

Efficacy of lipid nutrient supplements on growth and micronutrient status in infants

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University

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DEDICATION

To my wife, Forget, and our children, Delbert and Gabriella;

words cannot express how much I love you all.

“We are guilty of many errors and many faults but our worst crime is abandoning the children, neglecting the fountain of life. Many of the things we need can wait. The child cannot. Right now, is the time his bones are being formed, his blood is being made, and his senses are being developed.

To him we cannot answer 'Tomorrow.' His name is 'Today.'”

Gabriela Mistral (Lucila Godoy Alcayaga) (Chilean poet, 1889-1957)

DECLARATION

The PhD Promoters, and the Principal Investigators for the Tswaka study give permission to the candidate, Mr Tonderayi Matsungu, to include the article(s)/manuscript(s) as part of this PhD thesis for examination purposes. The first author was responsible for most stages of each manuscript, including literature searches, the collection of data, statistical analysis and interpretation of results and the writing of the articles.

The promoter(s) and principal investigator(s) contribution (advisory and supportive) was kept within reasonable limits, thereby enabling the candidate to submit this thesis for examination purposes.

This thesis, therefore serves as fulfilment of the requirements for the PhD degree in Nutrition at the Centre of Excellence for Nutrition, School of Physiology, Nutrition and Consumer Sciences, Faculty of Health Sciences at the North-West University, (Potchefstroom Campus), South Africa.



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“Science is thought to be a process of pure reductionism, taking the meaning out of mystery, explaining everything away, concentrating all our attention on measuring things and counting them up. It is not like this at all. The scientific method is guesswork, the making up of stories. The difference between this and other imaginative works of the human mind is that science is then obliged to find out whether the guesses are correct, the stories true. Curiosity drives the enterprise, and the open acknowledgement of ignorance.”

Lewis Thomas, *Sierra Club Bulletin*, March/April 1982, p. 52

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God, The Almighty,

I thank God, The Almighty, for having made everything possible by giving me strength and courage to complete this work. Grace makes everything possible in the Shona language we say *“Mwari ndewe munhu wese.”* As the Holy Bible says in 2 Timothy 4:7, *“I have fought the good fight, I have finished the race, and I have kept the faith.”*

Nonetheless, the responsibility is entirely my own if there should still be any errors or inadequacies that may remain in this work.

“We are like dwarfs on the shoulders of giants, so that we can see more than they, not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and raised up by their giant size”

John of Salisbury (1120-1180)

ABSTRACT

Efficacy of lipid nutrient supplements on growth and micronutrient status in infants

Background: Stunting or linear growth failure occurs as result of poor maternal nutrition and suboptimal health and/or feeding practices. Small-quantity lipid-based nutrient supplements (SQ-LNS) are promising home fortificants used to prevent stunting and micronutrient deficiencies in children aged 6-23 months, but evidence so far is inconclusive.

Aim: The study investigated the factors associated with stunting at age 6 months and evaluated the efficacy of SQ-LNS A and SQ-LNS B on linear growth and iron status among 6-month old infants followed for 6 months.

Methods: Baseline variables were explored to determine the factors associated with stunting at age 6-months old. The randomised controlled trial (RCT) was conducted between September 2013 and July 2015 in North West Province, South Africa. The infants were randomised to SQ-LNS A with essential fatty acids (EFAs), SQ-LNS B with EFAs, docosahexaenoic acid (DHA) and arachidonic acid (ARA), *phytase*, powder milk and lysine and a control group. Home visits to monitor adherence and morbidity were conducted weekly. Length-for-age (LAZ), weight-for-length (WLZ) and weight-for-age (WAZ) z-scores (WHO classification) were determined at baseline and age 8, 10 and 12 months. Blood samples (4ml) were analysed for haemoglobin (Hb), plasma ferritin (PF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), alpha-2 acid glycoprotein (AGP), at baseline and end. Socio-economic, breastfeeding and complementary feeding practices were assessed by questionnaire. Generalised linear, quantile, linear splines and logistic regression analysis were used.

Results: At baseline stunting, underweight, wasting and overweight affected 28.5%, 11.1%, 1.7% and 10.1% of infants respectively. Stunting was the predominant form of malnutrition and was inversely associated with birth weight (kg) (OR 0.12, 95% CI 0.07 to 0.20, $P<0.001$), and maternal height (cm) (OR 0.94, 95% CI 0.91 to 0.98, $P=0.001$) while male sex was significantly associated with higher odds of stunting (OR 1.73, 95% CI 1.10 to 2.70, $P=0.017$). The RCT show that SQ-LNS B had overall positive effects on LAZ ($P=0.036$) compared to the control, this was mainly driven by significant intervention effects at age 8 and 10 months, as at age 12 months (trial end) the intervention effect disappeared. There were positive effects on Hb for both SQ-LNS A ($P=0.027$) and SQ-LNS B ($P=0.005$) groups. The results also show that the risk of anaemia, iron deficiency

and iron deficiency anaemia was significantly lower in SQ-LNS A and SQ-LNS B groups compared to the control.

Conclusions: The cross-sectional results showed that stunting (28.5%) was associated with lower birth weight, shorter maternal height and male sex. The intervention showed that both SQ-LNS A and SQ-LNS B did not show an effect on growth at 12 months of age. However, SQ-LNS B showed better linear growth at age 8 and 10 months old compared to the control. In addition, both SQ-LNS A and SQ-LNS B significantly decreased the risk of infants for iron deficiency and iron deficiency anaemia. This trial was registered at <http://clinicaltrials.gov> as INTC01845610.

Keywords: stunting, lipid-based nutrient supplements, complementary feeding, anaemia, iron deficiency, ferritin, linear growth, malnutrition, South Africa.

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LIST OF ABRREVIATIONS AND ACRONYMS

ABR/ACR	Description
$\leq$	equal to or less than
$\geq$	Equal to or more than
AE	adverse event
AGP	alpha-1 acid glycoprotein
AI	adequate intake
ALA	alpha linolenic acid
ANOVA	analysis of variance
ARA	arachidonic acid
ATP	adenosine tri phosphate
BMI	body mass index
CF	complementary foods
CFS	complementary food supplements
CRP	c-reactive protein
DHA	docosaheaxaenoic acid
DRI	dietary reference intake
EAR	estimated average requirement
EDTA	ethylene-diamine-tetra-acetic
EFA	essential fatty acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaeonic acid
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FA	fatty acids
FAO	food and agricultural organisation
FFQ	food frequency questionnaire
FSP	fortified fat-based paste made from soy
FTL	field team leader

FTU	phytase unit
FW	fieldworker
g/dl	grams per decilitre
GH	pituitary growth hormone
GLA	gamma linolenic acid
HACCP	hazard analysis and critical control points
Hb	haemoglobin
HC	head circumference
HCZ	head circumference z-scores
HIV+	human immunodeficiency virus positive
IGF-1	insulin-like growth factor-1
IUGR	intrauterine growth restriction
IV	intravenous
LA	linoleic acid
LA	linoleic acid
LAZ	Length-for-age z-scores
LAZ	length-for-age z scores
LBW	low birth weight
LCPUFA	long chain polyunsaturated fatty acids
LNS	lipid-based nutrient supplement
MDGs	millennium development goals
mg	milligram
ml	millilitres (.001 litres)
MNP	micronutrient powder
SAMRC	South African Medical Research Council
MUAC	mid-upper arm circumference
MUACZ	mid upper arm circumference z-scores
MUFA	monounsaturated fatty acid
NCDs	non-communicable diseases
NDOH	National Department of Health
NFCS	National Food Consumption Survey

NFCS-FB-I	National Food Consumption Survey Fortification Baseline
NWU	North-West University
PACTR	Pan African Clinical Trials Registry
PER	protein efficiency ratio
PUFA	polyunsaturated fatty acids
RBC	red blood cell
RDA	recommended dietary allowance
RNI	recommended nutrient intake
SAE	serious adverse event
SDG	sustainable development goals
SMB	safety monitoring board
SPSS	Statistical Package for the Social Sciences
SUN	scaling up nutrition
UNICEF	United Nations Children's Fund
WAZ	Weight-for-age z-scores
WHA	World Health Assembly
WHO	World Health Organisation
WLZ	Weight-for-length z-scores

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Background, rationale and aims of the study



Top Left the Author at busy at the field station and **Top Right** the Author with PhD Promoter (Prof Marius Smuts).

Bottom Left the Author (back right) with fellow PhD candidates from CEN and **Bottom Right** the Author (front) with field team at the study site.

CHAPTER 1: BACKGROUND, RATIONALE AND AIMS OF THE STUDY

1.1 Background

Since the 1992 International Conference on Nutrition (ICN) and the 1996 World Food Summit there have been significant achievements, but slow and uneven progress in reducing hunger and malnutrition (WHO & FAO, 2014; Haddad *et al.*, 2015). According to the International Food Policy Research Institute (IFPRI), the prevalence of those suffering from undernutrition has declined, but remains unacceptably high, affecting over 800 million people, with the majority in South Asia and Sub-Saharan Africa (Haddad *et al.*, 2015).

However, stunting still affects approximately 178 million children under the age of five years worldwide and an important proportion of these children are in Sub-Saharan Africa and South-central Asia (Black *et al.*, 2008). It is projected that in 2025 about 127 million children under five years will be stunted if no meaningful action is taken to prevent stunting within the 1000-day window period (WHO, 2014a). Available evidence shows that growth faltering or intrauterine growth restriction (IUGR) occurs in the uterus (WHO, 1995), while stunting starts around age six months, as a result of the transition from exclusive breastfeeding to consumption of complementary foods of poor nutritional quality (WHO & UNICEF, 2003; WHO, 2013a).

Interventions to improve maternal nutrition, promotion of exclusive breastfeeding for the first six months of life, promotion of appropriate complementary feeding with continued breast feeding for children aged six to 23 months, and prevention and control of infections can help address stunting, wasting and micronutrient deficiencies in children (Bloem, 2013; WHO, 2013a). The actions to address multiple forms of malnutrition have been illustrated in the Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition endorsed by the World Health Assembly (WHA) in 2012 (WHO, 2014a). This plan emphasises the importance of addressing malnutrition among women, infants and young children (WHO & FAO, 2014). This strategy helps to break the cycle of malnutrition if the focus is on the first 1000 days of life (WHO, 2014a). There are proven strategies to improve maternal, infant and young child nutrition within the first 1000 days, as

outlined in the WHO package of effective direct nutrition interventions (WHO, 2013a) and strategies for infant and young child feeding (IYCF) (WHO & UNICEF, 2003; WHO, 2013a).

Therefore, investments should be made in nutrition-specific interventions in three key areas and these should be scaled up: optimal IYCF, addressing micronutrient deficiencies and improving maternal nutritional status before and during pregnancy (WHO & FAO, 2014).

