOOD GROUP WHERE 1 WAS NOT CIRCLED. ASK THE FOLLOWING QUESTION, CIRCLE '1' FOR YES AND '2' FOR NO:
   Yesterday during the day or night did the baby eat/drink any (FOOD GROUP ITEMS)?

OTHER FOODS: PLEASE WRITE DOWN FOODS THAT ARE MENTIONED BUT ARE NOT ON THE LIST BELOW:

NO.	FOOD GROUPS	CODING CATEGORIES			
			YES	NO	
A	Porridge, Phuthu, Samp, bread, rice, pasta, ijeqe, noodles		1	2	
B	Infant cereal eg Purity cereal, Nestum, Cerelac etc		1	2	
C	Potato, white fleshed sweet potato,amadumbe		1	2	
D	Pumpkin, butternut, orange fleshed sweet potato, carrots		1	2	
E	Dark green leafy vegetables eg Spinach, imifino		1	2	
F	Ripe mangoes , pawpaws		1	2	
G	Any other fruit or vegetables		1	2	
H	Liver, kidney, heart, or other organ meats		1	2	
I	Beef, lamb, pork, chicken or fish		1	2	
J	Polony or Vienna sausages		1	2	
K	Eggs		1	2	
L	Dried beans (Ubhontshisi) or peanut butter		1	2	
M	Cheese, maas or yoghurt		1	2	
N	Oil or margarine		1	2	
O	Purity Jar foods . Flavour_____		1	2	

8. How many times did the baby eat solid, semi solid, or soft foods other than liquids yesterday during the day or at night? ☐☐ TIMES

APPENDIX I: MACE UKZN BREC APPROVAL

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

15 June 2015

Dr. Rajen Naidoo
Discipline of Occupational and Environmental Health
University of KwaZulu-Natal
Howard College
Durban
4041

PROTOCOL: The Mother and Child in the Environment (THE MACE STUDY). REF:BF263/12.

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	22 April 2015
Expiration of Ethical Approval:	21 April 2016

I wish to advise you that your application for Recertification dated **06 May 2015** for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The approval will be **ratified** by **a** full Committee at a meeting to be held on **14 July 2015.**

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

APPENDIX J: KZN DEPARTMENT OF HEALTH APPROVAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM 048/13
Enquiries : Mr X Xaba
Tel : 033 – 395 2805

Dear Dr R. Naidoo

Subject: Approval of a Research Proposal

1. The research proposal titled **'The mother and Child in the environment (The MACE Study)'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Ante-natal clinics at Wentworth, Prince Mshiyeni, King Edward VIII hospital, Addington, Mahatma Gandhi and King George V hospitals.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 12/04/2013

APPENDIX K: MACE CONSENT FORM

a) The Mother and child in the Environment (MACE cohort)Consent Form

Mother Identification no._____

1. **Title of research project**
   Mother and Child in the Environment

2. **Purpose of the research**
   The University of KwaZulu-Natal, Department of Occupational and Environmental Health is conducting this study of the health effects of air pollution in the eThekwini municipality. In this study we want to learn whether pollution has an effect on the unborn child, and whether these effects are likely to create health problems for the child has he or she grows. The study has the support of the local industry, community groups concerned about these sorts of health problems, and the City Health Department. We are studying this community because of its location near sources of air pollutants such as oil refineries and because of health concerns expressed by teachers and students at schools and by the larger community. The purpose of the study is to figure out whether any health problems are being caused by air pollution in the community, its effect on pregnant mothers and the unborn child, and, if so, to make recommendations to improve the situation.

3. **Description of the research project**
   If you agree to participate, you will be interviewed during your pregnancy, and we will ask you to provide us with blood samples while you are pregnant. We will do a home visit during the pregnancy and conduct indoor air assessments in your home. We will also ask you to allow us to take a sample of blood from the birth cord after the delivery of your child, and for permission to take a spot of blood from your newborn baby and follow-up the baby until 5 years old.