In South Africa poverty and poor nutritional intake remain significant causes of high levels of poor infant and child physical growth and development (Chopra *et al.*, 2009). There is a high prevalence of stunting and underweight, particularly among black and coloured children in South Africa (Kruger *et al.*, 2012) and this is associated with corresponding high rates of nutrition-related infant morbidity and mortality (NDOH, 2008; Labadarios *et al.*, 2011; Shisana *et al.*, 2014). In addition, micronutrient deficiencies are also of public health importance, particularly insufficient vitamin A, iron and zinc (Faber, 2005; Shisana *et al.*, 2014). This reveals the presence of a cycle of malnutrition in South Africa, which undermines the development of a healthy productive population. The malnutrition cycle begins when women are unable to meet their nutritional requirements during pregnancy, resulting in poor birth outcomes (Tsimbos & Verropoulou, 2011; Yadav *et al.*, 2011; Adair *et al.*, 2013).

Complementary feeding interventions are usually targeted at the age range of six to 24 months, which is the time of peak incidence of growth faltering, micronutrient deficiencies and infectious illnesses in developing countries (Dewey & Adu-Afarwuah, 2008; WHO, 2013a). Such feeding interventions are the most effective and sustainable intervention of choice compared to programmes targeting individual nutrient deficiencies. Studies in South Africa have found that complementary feeding starts early, consists of a small variety of low-energy-dense foods and is associated with growth faltering and increased infections (Faber *et al.*, 1997; Chopra *et al.*, 2009). Therefore in South Africa there is a need to have nutrition intervention programmes that are effective at community level (Chopra *et al.*, 2009).

The National Food Consumption Survey (NFCS) of 1999 reported that in general one out of two children in South Africa had an intake of less than half of the recommended level of energy and a number of important micronutrients (Labadarios *et al.*, 2007). This pattern of intake is still worst in

low-income areas and directed the focus of the present study in the Jouberton area (Klerksdorp) in the North West Province of South Africa.

1.2 Rationale for the study

There is evidence that 45% of the deaths for children under five years can be traced back to undernutrition, this translates to 3.1 million of the 6.9 million child deaths in 2011 (Black *et al.*, 2013). In addition, intra uterine growth restriction (IUGR) and suboptimum breastfeeding are linked to more than 1.3 million deaths, or 19.4% of all deaths of children younger than 5 years, representing 43.5% of all nutrition-related deaths (Black *et al.*, 2013). Therefore, addressing under nutrition and micronutrient deficiencies for children under five years, should be scaled up in low to middle income countries as it will result in achievement of the sustainable development goals (SDGs).

The predominantly cereal- and legume-based diets in most developing countries do not supply adequate daily nutrient supply for optimum growth and development of infants and young children (Maleta *et al.*, 2015). Six months is the age when the introduction of complementary feeding is recommended (WHO & UNICEF, 2003; WHO, 2013a). Children aged six to eleven months consuming an average amount of breast milk need only 200 to 300 additional kcal from complementary foods (Agostoni *et al.*, 2008; Agostoni *et al.*, 2009).

Several complementary food supplements (CFS), including small quantity lipid-based nutrient supplements (SQ-LNS), have been developed and tested worldwide. The LNS efficacy trials (Adu-Afarwuah *et al.*, 2007; Adu-Afarwuah *et al.*, 2008; Dewey & Adu-Afarwuah, 2008; Iannotti *et al.*, 2014; Hess *et al.*, 2015) produced mixed results, but also demonstrated the potential for LNS to contribute to improved nutrition among infants during the complementary feeding period. However, some questions still need to be answered. The contribution of SQ-LNS to the prevention of growth faltering and the improvement of the micronutrient status of infants is only beginning to be understood. A study in Burkina Faso (Hess *et al.*, 2015) reported that SQ-LNS supplementation resulted in improved growth and reduced stunting, wasting and anaemia in children. In contrast, the findings of a trial in Malawi (Maleta *et al.*, 2015) failed to support the hypothesis that SQ-LNS supplementation for infants and children promotes linear growth or prevents growth faltering

between six and 18 months of age. Therefore, further evidence on the benefits and/or absence of adverse effects is needed to assess the feasibility of LNS in different community settings.

Compared to rice or wheat, maize has higher levels of phytates (Sandberg, 2002), which bind trace elements such as iron and zinc, and inhibit their absorption. In South Africa, maize is widely used as complementary food, yet the impact of LNS in the context of a maize-based diet has not been well investigated. Therefore, testing the efficacy of these newly developed SQ-LNS that contain both docosahexaenoic acid (DHA) and arachidonic acid (ARA) and one with added phytase (150 FTU) to improve iron and zinc bioavailability (Troesch *et al.*, 2011) is of particular interest in the context of South Africa, of which maize is the staple food.

The present study assessed the efficacy of low-calorie SQ-LNS in improving infant growth and micronutrient status. Low-energy SQ-LNS will complement and not replace breastfeeding and leave space for consumption of an additional variety of local foods, including animal-source foods, fruit and vegetables. If found to be effective, these SQ-LNS will cost less to produce when compared to high-energy-containing supplements with similar formulations and will thus be more affordable for low-income consumers.

1.3 Research aim

The aim of this study is to investigate the effects of a fortified fat-based paste containing essential fatty acids (SQ-LNS A) and a fortified fat-based paste containing powder milk, DHA, ARA, lysine and *phytase* in addition to essential fatty acids (SQ-LNS B) on the growth and micronutrient status of infants from age six to 12 months compared to a control group. The control group became a delayed intervention group after the efficacy trial in a post-intervention study that compared the effects of early vs. late introduction of SQ-LNS on linear growth outcome.

1.4 Specific objectives

The specific objectives of the study were as outlined below:

1. Investigate the prevalence and factors associated with stunting in infants at age 6 months,
2. Investigate the effect of SQ-LNS A and SQ-LNS B on growth in infants from age 6-12 months old when compared to controls,

3. Evaluate the effect of SQ-LNS A and SQ-LNS B on anaemia and iron status of infants from age 6-12 months old when compared to controls.

1.6 Hypothesis

The central hypothesis was that infants randomised to receive low-energy; fortified small quantity lipid-based nutrient supplements (SQ-LNS A and SQ-LNS B), will improve linear growth, anaemia and iron status compared to a control group.

1.7 Randomisation of infants to the three groups

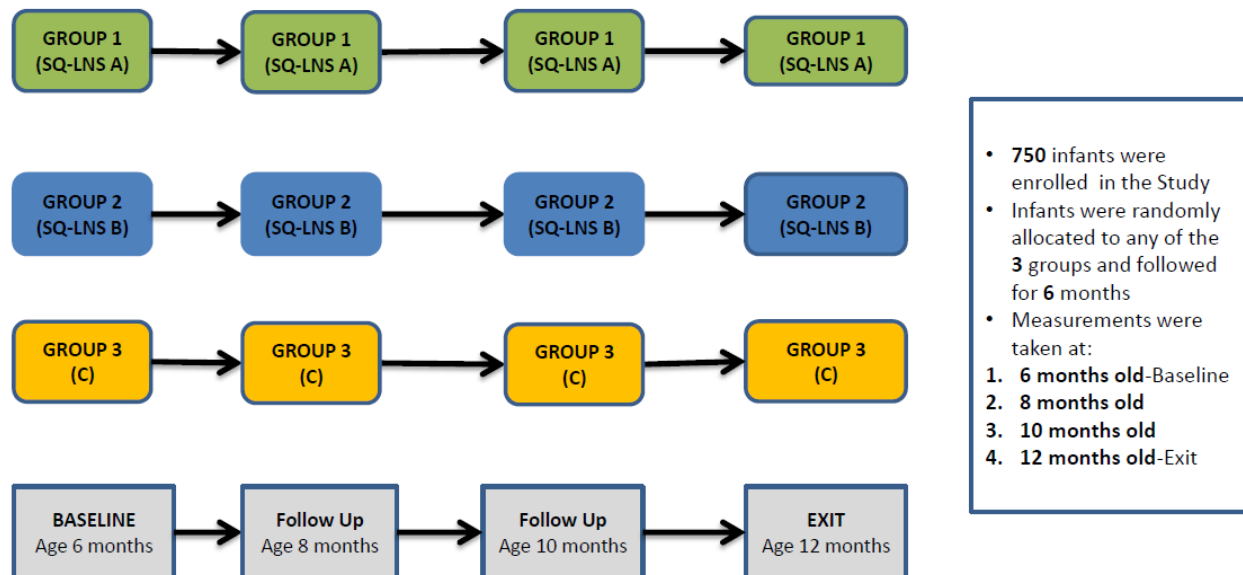


Figure 1. 1: Randomisation of infants to the three groups

SQ-LNS = Small quantity lipid-based nutrient supplements. **SQ-LNS A** is a fortified fat-based paste (without DHA, ARA); **SQ-LNS B** is a fortified fat-based paste containing essential fatty acids (with DHA and ARA and *phytase*). Both products are made from soy. **C** = the control. This group received their LNS supplements after the end of the trial (age 12-18 months, delayed intervention). Group 1 = SQ-LNS A, Group 2 = SQ-LNS B and Group 3 = control.

The study design for the current study was a randomised, controlled, parallel-group efficacy trial with 750 infants enrolled and randomly allocated to one of the three groups (SQ-LNS A, SQ-LNS B and control). The trial duration was six months with enrolment/baseline at age six months old and exit from the trial at 12 months old. Anthropometric measurements were taken when infants were six months, eight months, 10 months and 12 months old, whereas blood samples were taken only at

six months and 12 months of age. The study was embedded in a larger research project entitled “Randomized controlled trial in South Africa comparing the efficacy of complementary food products on child growth” (*Tswaka study*).

This study was carried out in the peri-urban Jouberton and Alabama areas of the greater Matlosana (Klerksdorp) municipality, Dr Kenneth Kaunda district, North West Province of South Africa. The 2011 South African census revealed that the greater city area of Klerksdorp had a population of 186 515, with a racial makeup of black African (74.0%), coloured (6.4%), Indian/Asian (1.3%), white (18.0%) and other (0.3%). The first languages were Setswana (42.7%), Afrikaans (23.8%), Xhosa (11.7%) and Sotho (10.7%) (SSA, 2012). In 2012 South Africa had a total population of 52 386 000, life expectancy at birth for men and women of 56 and 62 years, respectively, and an under-five mortality rate (per 1000 live births) of 47 for both sexes (WHO, 2013b).

Ethical approval was obtained from the Ethics Committees of North West University (NWU) (NWU-00001-11-A1) and the South African Medical Research Council (SAMRC), (EC-01-03/2012). After institutional ethical approval, the project was reviewed by local authorities. The provincial, district and community’s approval to conduct the study was sought through an engagement process with relevant stakeholders

1.8 Structure of the thesis

This thesis consists of six main parts as indicated in **Figure 1.1**. The six chapters for the thesis and the contents of each chapter are outlined as follows: **Chapter 1** covers the introduction, problem statement and aims of the study, **Chapter 2** explores the literature review. **Chapter 3**. Research article entitled: *The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community*. **Chapter 4**. Research article entitled “*Effects of small-quantity lipid-based nutrient-supplements on growth in 6- to 12-month-old South African infants: a randomised control trial*”. **Chapter 5**. Research article entitled *Effect of small-quantity lipid-based nutrient supplements on anaemia and iron status in 6-month-old infants from a peri-urban South African community: a randomised controlled trial*. **Chapter 6** presents the summary, conclusions and recommendations. Chapter 6 is followed by a list of appendices.

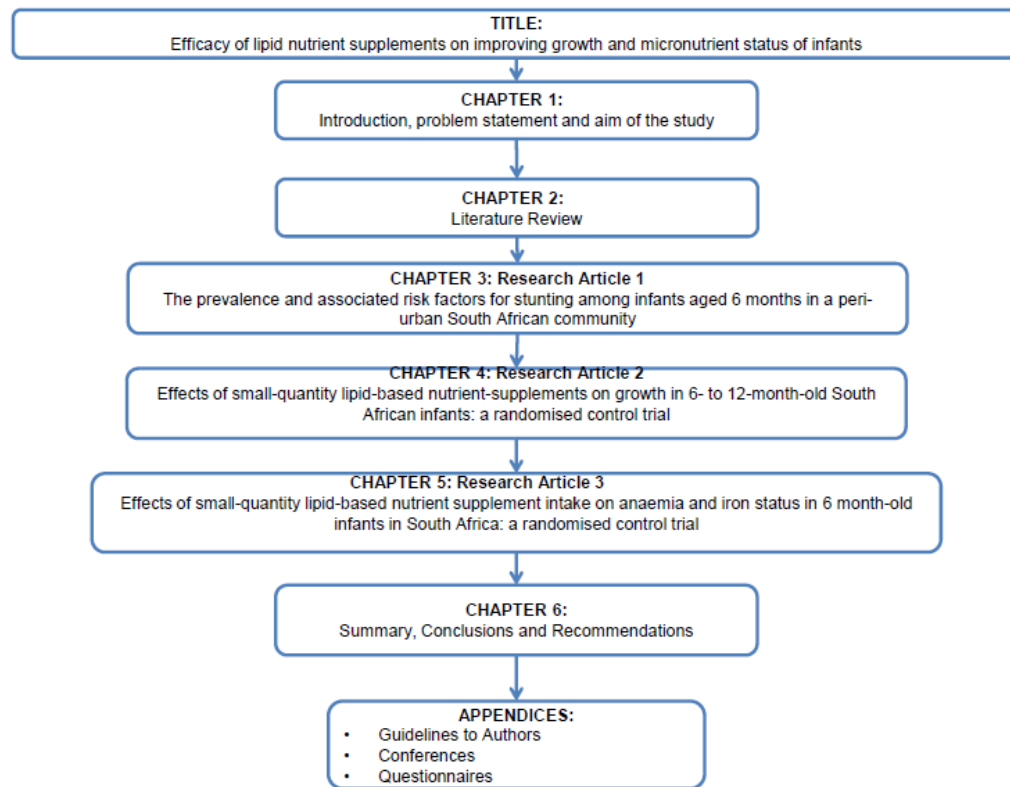


Figure 1. 2: Structure of the thesis

This thesis is submitted in article format, as approved by the senate of the North-West University (NWU) (Potchefstroom Campus), according to the 2012 Guidelines for Postgraduate Studies. Chapters 1, 2 and 6 have been written according to the prescribed reference style of the NWU. The articles have been prepared according to the guidelines to authors for publication in accredited peer-reviewed journals; *Maternal and Child Nutrition* for article 1 (appendix F) and *The American Journal of Clinical Nutrition* for articles 2 and 3 (appendix G). For the purpose of uniformity and examination, the font and spacing is kept the same throughout the thesis. The tables and figures are also placed in between the text and not at the end of each article. The results of the research articles in Chapters 3-5 are presented and interpreted in each chapter respectively.

1.9 Research team

Table 1. 1: List and responsibilities of the research team

Members	Respective roles
Tonderayi. M Matsungu¹	PhD student and co-study coordinator of the Tswaka trial and was involved in the protocol development of this study. Involved in study design protocol writing, ethical approval process, supervising field data collection, data quality control, qualitative data analysis and statistical analysis, interpretation of results and writing of the literature review and leading author on all manuscripts.
Prof. Marius Smuts¹	Guidance regarding study design, protocol development, review of dissertation components, interpretation of results and co-author of all manuscripts. Principal investigator of Tswaka trial. Promoter of PhD thesis.
Prof. Salome Kruger¹	Guidance regarding study design, protocol development, review of dissertation components, interpretation of results and co-author of all manuscripts. Provided training on anthropometric measurements and analysis; standardisation of anthropometry measurement. Co-promoter of PhD thesis.
Prof Mieke Faber²	Co-principle investigator of the Tswaka study; training, guidance on data collection, quality control and analysis of dietary and feeding practices, academic input and review of manuscripts and co-author of all manuscripts.
Marinel Rothman¹	Co-study coordinator of Tswaka trial supervising field data collection and data quality control of feeding practices and psychomotor development assessments. Co-author of all manuscripts.
Carl Lombard²	Statistician who provided guidance regarding statistical analysis for manuscripts. Co-author on two manuscripts.

Notes:

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²The South African Medical Research Council (SAMRC), Non-communicable Diseases Research Unit, P.O Box 19070, Tygerberg 7505, South Africa.

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Top Left the Author (right) with Co-Principal Investigator (Prof Mieke Faber). **Top Right** the Tswaka Study Nurse and Site Manager (Sr. Linda Lemmer)

Bottom Left the field team busy with paper work (source data) at the study site and **Bottom Right** the field team at the study site.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Worldwide malnutrition has been responsible, directly or indirectly, for 60% of the 10.9 million deaths annually among children under five years. Well over two thirds of these deaths, which are often associated with inappropriate feeding practices, occur during the first year of life (WHO & UNICEF, 2003). Furthermore, undernutrition has been shown to be associated with more than one third of the global disease burden for children under five years worldwide (WHO & UNICEF, 2003).

Malnutrition at age six to 23 months can result in long-term physical and mental damage that may be irreversible. This is an important stage in the development of children, as they are susceptible to infections and at high risk of developing undernutrition, usually in developing countries (Bhutta *et al.*, 2013). Therefore, nutrition interventions during this period may help to prevent these negative outcomes. Appropriate IYCF should help to improve child survival and promote healthy growth and development life (WHO & UNICEF, 2003). This calls for evidence-based, innovative and affordable interventions and political commitment in order to address this complex problem of malnutrition.

In order to improve growth and development of children from birth to 23 months old, it is necessary to supplement the traditional complementary foods with appropriate nutrients. This early childhood stage offers a window of opportunity for nutrition-related interventions (SUN, 2012). It is during this period that interventions will have the greatest impact (Branca & Ferrari, 2002; Jones *et al.*, 2003; Chopra *et al.*, 2009). In South Africa, this was also evident from food consumption surveys (1999 and 2005) (Labadarios *et al.*, 2005; Labadarios *et al.*, 2007; NDOH, 2008). In addition, studies in South Africa have shown that the majority of infants receive foods other than breast milk even before the age of four months (Bergström *et al.*; Steyn *et al.*, 1993), and that cereal (maize and wheat) based complementary foods are also given to infants as early as two to three months of age (Bourne *et al.*, 2007). Interestingly, when compared to rice or wheat, maize has higher levels of phytates, which reduce the bio-availability of trace elements such as iron and zinc (Sandberg, 2002).

This translates to increased risk of developing micronutrient deficiencies for children in South Africa.

Therefore, the current efficacy trial investigated the impact of lipid nutrient supplements (SQ-LNS) on improving growth and the micronutrient status of children from age six to 12 months in the context of a maize-based diet. This impact has not been well investigated. Although the efficacy trials (Adu-Afarwuah *et al.*, 2007; Adu-Afarwuah *et al.*, 2008; Dewey & Adu-Afarwuah, 2008) of CFS in the form of SQ-LNS have demonstrated potential for this new category of products to contribute to improved nutrition among infants during the complementary feeding stage (six to 23 months), many questions still remain. The current efficacy trial on newly developed high-quality products that contain DHA and ARA to improve vision and cognition (Hoffman *et al.*, 2009), and *phytase* (150 FTU) to improve iron and zinc bioavailability (Troesch *et al.*, 2011), will be of particular interest and justified in the context of South Africa's maize-based complementary feeding diet. There is evidence (Adair *et al.*, 2013; Bhutta *et al.*, 2013; UNICEF, 2014) that improvements in nutrition will result in decreased undernutrition and help improve the lives of children worldwide. Therefore, this current study is an important milestone in efforts to address problems of undernutrition in infants in South Africa and beyond.

This chapter covers an extensive review of literature on early child growth and development and the role of LNS as a class of CFS on child growth and development. The review starts with an overview of recommended IYCF practices in the context of the first 1000-days initiative. This leads to a description of LNS and studies done on LNS in developing countries. The rest of the review addresses contextual issues concerning LNS and the growth and development of children, SQ-LNS and the micronutrient status of children, LNS and IGF-1, the relationship between socio-demographic factors and anthropometric indicators of children, the relationship between pro-inflammatory cytokines and markers of future cardiovascular risk, C-reactive protein (CRP) and anthropometric indicators of children. A summary and recommendations are outlined at the end of the review.

2.2 The first 1000 days and scaling up nutrition

The period from conception through pregnancy and a child's first two years of life, also referred to as the "*first 1000 days of life*" is considered a "*critical window of opportunity*" for prevention of

growth faltering (WHO & UNICEF, 2003). Optimal nutrition during this period helps to shape a healthier and more prosperous future for a child through reduced morbidity and mortality and a lower risk of chronic diseases, and it promotes healthy growth and development for children.

Optimal breastfeeding is so critical that it could save about 800 000 lives of under-five children every year. In countries where stunting is highly prevalent, promotion of breastfeeding and appropriate complementary feeding prevents about 220 000 deaths among children under five years of age (UNICEF, 2011). The *Innocenti Declaration on the Protection, Promotion and Support of Breastfeeding* (UNICEF, 2006), the 2002 Global Strategy for Infant and Young Child Feeding (WHO & UNICEF, 2003) and the millennium development goals (MDG), which have now paved the way for sustainable development goals (SDG), recognise that inappropriate IYCF practices, sub-optimal or no breastfeeding and inadequate complementary feeding are significant threats to child health (Haddad *et al.*, 2015; Wüstefeld *et al.*, 2015).

Therefore, it is now generally agreed that improving nutrition during the critical 1000-day window is one of the best investments that can be made to achieve lasting progress in improving infant and young child nutrition and has a long-term impact on global health and development (Gruszfeld & Socha, 2013; WHO & FAO, 2014; Haddad *et al.*, 2015). The *Lancet maternal and child nutrition series* published in 2008 and 2013 (Bhutta *et al.*, 2008; Black *et al.*, 2008; Victora *et al.*, 2008; Adair *et al.*, 2013; Bhutta *et al.*, 2013; Black *et al.*, 2013; Gillespie *et al.*, 2013; Nabarro, 2013; Ruel & Alderman, 2013) emphasised the adoption of cost-effective interventions within the 1000-day window of opportunity in order to yield high returns for cognitive development, individual adult earnings and economic growth. This policy brief became known as the scaling up nutrition (SUN) framework (Nabarro, 2013), which set the stage for the transformation that is now happening in global nutrition. SUN is a unique movement launched in 2010, on the principle that all people have a right to food and good nutrition. Its principles are outlined in the SUN Framework and Road Map (SUN, 2012). The movement includes national governments, civil society, the United Nations, donors, businesses and researchers working to improve nutrition worldwide. The SUN approach focuses on increasing people's access to affordable nutritious food and other determinants of nutritional status such as clean water, sanitation, healthcare, social protection and initiatives to empower women.