   Interviews: You will be interviewed by a trained interviewer from our research team. This interview will be done at the clinic, in the complete privacy of one of the consulting rooms at the clinic. You can choose the language in which you will prefer to be interviewed. During the course of this interview you will be asked many questions about your pregnancy, diet, work exposures etc. You may refuse to answer any questions that you feel uncomfortable about answering. If during the course of the interview, you wish to stop and continue at a later stage, you may do so.

   Blood tests: Trained medical staff will take samples of blood from you. This blood will be analysed for your body's reaction to external environmental factors (referred to as "oxidative stress"), assessing your allergy status and potential, vitamin analysis and genetic analysis will be done on the blood prenatally. 4 Tablespoons of blood will be taken during your pregnancy. A home visit to assess indoor air quality will be done twice during the study period. You will be informed prior to these visits. Similarly, a sample of blood will be taken from the umbilical cord, once this is removed after the birth of your child and a heel prick will be done on your baby within 6 days of birth. These tests will then be repeated. Only these specified tests will be conducted on this blood sample. We will store a sample of your DNA so that we can check for new genes that are discovered in the future that might make your child more likely to develop lung conditions such as asthma. The total amount of blood taken will not exceed more than four tablespoons. YOU WILL NOT BE INJECTED WITH ANY SUBSTANCE/MEDICATION.

   During the 5 year follow-up period we will assess your child at different time points where lung functions tests will be performed and blood samples will be taken. However you will be informed in advance when this will occur.

4. **Confidentiality of information collected**
   The interview and blood test information we collect about you is completely confidential and will never be seen by anyone other than the personnel conducting the study without your written consent. The results of the overall study, which will be made available to the local government and the community, will presented so as to protect the identity of individual participants.

5. **Risks and discomforts of the research**
There are no risks from the interviews and the blood tests.

6. **Expected benefits to you and to others**
You will be given a written copy of all your test results along with an explanation of what they mean. You may wish to show these to your doctor if you are having any problems. If problems are found in the community, we may be able to make specific recommendations about air pollution to improve the situation. What we learn from this study may help to protect people in South Africa and other parts of the world from problems caused by air pollution.

7. **Costs to you resulting from participation in the study**
The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

8. **Voluntary nature of participation**
You are free to decline to participate or to withdraw from the study at any time without suffering any penalty or disadvantage.

9. **Contact person**.
You may contact the Project Manager, Kareshma Asharam (telephone no. 031-2604523 or the Principal Investigator of the study Dr Rajen Naidoo (telephone no: **031 260 4385**) for answers to further questions about the research. For reporting of complaints/problems, you may contact the Biomedical Research Ethics Committee, whose details are shown below:

Contact details of BREC Administrator or Chair –
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
University of KwaZulu-Natal
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

10. **Consent of the participant**
I have read the information given above, or it has been read to me. I understand the meaning of this information, Dr./Mr./Ms. _____
has offered to answer any questions concerning the study. By signing this form, I hereby consent to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

11. **Documentation of the consent**
One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

_____ _____
Printed name of participant Signature, Mark, or Thumb Print

_____
_____
Interviewer's name (Print) Signature

DATE: _____

b) The Mother and Child in the Environment Study
Informed Consent: Genetic Study (Mother and baby)

MID:: _____

If you do not understand any part of this document, please ask for an explanation from a member of the study team before giving consent

Your consent is required so that you and your baby can participate in a research study conducted by the Discipline of Occupational and Environmental Health, at the University of KwaZulu-Natal. We are investigating genetic influences on the health effects of environmental pollution. This may reveal information about improving the health of children living in polluted areas.

I hereby consent to donate a small quantity (5mls) of my babies cord blood at delivery and a heel prick to be done on my baby that will to be used in this project. This will be taken by trained medical staff and has minimal risks to us both.

I am aware that the genetic information arising from this project will not be used for any discriminatory purpose. I am aware that the results and information that is collected from my baby and I will remain completely confidential. The overall results of the project will be made available to the community but my name and identity will be protected and not revealed. I know that this study is done at no cost to me.

I am aware that the biological samples taken during this study will be stored at the Department of Medical Biochemistry at the Nelson R Mandela Medical and may be used for future genetic research. This research will restricted to respiratory and other health effects linked to air pollution.