This initiative was enshrined in the *UNICEF Strategy for improved nutrition of children and women in developing countries* (UNICEF, 1990) and summarised in the form of the UNICEF conceptual framework to deal with the causes of malnutrition and death. The framework shows that malnutrition has multiple causes (UNICEF, 1990) and as such requires multifaceted interventions that encompass a broad array of stakeholders, not just from the nutrition and health fields. Recognising this, the SUN Movement (SUN, 2012) and the Lancet Series (Ruel & Alderman, 2013) recommend the implementation of nutrition-specific interventions and/or nutrition-sensitive approaches to combat undernutrition, particularly in women and children. Therefore, in order to address growth faltering and micronutrient deficiencies and break the cycle of undernutrition, efforts should be aimed at reducing the number of infants with low birth weight (LBW) and of children who are stunted, wasted or deficient in micronutrients, and prioritise the nutrition of all women of child-bearing age (SUN, 2012). The conclusions of a new *Lancet series on breastfeeding* were that breastfeeding can prevent the deaths of 823 000 children and 20 000 mothers each year. This offers economic benefits, has the potential to save US \$300 billion (Lancet, 2016) and adds more support to continued focus on the 1000-days window period.

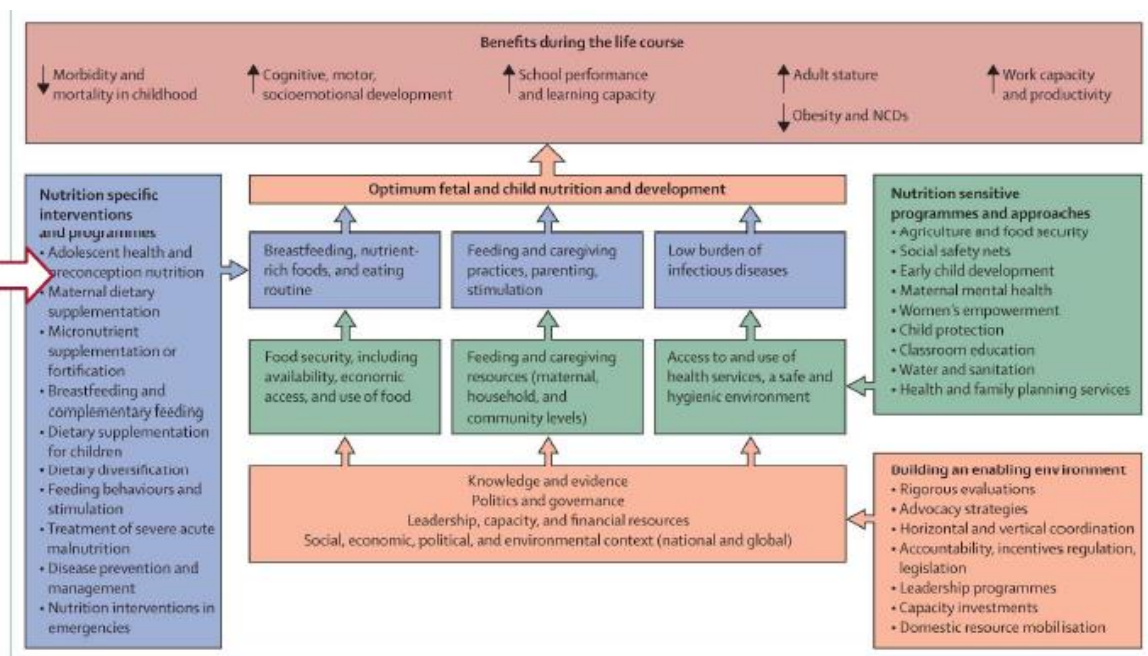


Figure 2. 1: Framework for actions to achieve optimum foetal and child nutrition and development¹

¹Source: (Bhutta et al., 2013).

Nutrition-specific interventions (Ruel & Alderman, 2013) target the immediate causes of malnutrition and include: (1) support for exclusive breastfeeding up to six months of age and continued breastfeeding, together with appropriate and nutritious food, up to two years of age; (2) fortification of foods; (3) micronutrient supplementation; and (4) treatment of severe acute malnutrition (SAM). Nutrition-sensitive approaches address the underlying causes of malnutrition and include: (1) agriculture: making nutritious food more accessible to everyone, and supporting small farms as a source of income for women and families; (2) clean water and sanitation: improving access to reduce infection and disease; (3) education and employment: making sure children have the energy that they need to learn and earn sufficient income as adults; (4) health care: improving access to services to ensure that women and children stay healthy; (5) support for resilience: establishing a stronger, healthier population and sustained prosperity to endure emergencies and conflicts better; and (6) women's empowerment: at the core of all efforts, women are empowered to be leaders in their families and communities, leading the way to a healthier and stronger world (SUN, 2012). These interventions can be scaled up and effectively implemented if countries put the right policies in place, promote collaboration with partners to implement programmes with shared nutrition goals, and mobilise resources to scale up nutrition effectively, with the core focus on empowering women.

2.3 Child growth: six-to-12-month period

The health and wellbeing of children are determined by the interaction between genes and the external environment (adequacy of nutrition, safety of the environment, social interaction and stimulation) (Singh, 2004). Therefore, nutritional status as reflected in the ability of children to achieve optimal growth and development can be used as an indicator of socio-economic development and advancement of societies (De Onis, 2008). It is therefore becoming increasingly important to monitor the quality of life of infants and young children. Furthermore, child growth is now regarded as an indicator of the physical well-being of children because poor feeding practices and infections are major factors that affect physical growth and mental development in children worldwide. Poor child growth is the consequence of a range of factors that are closely linked and multifaceted (UNICEF, 1990). This calls for holistic interventions or strategies to address problems of growth faltering in children (De Onis, 2008).

Inadequate food intake in the first two years of life is responsible for stunting and poor weight development in millions of children worldwide (De Onis & Who, 2006). Specifically, impaired growth is a response to limited nutrient availability or utilisation at the cellular level. In the past growth faltering was thought to be associated only with inadequate protein-energy intake. There is increasing evidence of the important role that micronutrient (iron, zinc and vitamin A) deficiency plays in child growth and development (Rivera *et al.*, 2003). This is usually a result of poor breastfeeding and complementary feeding practices for infants and young children in most developing countries.

In children, the three most commonly used indicators to assess growth status are weight-for-age (WA) (underweight), length/height-for-age (stunting), and weight-for-length/height (WL) (wasting and overweight) (De Onis, 2008). Other commonly used anthropometric indicators include mid-upper arm circumference (MUAC), body mass index (BMI), skinfolds, and head circumference. According to (De Onis, 2008), the major outcomes of poor growth in children can be classified based on mortality, morbidity (incidence and severity), and psychological and intellectual development (De Onis, 2008).

Stunting is the most prevalent condition affecting infants and young children. It normally has origins from pregnancy as a consequence of intrauterine growth restriction (IUGR that manifests as an LBW baby. IUGR or growth failure can occur *in utero*, as early as the second trimester (WHO, 1995), and stunting most often emerges at about six months when children enter into the complementary feeding period (six to 23 months), characterised by poor complementary feeding practices and increased exposure to infections (Caulfield *et al.*, 2006).

Stunting is part of a complex syndrome also involving reduced immune function, retarded development and impairment of cognitive function, as well as other metabolic disturbances that might affect the individual either immediately or in the long term (Branca & Ferrari, 2002). The occurrence of IUGR is normally higher in stunted young pregnant women and this creates an inter-generational cycle of stunting (**Figure 2.2**)

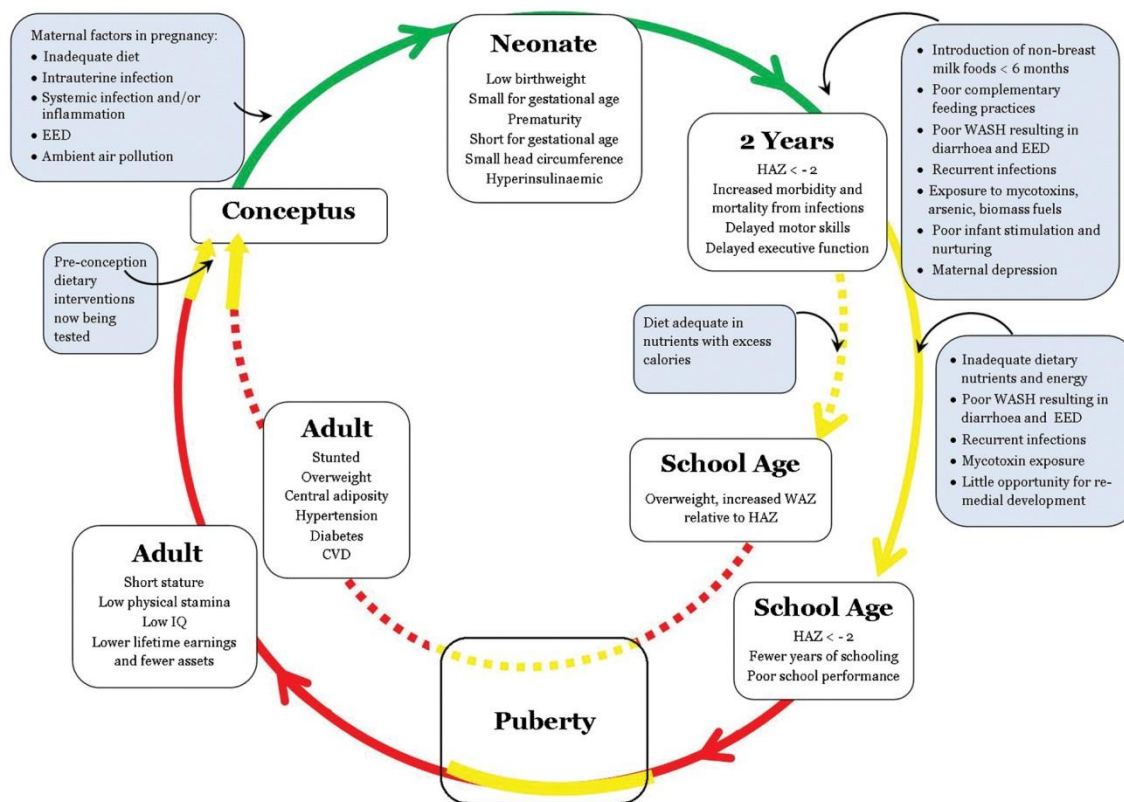


Figure 2. 2: The poor nutrition cycle¹

¹Figure 2.2 shows; “the stunting syndrome, the green pathway denotes the period between conception and 2 years (‘the first 1000 days’) when stunting and probably all associated pathology is most responsive to, or preventable by, interventions. The yellow pathway denotes periods between age 2 years and mid-childhood and during the adolescent growth spurt when some catch-up in linear growth may occur, though effects during these periods on other components of the stunting syndrome (e.g. cognition and immune function) are less clear. The short yellow pathway before Conceptus reflects evidence that dietary interventions targeting stunted women during the pre-conception period improve birth outcomes. The red pathway denotes periods when the stunting syndrome appears unresponsive to interventions. Blue boxes list age-specific causative or aggravating factors. White boxes describe common age-specific outcomes. Between 2 years and adulthood, the pathways diverge to denote: dashed line, a stunted child whose environment becomes more affluent with abundant access to food, causing excessive weight gain; solid line: a stunted child whose environment remains resource-constrained/food insecure” (Prendergast & Humphrey, 2014).

It has been shown that there are differences in growth patterns for breastfed vs. bottle-fed infants and young children (Agostoni *et al.*, 1999) and these are more pronounced during the period birth to six months of life. Furthermore, from six to 12 months of life, breastfed infants showed a progressive decline in growth rate (particularly in those breastfed for 12 months), while in the formula-fed group there was a continuous increase in the growth rate (Agostoni *et al.*, 1999). These findings were also reported earlier (WHO, 1995), where major growth differences in terms of

reduced WA and WL accretion of 12-month-old breastfed infants had been recorded also in the second part of the year.

Although remarkable increases in body size and length occur during organismal growth, very little is known about the mechanism of the growth process (Sarma, 2009). Linear growth occurs through a process of cell proliferation, the addition of new cells to the growth plate of the bone and hypertrophy, resulting in the expansion of the growth plate (Loveridge & Noble, 1994). Human growth rate is determined by a complex interaction of physical, endocrine and nutritional factors, of which growth hormone (GH) and nutrition are the key determinants of child growth (Lampl & Johnson, 1997). GH releasing hormone (GHRH) stimulates GH production from the pituitary gland into the bloodstream. This in turn stimulates IGF-1, which has growth-promoting effects of GH resulting in growth (GH-IGF-1 axis) (Bogin, 1999). IGF-1 itself stimulates synthesis of collagen and proteoglycans (Rivera *et al.*, 2003) and these physiological functions explain the role of IGF-I in linear growth.

Balanced age-appropriate diets that include the essential nutrients, especially micronutrients, have a positive influence on child growth (Branca & Ferrari, 2002; Rivera *et al.*, 2003; Ekweagwu *et al.*, 2008; Ramakrishnan *et al.*, 2009; Sarma, 2009; Souganidis, 2012). A deficiency of growth-promoting macro- and micronutrients (Rivera *et al.*, 2003) can result in growth faltering, usually presenting in the form of stunting. This usually occurs because of the impact of a deficiency of specific nutrients on the GH-IGF-1 axis.

2.4 Complementary feeding: six-to-23-month period

Complementary foods are consumed to complement breastfeeding, from the age of six to 23 months, and at this stage the nutrient requirements for infants are very high as a result of rapid growth and development. This is exacerbated by the presence of infections and illness (WHO, 2013a). This means that breast milk or formula milk alone will no longer be adequate to meet nutritional requirements. However, it is a big challenge to meet the additional nutrient needs via complementary feeding. In particular, there is increased demand for energy, protein, iron, zinc and vitamins A and D. Children enter into the complementary feeding period (six to 23 months) when they start eating in a manner consistent with the culture into which they are born, ensuring adequate nutrition.

The global strategy on IYCF (WHO & UNICEF, 2003) is based on the achievements of particularly the Baby-friendly Hospital Initiative (1991), the International Code of Marketing of Breast-milk Substitutes (1981) and the Innocenti Declaration on the Protection, Promotion and Support of Breastfeeding (1990). National government policies on nutrition and child health should consequently be grounded in this strategy and be consistent with the principles of the World Declaration and Plan of Action for Nutrition that was agreed on at the ICN of 1992 (FAO, 1992). In addition, attention should be paid to including guidelines on ensuring appropriate feeding of infants and young children in exceptionally difficult circumstances, and the need to ensure that all health services protect, promote and support exclusive breastfeeding and timely and adequate complementary feeding with continued breastfeeding (WHO & UNICEF, 2003).

In most developing countries, the prevalence of undernutrition and micronutrient deficiencies is high among infants and young children aged six to 24 months (Bhutta *et al.*, 2013; Prentice *et al.*, 2013). This period of transition from exclusive breastfeeding to consuming a wide range of foods in addition to breast milk is the longest part of the “1000-days” window of opportunity for preventing and addressing undernutrition. Promoting adequate nutrition during this period should be a major global health priority (Dewey & Brown, 2003; WHO & UNICEF, 2003). The ideal complementary feeding diet should include different types of animal-source and/or fortified foods per week in addition to plant-source foods (WHO & UNICEF, 2003); unfortunately, such an ideal diet is usually too costly for families in developing countries.

As a result undernutrition gets worse, particularly from age six to 24 months, since most children in developing countries are fed nutritionally inadequate cereal-based diets, which are deficient in energy and micronutrients (particularly iron and zinc) and have poor mineral bioavailability (Dewey, 2013). Therefore, strategies for achieving adequate nutrition for infants and young children in developing countries have to address the challenge of meeting nutrient needs from largely cereal-based diets. The recommendations for improving the nutritional status of children in this period of complementary feeding are to feed them locally available micronutrient-rich foods (dietary diversification) and to encourage local production of low-cost, industrially processed, fortified cereal-based complementary foods (Nestel *et al.*, 2003; Dewey, 2013).

2.5 Nutrients and their effects on growth of infants and young children six to 23 months

From six months of age the WHO recommends that a combination of solid foods and breast milk should be given in order to complement nutrients that are insufficient if supplied only with human milk, particularly energy, protein, iron, zinc and fat-soluble vitamins (WHO, 1981; WHO & UNICEF, 2003).

The complementary feeding period is a high-risk period for developing malnutrition if the complementary feeding practices are poor, as infants and toddlers have high nutritional requirements relative to body size, but consume small amounts of food. Therefore, from six to 23 months of age there is a need for nutrient-dense complementary foods to prevent growth faltering and micronutrient deficiencies. It is commonly assumed that an increased need for energy and protein is the primary factor dictating complementary feeding. Some micronutrients are likely to become limited sooner than the macronutrients and deficiency of these nutrients will consequently affect growth and result in growth faltering (Dewey, 2001). However, the effect of nutrients on linear growth remains largely controversial, with conflicting evidence (Branca & Ferrari, 2002; Rivera *et al.*, 2003; Ekweagwu *et al.*, 2008; Ramakrishnan *et al.*, 2009; Sarma, 2009; Souganidis, 2012).

In general, linear growth, faltering is thought to be regulated by the expression of GH receptors on the growth plates. Stunting occurs as result of repeated insults to the growth plate, with reduced chondrocyte proliferation and maturation (Branca & Ferrari, 2002). It is hypothesised that this mechanism is dependent on the interaction between concurrent nourishment (**Table 2.1**) and the nominal growth rate set during pregnancy (Branca & Ferrari, 2002).

Table 2. 1: Classification of nutrients in relation to their effect on growth¹

Characteristics and effects on growth		
Type 1		Type 2
Initial normal growth		Growth retardation
Decreased tissue concentration		Normal tissue concentration
Specific deficiency signs		Absence of specific deficiency signs
Used in specific pathways		Ubiquitously used
Stored in the body		Limited body stores
Not interdependent		Control each other's balance
Little excretory control		Sensitive physiological control
Unlikely to influence linear growth		Likely to influence linear growth
List of nutrients for Type 1 and Type 2		
Iodine	Ascorbic acid	Energy
Iron	Vitamin A	Water
Copper	Vitamin E	Protein [nitrogen, essential amino acids; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine]
Calcium	Vitamin B12	
Manganese	Vitamin K	
Selenium	Vitamin D	
Thiamine		<i>Micronutriments :</i>
Riboflavin		Zinc, potassium, phosphorous, sulphur, magnesium

¹Adapted from (Branca & Ferrari, 2002)

Deficiencies of the type 1 nutrients result in clinical symptoms; these nutrients include vitamins, calcium, iron, iodine and selenium (Golden, 2009). Requirements for these nutrients are thought to be higher than recommended nutrient intakes (RNI) for children in low-income countries (Golden, 2009), while type 2 nutrients are those required for growth of lean tissues, such as sulphur (mostly from protein), magnesium, phosphorus, potassium and zinc (Golden, 2009). Zinc and magnesium are the only type 2 nutrients that have traditionally been included as fortificants in complementary foods or supplements (MIYCN, 2009). This may explain why previous complementary foods or supplements had no impact on growth. The inclusion of more Type 2 nutrients in newly formulated complementary food supplements such as LNS and micronutrient powders (MNP) should improve the outcomes.

The role of energy and proteins on physical growth is well established (Sarma, 2009), though there is still inconclusive evidence on the importance of micronutrients in enhancing the full growth potential (Branca & Ferrari, 2002; Rivera *et al.*, 2003; Ekweagwu *et al.*, 2008; Ramakrishnan *et al.*, 2009; Sarma, 2009; Souganidis, 2012). Therefore, the next sections will highlight the roles of essential fats or essential fatty acids (EFAs) and micronutrients (vitamin A, iron and zinc) that are linked to linear growth faltering that occurs in developing countries.

Poor dietary intake during childhood leads to undernutrition, which results in growth retardation, reduced work capacity and poor mental and social development (Souganidis, 2012). Therefore, the current efficacy trial used SQ-LNS, for “home-fortification” of local cereal-based foods as a daily ration of only 20g (four teaspoons). These SQ-LNS contain over 20 micronutrients including Zinc, iron and vitamin A, and essential fatty acids and a small amount of protein (Hess *et al.*, 2015). Small-quantity LNS provide less than 120 calories/day (Hess *et al.*, 2015). Therefore, these SQ-LNS are designed to enhance the nutrient content of local complementary foods, not to replace the local foods (Dewey & Arimond, 2012). This fact that SQ-LNS provide energy, protein, macro-minerals and essential fatty acids, in addition to micronutrients makes them superior over multiple micronutrient (MMN) supplements like micronutrient powders (MNP) to improve growth and development on infants and children.

2.5.1 Multiple micronutrient (MMN) supplements

Results on multiple micronutrient supplementation have also been inconclusive (Souganidis, 2012). This demonstrates that the prevention and reversal of stunting is considerably more complex than previously thought. Although micronutrient interventions have received much attention as a cost-effective and promising strategy to improve child health, their efficacy in improving child growth remains unclear. In a meta-analysis of single and multiple nutrient interventions to improve micronutrient deficiencies (Ramakrishnan, 2002) found no evidence of efficacy from single nutrient interventions including iron and vitamin A. This led to the shift towards multiple micronutrient supplementation approach (Souganidis, 2012) as this was assumed that it will be more significant than single nutrient supplementation in populations with multiple micronutrient deficiencies.

However, the results of multiple micronutrient intervention studies have also been inconclusive (Souganidis, 2012). This emphasises the need for new multifaceted approaches that also encompasses improving complementary feeding taking into account socioeconomic factors such as disease and poverty (Bhutta *et al.*, 2000) in order to address problems of undernutrition and growth faltering. This may lead to the development of LNS usually in form of SQ-LNS which have an advantage of providing energy, protein, macro-minerals and essential fatty acids, in addition to micronutrients (Hess *et al.*, 2015).