I am also aware that should I change my mind, this consent can be withdrawn at any time without suffering any penalty or disadvantages.

Consent: I have read and understood the meaning of this form.
Dr./Mr./Ms. _____ has offered to answer any questions concerning the study. I hereby consent to participating in the study.

Documentation of the consent. One copy of this document will be kept together with our research records on this study. A second copy will be given to you to keep.

Contact person. You may contact Project Manager, Kareshma Asharam (telephone no. 031-2604523 or the Principal Investigator of the study Dr Rajen Naidoo (telephone no: **031 260 4385**) for answers to further questions about the research. For reporting of complaints/problems, you may contact the Biomedical Research Ethics Committee, whose details are shown below:

Contact details of BREC Administrator or Chair –
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Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

_____ _____ _____
Printed name of participant Signature

_____ _____
Interviewer's name (Print) Signature

c) The Mother and Child in the Environment Study
Storage of Genetic Samples (Mother and baby)

MID:_____

If you do not understand any part of this document, please ask for an explanation from a member of the study team before giving consent

I am aware that my and my baby's genetic material will be securely stored at the Department of Medical Biochemistry at the Nelson R Mandela Medical (UKZN) and used in future research. The genetic material collected from me and my baby will be used for the purpose of respiratory related research only by members of the study team. I am aware that my and my baby's genetic material will be stored for a maximum period of 5 years after which it will be destroyed and disposed using methods of medical waste disposal.

For future genetic research, please sign one of the two clauses below.

Should the analysis of my or my baby's genetic material result in financial gain / probability of financial gain for the researchers concerned,

1.1. I hereby waive (give up) my right to being informed of the results of such research and accompanying financial gain / probability of financial gain which may arise from such research, and forfeit (give up) any legal claim to such genetic material and any financial benefit which may arise from research on such material. By doing so, I do not waive my rights to confidentiality related to the research on, and subsequent publication of, (if any), such material.

Signed _____ OR

1.2. I hereby declare my intention to being informed of the results of such research and do not waive (give up) my rights to such genetic material and any financial benefit which arise / may arise from research on such material. By doing so, I do not waive (give up) my rights to confidentiality related to the research on, and subsequent publication of (if any), such material.

Signed _____

I am ensured privacy and confidentiality in the recording, storage and release of any future research resulting from use of my and my baby's genetic material. No predictive disease tests on stored samples may be linked to me without my consent. I am also aware that should I change my mind, this consent for storage of genetic material can be withdrawn at any time without suffering any penalty or disadvantages.
I have read and understood this form.
Dr./Mr./Ms. _____ has offered to answer any questions concerning the study. I hereby consent to participating in the study.

Documentation of the consent. One copy of this document will be kept together with our research records on this study. A second copy will be given to you to keep.

Contact person. You may contact Project Manager, Kareshma Asharam (telephone no. 031-2604523 or the Principal Investigator of the study Dr Rajen Naidoo (telephone no: **031 260 4385**) for answers to further questions about the research. For reporting of complaints/problems, you may contact the Biomedical Research Ethics Committee, whose details are shown below:
Contact details of BREC Administrator or Chair –
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
University of KwaZulu-Natal Research Office, Westville Campus
Govan Mbeki Building Private Bag X 54001, Durban, 4000 KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609 Email: BREC@ukzn.ac.za

_____ _____ _____
Printed name of participant Signature

_____ _____
Interviewer's name (Print) Signature

APPENDIX L: MSc DIETETICS BREC APPROVAL

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 January 2016

Mrs PA Jarvie (822827665)
Discipline of Dietetics and Human Nutrition
School of Agriculture, Earth and Environmental Science
jarviep@ukzn.ac.za

Dear Mrs Jarvie

Protocol: In utero and post-natal factors associated with stunting and overweight in infants enrolled in the Mother and Child in the Environment (MACE) study.
Degree: MSc
BREC reference number: BE479/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 09 November 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 19 January 2016 to queries raised on 24 December 2015 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval.**

This approval is valid for one year from **28 January 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **08 March 2016.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: veldmanf@ukzn.ac.za
cc postgrad: manjoom@ukzn.ac.za