2.5.2 Key micronutrients in child growth

Worldwide, there are widespread deficiencies of micronutrients resulting in iron deficiency anaemia, iodine deficiency disorders and milder forms of vitamin A deficiency (Singh, 2004). This affects mostly women and children who usually suffer from multiple micronutrient deficiencies. In children micronutrients are required for promotion of physical growth, sexual maturation and psychomotor development (Singh, 2004), therefore deficiencies of these micronutrients will result in growth retardation and stunting. Key among them is the deficiencies of iron, zinc, iodine, vitamin A which are linked to growth faltering in children (Pedraza & de Queiroz, 2011).

Many trials are done on the effect of single-nutrient supplements on the nutrition status and growth and development of infants and children. In most studies supplementation with specific nutrients improved body stores of the nutrient. However, there are still many unanswered questions on the effect of micronutrient supplementation on functional outcomes such as growth, vision equity, morbidity or mortality, or cognitive and motor development (Allen & Gillespie, 2001). In this section the focus will be on the impact of supplements on growth, focussed on linear growth. The roles of selected micronutrients on child growth are summarised in **Table 2.2**.

Table 2. 2: Role of micronutrients in growth of children¹

Micronutrient	Role in growth
Vitamin D and Calcium	Deficiency affects bone development, which manifests as rickets
Potassium, Zinc, Copper	Deficiency disturbs the GH-IGF-1 system and affects growth
Manganese	Deficiency leads to skeletal abnormalities including retarded growth, which may be mediated through defects in proteoglycan physiology in the growth plate
Iron and Iodine	Help in cognitive development and growth
Vitamin A	Indirectly helps in growth
Zinc	Overall growth
Vitamin E	Muscle development

¹Table was adapted from (Sarma, 2009)

The WHO Expert Committee identified some “essential” micronutrients whose deficiencies are linked to linear growth faltering. These include iodine, zinc, selenium, copper, molybdenum,

chromium, vitamin A and calcium (WHO, 1996). Micronutrient deficiencies before and during pregnancy could result in foetal programming that leads to increased risk of stunting in infancy and childhood (Bhandari *et al.*, 2001). Although MMN supplementation is now considered to have greater impact on length compared to single micronutrients (Bhandari *et al.*, 2001), these findings are still too inconclusive to warrant adoption of recommendations at population level.

In summary, zinc and iron seem to have some effect on linear growth in deficient populations (Bhandari *et al.*, 2001). Therefore, zinc deficiency affects linear growth in children (Pedraza & de Queiroz, 2011), resulting in nutritional stunting. Despite the strong evidence linking zinc deficiency to stunting, the results of studies on zinc supplementation have been inconclusive (Brown *et al.*, 2002; Souganidis, 2012). In addition, more work can be done on assessing the impact of iron supplementation in non-anaemic children (Bhandari *et al.*, 2001).

On the contrary, despite numerous cross-sectional studies linking vitamin A deficiency to a greater risk of stunting, there is still inconclusive evidence on the causal relation between vitamin A deficiency and poor child growth (Bhandari *et al.*, 2001). Studies have found vitamin A supplementation to either improve linear growth (Arroyave, 1979; Muhilal *et al.*, 1988) or to have no significant effect (Lie *et al.*, 1993; Ramakrishnan *et al.*, 1995) on child growth. The evidence available on routine vitamin A supplementation suggests that this has little or no direct impact on linear growth. More work can be done on investigating the impact of vitamin A in children with clinical or biochemical deficiency (Bhandari *et al.*, 2001).

There are limited randomised iodine supplementation trials on linear growth, probably due to the adoption of universal salt iodization in deficient populations (Bhandari *et al.*, 2001). Nonetheless, evidence from observational studies seems to suggest that iodine deficiency is associated with linear growth retardation presenting in the form of cretinism (Zimmermann *et al.*, 2008). The results from copper supplementation are not sufficient to make conclusions on the impact of the micronutrient on linear growth (Bhandari *et al.*, 2001) and more studies are required in this area.

Evidence suggests that linear growth faltering is associated with MMN deficiencies, although some studies (Smuts *et al.*, 2005) observed no significant impact on growth through MNN supplementation. It is now generally agreed that MNN supplementation should be the preferred method of choice over single-micronutrient supplementation strategies (Allen & Gillespie, 2001) to

prevent growth faltering and improve the micronutrient status of infants and children. Although most of the effect on growth from SQ-LNS supplementation trials has been attributed to the protein, energy and micronutrient content, less is known about the impact of the EFA content of this regime of supplements.

2.5.3 Essential fatty acids

The complementary feeding diets for children in most low-income countries are deficient in EFAs or contain an inappropriate ratio of omega-6 (n-6), (linoleic) to omega-3 (n-3) (alpha-linolenic) fatty acids. Therefore consumption of foods rich in or supplemented with n-3 fatty acids should be recommended in these settings (MIYCN, 2009). Thus, oils containing both linoleic (n-6) and alpha linolenic acid (n-3), such as soybean or canola oil, are better choices over other oils such as corn, peanut and palm oil, which contain little n-3 fatty acids. Fatty fish and full-fat soy flour also contain high levels of alpha-linolenic acid (n-3).

EFAs are unsaturated fats that exists in the form of and/or can be derived from linolenic acid (LNA) and linoleic acid (LA) (Gropper & Smith, 2012). Although EFAs are important for human health, the human body cannot synthesise them and they have to be obtained from food. These EFAs are found in plant-based foods and are required to form omega-3 and omega-6 fatty acids, which are important in the normal functioning of all tissues of the body (Gropper & Smith, 2012). Omega-6 (n-6) fats are derived from LA and omega-3 (n-3) fats are derived from LNA. To promote optimal health the ratio of n-6 to n-3 fatty acids should be between 1:1 and 4:1 (Simopoulos, 2008). Omega-6 fatty acids include ARA and gamma-linolenic acid (GLA). The main n-3 is alpha-linolenic acid (ALA), which can be broken down to eicosapentaenoic acid (EPA) and DHA by the body (Connor, 2000). This makes ALA the only essential n-3 fatty acid (Gropper & Smith, 2012).

The deficiency of EFA leads to an array of nutrition-related complications in the liver and the kidneys, resulting in growth faltering, decreased immune function and dryness of the skin, particularly in children (Gropper & Smith, 2012). Therefore, EFA should be supplied in correct amounts from conception through pregnancy and infancy in the context of the 1000-days initiative. This explains the inclusion of long-chain polyunsaturated fatty acids (LCPUFAs), DHA (n-3) and ARA (n-6) as the main fatty acids in the supplements used in the current study of infants. Before birth, all of the n-6 and n-3 fatty acids accumulated by the foetus must originate from the maternal

circulation through placental transfer, and after birth all must be derived from the milk or formula diet and later from complementary foods.

Most of the studies (Leaf *et al.*, 1992; Auestad *et al.*, 2001; Innis, 2003; Agostoni, 2008) that were done on DHA and ARA have focused on their role in visual function and neurodevelopmental outcome, including cognitive development. The physiologic significance of a dietary supply of the LCPUFAs in the form of DHA and ARA has been an intensively studied area of infant nutrition (Innis *et al.*, 2002; Clandinin *et al.*, 2005; Hoffman *et al.*, 2008). Limited studies have evaluated the impact these LCPUFAs have on linear growth and/or prevention of growth faltering. These studies (Innis *et al.*, 2002; Clandinin *et al.*, 2005; Hoffman *et al.*, 2008), produced inconclusive results. However, there is growing evidence suggesting that low intake of EFAs is linked with growth faltering (Uauy & Castillo, 2003; Hoffman *et al.*, 2004; Hoffman *et al.*, 2008). It is hypothesised that increasing the EFA content of complementary foods will yield positive outcomes.

In several studies on term infants' researchers used soy-based formula supplemented with DHA and observed that these supported normal growth (Innis *et al.*, 2002; Clandinin *et al.*, 2005; Hoffman *et al.*, 2008). Therefore, the current study in which DHA and ARA were ingredients of the investigational SQ-LNS can help establish more conclusive findings about the roles of DHA and ARA supplementation on promoting growth in infants.

2.6 Nutritional status of children (<2 years) in South Africa

Nutritional status can be assessed by dietary, anthropometric, biochemical and clinical methods. Ideally, a combination of methods should be used when assessing nutritional status using standardised techniques. Faber also emphasised (Faber & Wenhold, 2007) that to do meaningful interpretation of nutritional assessment, indicators of socio-economic conditions, cultural practices, health statistics, food-related behaviour, and knowledge, attitudes and practices should be considered as well. In South Africa the most significant national data on the nutritional status of South African children is from the South African Vitamin A Consultative Group (SAVACG) study conducted in 1994 (Labadarios & Van Middelkoop, 1995) and the NFCS of 1999 (Labadarios *et al.*, 2001), the National Food Consumption Survey–Fortification Baseline (NFCS-FB) of 2005 (Labadarios *et al.*, 2007) and the 2012 South African National Health and Nutrition Examination Survey (SANHANES), (Shisana *et al.*, 2014).

The available evidence indicates that the most prevalent nutritional concerns in South Africa are stunting, moderate and severe acute malnutrition (MAM, SAM) and micronutrient deficiencies or ‘hidden hunger’. Micronutrient deficiencies, namely vitamin A deficiency, iron deficiency anaemia and zinc deficiency, tend to interact and cluster and often occur in the same child (Faber & Wenhold, 2007).

2.6.1 Anthropometric status of South African children (<2 years)

Growth trajectories are important in determining life course epidemiology and help to point out specific indicators of prenatal or childhood development, predicting potential determinants of adult health outcomes as well. In South Africa data from national studies revealed that at the national level there is a low prevalence of wasting (<5.0%), a low (<10.0%) prevalence of underweight, and a medium (10-19%) prevalence of stunting (Shisana *et al.*, 2014), according to criteria defined by the World Health Organisation (WHO) (WHO, 1995; De Onis & Who, 2006).

2.6.1.1 Stunting (height-for age/length-for age)

Moderate and severe stunting occurs when a child is below minus two SD from median length/height for age z-scores (LAZ) of a reference population. This is a common problem of the paediatric population in developing countries.

The SAVACG survey conducted in 1994 on children aged six to 71 months found that the prevalence of stunting was 16.7% (six to 11 months) and 23.4% (12 to 23 months) respectively (Labadarios & Van Middelkoop, 1995). It appears as if the incidence of stunting is high among the six-to-11-months age group. The 1999 NFCS on children aged one to nine years found a stunting prevalence based on secondary analysis of data from the 1999 NFCS reported of 24.4% among one-to-three-year-olds (Steyn *et al.*, 2005). It is difficult to determine a pattern over the years. However, there seemed to have been no meaningful impact on stunting in children younger than two years between 1994 and 1999 in South Africa.

Findings of the South African Demographic Health Survey (SADHS) of 2003 (NDOH *et al.*, 2007) revealed that 27.0% of children below five years were stunted. Then the 2005 NFCS-FB also reported that the incidence of stunting was highest in rural farming areas (24.5%), compared to tribal areas (19.5%) and urban informal areas (18.5%) and was highest in children one to three years

old (25.5%) (Labadarios *et al.*, 2007). Based on the 2012 SANHANES (Shisana *et al.*, 2014), in children from birth to 14 years the prevalence of stunting was highest among those from birth to three years of age, affecting 26.9% of boys and 25.9% of girls respectively.

In a review of studies and surveys that reported the prevalence of stunting from 1970 to 2013 in South Africa, researchers concluded that stunting remains high and prevalent in South Africa despite improvements in the economy and social and political commitment to address the problem (Said-Mohamed *et al.*, 2015). It is recommended that the National Department of Health (NDOH) should adopt a multi-sectoral and public health approach to improve the monitoring of stunting and focus interventions on the first 1000-days window of opportunity in order to improve the nutritional status of pregnant women and promote appropriate infant-feeding practices (Said-Mohamed *et al.*, 2015).

2.6.1.2 Wasting (weight-for height)

Wasting (moderate and severe) occurs when a child falls below minus two SD from the median weight for height z-scores (WLZ) of the reference population and is one of the widely-used indices for describing the prevalence of malnutrition in childhood. The SAVACG survey among children aged six to 71 months found a prevalence of wasting of 3.3% (six to 11 months) and 3.6% (12 to 23 months) respectively (Labadarios & Van Middelkoop, 1995). The prevalence was reported as 3.4% for children aged one to three years in the 1999 NFCS (Labadarios *et al.*, 2001). Wasting based on the findings of the 2003 SADHS was reported as 5.0% for children below five years in South Africa (NDOH *et al.*, 2007). Then the 2005 NFCS-FB revealed a wasting prevalence of 4.5% (Labadarios *et al.*, 2007) among children of one to nine years in South Africa. Based on the 2012 SANHANES (Shisana *et al.*, 2014) in South Africa the prevalence of wasting and severe wasting is 3.8% among children from birth to three years, with boys being more wasted (thinner) than girls. Because of differences in age groups and references used in the different studies, it is difficult to outline a trend in wasting. Nonetheless, the findings show that for South African children below two years the prevalence of wasting is low, based on WHO criteria.

2.6.1.3 Underweight (weight-for age)

Children are classified as moderately underweight when they are below minus two SD from median weight for age z-scores (WAZ) of a reference population; severely underweight if below minus

three SD from median WAZ of reference population. The SAVACG for children aged six to 71 months found a prevalence of underweight of 7.1% and 9.0% among children aged six to 11 months and 12 to 23 months respectively (Labadarios & Van Middelkoop, 1995). The 1999 NFCS on children aged one to nine years found a prevalence of 11.4% among children one to three years old (Steyn *et al.*, 2005). This revealed a slight increase when compared to the SAVACG results. The 2003 SADHS reported that 12% of South African children below five years were underweight (NDOH *et al.*, 2007). The 2005 NFCS-FB, on the other hand, presented a prevalence of underweight of 11.4% among one-to-three-year-olds (Labadarios *et al.*, 2007). Then the 2012 SANHANES-1 (Shisana *et al.*, 2014) reported a prevalence of underweight of 8.2% among children from birth to three years of age, stating that boys were more underweight than girls. This may indicate that the prevalence of underweight decreased over the years from 2005 to 2012. A reduction in underweight should have a substantial impact on child mortality (Nannan *et al.*, 2007) and this may indicate an improvement in the child health situation in South Africa.

2.6.1.4 Overweight and obesity

Overweight and obesity occur in children when their weights are above plus 2 SD of the median weight for length z-scores (WLZ) and obese when their weights are above plus 3 SD of the median WLZ. The 1999 NFCS in South Africa reported overweight and obesity in 23.7% among children one to three years of age (Labadarios *et al.*, 2001). The next NFCS of 2005 revealed that overweight affected 10% (based on international BMI cut-offs) children of one to nine years and was highest (5.5%) in urban formal areas, while 4% were obese (Labadarios *et al.*, 2007). The prevalence of overweight in SANHANES-1 among two-to-five-year-olds was higher (18.2% vs 10.6%) than that of children of one to three years old in the NFCS–2005 and that of obesity in the SANHANES-1 was about the same as in the NFCS–2005 (4.7% vs 4.5%), (Shisana *et al.*, 2014).

In general, it was also observed that the overweight and obesity rates were significantly higher among girls than boys and more pronounced in urban areas and among rural communities. In addition, although there has always been a problem of undernutrition among South African children, the findings from SANHANES-1 seem to indicate an upward trend in the prevalence of overweight and obesity, which is reminiscent of a country in transition. This notion is supported by (Bourne *et al.*, 2002), who indicated that South Africa is considered to be one of the countries in Sub-Saharan

Africa that is undergoing rapid demographic and nutritional transition. As such one expects the overweight and obesity rates to increase slowly with continued high rates of undernutrition.

2.6.1.5 Combined stunting and overweight (double burden of malnutrition)

In South Africa observations by several researchers (Mamabolo *et al.*, 2005; Steyn *et al.*, 2005; Timaeus, 2012; Kimani-Murage, 2013) have indicated the co-existence of under- and over-nutrition in South African children. There is evidence that South Africa is indeed a country in transition. This is usually characterised by moderate stunting co-existing with overweight and obesity, particularly among girls (Kimani-Murage, 2013). Research suggests that patterns of under- and over-nutrition in South African children are changing and indicates the occurrence of the early stages of a complex nutritional transition. Therefore, it may be necessary to review the Integrated Nutrition Programme (INP) of the Department of Health to be prepared for the double burden of malnutrition and shift the focus from undernutrition to monitoring overweight/obesity as well in order to reduce the risk of developing non-communicable diseases in adulthood.

2.6.1.6 Summary

In order to compare the SANHANES-1 with regional and global data, the prevalence of stunting, wasting and underweight was calculated for children under five years; the respective values were 21.6%, 2.5%, 5.5% (Shisana *et al.*, 2014). Compared to the 2005 NFCS-FB (Labadarios *et al.*, 2007), there was a slight increase in stunting, but a clear decrease in wasting and underweight among children younger than five years in South Africa. In the global context, the prevalence may be classified as of medium severity for stunting and low for wasting and underweight (WHO, 1995; WHO & UNICEF, 2003).

2.6.2 Micronutrient status of South African children (<2 years)

The deficiency of micronutrients in developing countries remains a problem of public health significance. This occurs mainly as a result of inadequate food intake, poor dietary quality, poor bioavailability (foods containing inhibitors, poor mode of preparation, and nutrient-nutrient interactions), and/or underlying infections and disease. Worldwide deficiencies of vitamin A, iron and iodine remain significant public health concerns. It is important also to recognise other important micronutrient deficiencies such as zinc and folate; these usually present as multiple micronutrient malnutrition (MMM) in one individual.

Africa has a high prevalence of micronutrient deficiencies, with iron deficiency anaemia and vitamin A deficiency being of major public health relevance (Muthayya *et al.*, 2013). The combined deficiencies of vitamin A and zinc caused the largest burden of disease, estimated to be responsible for 0.6 million and 0.4 million deaths respectively, and 9% of global childhood disability adjusted life years (DALYs) (Black *et al.*, 2008). Therefore, sustained effort is needed to reduce their burden further. On the contrary, combined iron and iodine deficiencies were linked to fewer child deaths, and accounted for about 0.2% of global childhood DALYs (Black *et al.*, 2008). This low burden can be attributed to successful interventions, such as the routine vitamin A supplementation via the WHO Expanded Programme on Immunisation (EPI) and universal salt iodisation (WHO *et al.*, 2007; WHO & FAO, 2014), but more work still needs to be done.

In South Africa, there is a crucial need for updated monitoring and surveillance data on micronutrient deficiencies via the INP of the NDOH in order to improve the efficiency of intervention programmes. This can also be used to advocate for increased commitments to scale up effective nutrition interventions by national government.

2.6.2.1 Vitamin A deficiency

Vitamin A deficiency (VAD) is a public health problem worldwide, affecting young children and pregnant women. Every year, VAD may contribute to 1.3-2.2 million preventable deaths in children younger than five years from infections (WHO, 2009). Furthermore, VAD is the major cause of blindness among young children, with an estimated 500 000 children going blind every year; 20-70% (WHO, 2009) of these children die within months of the onset of blindness.

The incidence of VAD among South African children from birth to five years was 34.7% (serum retinol <20 micrograms/dl), based on findings from the 1995 SAVACG survey (Labadarios & Van Middelkoop, 1995). In the 2005 NFCS-FB, VAD was found in 63.6% of children one to nine years old (Labadarios *et al.*, 2007). The prevalence of VAD according to the 2012 SANHANES-1 showed that 43.6% of two to five-year-old children were vitamin A deficient (Faber *et al.*, 2014; Shisana *et al.*, 2014). This indicates a decrease in deficiency levels probably due to the micronutrient fortification programme introduced in 2003 by the South African government (NDOH, 2008).

Micronutrient malnutrition appeared to be widespread, with rural areas affected more than urban areas. The national vitamin A supplementation programme for children and the food fortification

programme introduced in South Africa in 2003 may have helped to decrease morbidity and mortality related to VAD. Continued monitoring of the effectiveness of these interventions is strongly recommended in South Africa (Nojilana *et al.*, 2007).

2.6.2.2 Anaemia and iron deficiency

Iron deficiency is one of the leading risk factors for disability and death, affecting an estimated 2 billion people worldwide (Zimmermann & Hurrell, 2007). Iron deficiency has substantial health and economic costs, among them poor pregnancy outcome, impaired school performance and decreased productivity. In children, it results in impaired growth and development. Anaemia is a major public health problem affecting 1.62 billion people globally (McLean *et al.*, 2009). The WHO Global Database on Anaemia for 1993–2005, covering almost half the world’s population, estimated the prevalence of anaemia worldwide at 25% (McLean *et al.*, 2009).

The South African NFCS-FB (2005) found anaemia in 27.9% of children, moderate anaemia in 6.4% and severe anaemia in 0.3% (Labadarios *et al.*, 2007). The results of the 2012 SANHANES-1 revealed that of children under five years of age, 10.7% were anaemic (Hb < 11 g/dl), 8.1% suffered from iron depletion (Hb $\geq$ 11 g/dl and ferritin < 12 ng/ml) and 1.9% had iron deficiency anaemia (IDA) (Hb <11 g/dl and ferritin < 12 ng/ml), (Shisana *et al.*, 2014). When compared to the 2005 NFCS-FB results, there was a decrease in anaemia (28.9% vs 10.7%), a slight increase in the prevalence of iron depletion (7.8% vs 8.1%) and a significant decrease in IDA (11.3% vs 1.9%) (Shisana *et al.*, 2014).

From these findings, it is evident that anaemia and iron deficiency are problems of public health importance and the government must intensify efforts to prevent and manage iron deficiency among South African children. Targeted iron supplementation and/or continued monitoring of the iron fortification programme can control iron deficiency in South African children.

2.6.2.3 Zinc deficiency

In recent years’ zinc, which is an essential trace element, has gained prominence and focus in relation to nutrition and the health of children. Zinc deficiency causes an array of health problems among children, of which the impairment of physical growth has been studied most (Hambidge, 2000). This occurs in children in the form of stunting or the syndrome of “nutritional dwarfism”, which can be used as an indirect indicator to estimate the risk of zinc deficiency at population level

(Brown *et al.*, 2001; Hotz & Brown, 2004). Zinc deficiency increases the risk of infants and children developing symptoms of diarrhoea, pneumonia and malaria (Black *et al.*, 2008). This has been reported in a number of randomised placebo-controlled trials that have been carried out worldwide (Bhutta *et al.*, 1999; Brooks *et al.*, 2004; Walker & Black, 2004; Sazawal *et al.*, 2007; Tielsch *et al.*, 2007).

Currently, few developing countries have information on the zinc status at national level. In South Africa, findings from the 2005 NFCS-FB revealed that 45.3% of South African children one to nine years old were zinc-deficient. The prevalence was highest among one-to-three-year-olds (51.3%), followed by four-to-six-year-olds (45.4%) (Labadarios *et al.*, 2007). Significant associations between the prevalence of stunting and zinc deficiency in children have been reported (Brown *et al.*, 2002; Hotz & Brown, 2004). The high prevalence of stunting among children from birth to three years of age, with boys at 26.9% and girls at 25.9% respectively (Shisana *et al.*, 2014), may also indicate a high prevalence of zinc deficiency among South African children.

Although limited direct national representative data on zinc deficiency are available in South Africa, these observations show that zinc deficiency is an important problem that requires national government prioritisation. Ideally, zinc should continue to be integrated into current intervention programmes for addressing micronutrient deficiencies (vitamin A, iron and zinc).

2.6.2.4 Iodine deficiency

Worldwide, 2 billion individuals have insufficient iodine intake, with those in South Asia and Sub-Saharan Africa particularly affected (Zimmermann *et al.*, 2008). Iodine deficiency has many adverse effects on the growth and development of children. The 2005 NFCS-FB report (Labadarios *et al.*, 2007) showed that under 15% of children from one to nine years had deficient iodine status (urinary iodine-UI<100 µg/L). The median urinary iodine (UI) among these children was 214.8 µg/L), and this indicates more than adequate iodine status and may imply that iodine deficiency has been eradicated among children in South Africa. This may be attributed to the compulsory iodisation of table salt that was introduced in South Africa in 1995 (Jooste *et al.*, 2001). Unfortunately, some households still use non-iodised salt (Jooste *et al.*, 2001). Therefore, there is a need for effective monitoring of the compulsory salt iodisation programme to prevent relapse.

2.7 Breastfeeding, infant and young child feeding and dietary practices of South African children (<2 years)

In South Africa the exclusive breastfeeding rate is very low and barriers to exclusive and continued breastfeeding include the perception of insufficient milk, compounded by fears of HIV transmission, marketing of breast milk substitutes, misinformation, breastfeeding problems, returning to full-time employment without supportive structures and lack of guidance and encouragement from health care personnel, among other factors (WHO, 1981; NDOH, 2008). Appropriate IYCF can be enhanced when women receive skilled support from health care personnel during antenatal, intra-partum, postnatal and follow-up care and reduction of child mortality can be achieved only when IYCF is prioritised in national policies and strategies (WHO *et al.*, 2000; WHO & UNICEF, 2003). To promote healthy growth and development of children, it is recommend that breastfeeding be initiated early and exclusive breastfeeding before age six months be promoted, as well as the introduction of nutritionally adequate and safe complementary foods from six months and continued breastfeeding up to two years of age or beyond (WHO & UNICEF, 2003).

According to (WHO & UNICEF, 2003), worldwide no more than 35% of infants were exclusively breastfed during the first four months of life; complementary feeding was frequently introduced too early or too late, and foods were often nutritionally inadequate and unsafe. Malnourished children who survive are more frequently sick and suffer the lifelong consequences of impaired development. Furthermore, the rising incidence of overweight and obesity in children is a matter of serious concern (WHO & UNICEF, 2003). Poor feeding practices are therefore major threats to social and economic development; they are among the most serious barriers to the optimum growth and development of children.

South Africa has one of the lowest exclusive breastfeeding rates in the world; only 8% of babies are exclusively breastfed for the recommended period from birth to six months (NDOH, 2013). Breastfeeding makes a major contribution to child health by protecting infants from morbidity and mortality associated with common infectious diseases (WHO & UNICEF, 2003). Most children younger than six months are fed a mixed diet (NDOH, 2013). This may be responsible for high rates of diarrhoea, infant malnutrition and mortality in South Africa. The main meal pattern for children of all ages in South Africa is primarily that of three daily meals and this pattern occurs irrespective of area of residence, in all provinces (Labadarios *et al.*, 2001; Shisana *et al.*, 2014). However, fruit

and vegetable consumption among children is low because of poor access and availability (Faber & Wenhold, 2007). Based on the 1999 NFCS (Labadarios *et al.*, 2001), dietary diversity is also low for South African children and this is often associated with poor child growth (stunting). In South Africa, maize meal and bread are staple foods and maize meal porridge, bread and cereals are commonly eaten for breakfast (Shisana *et al.*, 2014).

According to the 1999 NFCS, in one-to-nine-year-old children, micronutrients, including calcium, iron, zinc, vitamin A, C and E, niacin, riboflavin, vitamin B6 and folate, were deficient in the diet, particularly in rural children (Labadarios *et al.*, 2001; Labadarios *et al.*, 2005). This is explained by the findings that most commonly consumed foods, namely maize, sugar, tea and bread, are not good sources of the deficient nutrients (Steyn *et al.*, 2006). In addition, the secondary data analysis of the 1999 NFCS by (Steyn *et al.*, 2005) revealed that the food items most commonly consumed by one-to-five-year-old children were maize porridge (80.0%), sugar (76.0%), tea (44.0%), full-cream milk (39.0%) and white bread (24.0%). This served as benchmark for the national mandatory food fortification of staple foods maize meal and wheat flour (bread,) with vitamin A, iron, zinc, folic acid, thiamine, niacin, vitamin B6 and riboflavin in South Africa since October 2003 (NDOH, 2003; Labadarios *et al.*, 2005). The 1999 NFCS also showed that among South African children overall, one in two households (52%) experienced hunger, one in four (23%) was at risk of hunger and only one in four households (25%) appeared food-secure (Labadarios *et al.*, 2005).

The 1999 NFCS revealed that households that enjoyed food security consumed an average of 16 different food items over 24 hours, whereas poorer households spent less money on food and consumed fewer than eight different food items (Labadarios *et al.*, 2001). Moreover, these children had low mean scores for dietary diversity (3.58; SD: ± 1.37) and dietary variety (5.52; SD: ± 2.54) (Labadarios *et al.*, 2001).

Table 2. 3: Summary of key nutrition indicators for South African children under five years

Variable	Indicator	Status (%)	Source
Anthropometry	Stunting	15.4	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Wasting	2.9	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Underweight	5.8	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Overweight	18.1	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Low birth weight	12	SADHS, 2003 (NDOH <i>et al.</i> , 2007)
Micronutrients	Vitamin A deficiency (serum retinol <0.70 µmol/l)	43.6	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Anaemia and iron status		
	Anaemia (Hb < 11g/dl)	10.7	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Iron depletion (Hb ≥ 11 g/dl and ferritin < 12 ng/ml)	8.1	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Iron deficiency anaemia (Hb <11 g/dl and ferritin <12 ng/ml)	1.9	SANHANES-1, (Shisana <i>et al.</i> , 2014)
	Zinc (µmol/l)	45.3	NFCS-FB, (Labadarios <i>et al.</i> , 2007)
	Iodine (UI<100 µg/l)	< 15	NFCS-FB, (Labadarios <i>et al.</i> , 2007)
	Vitamin A supplementation coverage	29	SADHS, 2003, (NDOH <i>et al.</i> , 2007:1)
Breastfeeding practices	Timely initiation	61	SADHS, 2003, (NDOH <i>et al.</i> , 2007)
	Exclusive breast feeding birth to 6 months	8.3	SADHS, 2003, (NDOH <i>et al.</i> , 2007)
	Continued breastfeeding 12 to 24 months	0.4	SADHS, 2003, (NDOH <i>et al.</i> , 2007)

2.7.1 Summary

In South Africa the nutritional status of children is inadequate, as reflected in high rates of stunting, micronutrient deficiencies, hunger and food insecurity (Iversen *et al.*, 2011). In addition, there are coexisting factors such as HIV/AIDS and tuberculosis, together with nutrition transition and a double burden of malnutrition, which make the management of malnutrition complex.

However, the most prevalent nutritional concerns in South Africa are stunting and deficiencies of vitamin A, iron deficiency anaemia and zinc deficiency, which tend to interact, cluster and often occur in the same child. This indicates that South Africa has a problem of chronic malnutrition, rather than acute malnutrition. Childhood malnutrition starts early in life and often coincides with the introduction of complementary feeding with stunting and underweight, increasing twofold between the first and second years of a child's life (Faber & Wenhold, 2007).

2.8 Interventions to address growth faltering and micronutrient deficiencies within the 1000-day window period

Undernutrition, causing foetal growth restriction, stunting, wasting, and deficiencies of vitamin A and zinc, along with sub-optimum breastfeeding, underlies nearly 31 million deaths of children younger than five years annually world-wide, representing about 45% of all deaths in this group (Liu *et al.*, 2012). This can be prevented if evidence-based nutrition interventions are implemented within the first 1000 days of a child's life. The total number of deaths in children younger than five years can be reduced by 15% if populations can access 10 evidence-based nutrition interventions at 90% coverage (Bhutta *et al.*, 2013). Researchers have also suggested that accelerated gains are possible and about a fifth of the existing burden of stunting can be averted using these approaches, if access is improved in this way.

Solutions to improve child nutrition in the 1000-day window are available (WHO, 2014b) and are based on the realisation that the causes of malnutrition are multifaceted. The causes include factors generally associated with nutrition (nutrition-specific), as well as factors that affect the broader context of life and health (nutrition-sensitive). Addressing malnutrition within the 1000-day window period will therefore also require nutrition-specific interventions and nutrition-sensitive approaches (Bhutta *et al.*, 2013). Nutrition-specific interventions include support for exclusive breastfeeding up to six months of age and continued breastfeeding, together with appropriate and nutritious food, up to two years of age; fortification of foods; micronutrient supplementation and treatment of severe malnutrition (Bhutta *et al.*, 2013; WHO, 2014b). On the other hand, nutrition-sensitive approaches cover other sectors such as agriculture, clean water and sanitation, education and employment, health care, support for resilience and women's empowerment (Bhutta *et al.*, 2013; WHO, 2014b). In South Africa, the NDOH plays a key role in developing and implementing nutrition programmes and services and ensuring that nutrition and food security are specified and monitored, including other socio-economic programmes in the public and private sector.

The high prevalence of micronutrient deficiencies (vitamin A, iron, zinc) in South Africa, has led to the establishment of the sound regulatory and legislative frameworks pertaining to the prevention of micronutrient malnutrition. The South African government continues efforts to fight malnutrition through fortification with key vitamins and minerals of wheat flour, maize flour and retail sugar in

accordance with mandatory regulations that came into effect in 2003. This fortification of staple foods with a range of micronutrients and salt iodisation has worked to reduce the prevalence of folate and iodine deficiencies among children in South Africa (NDOH, 2010).

The (NDOH, 2010) has reported the achievement of virtual elimination of iodine deficiency disorders (IDD) in South Africa, which came about as a result of the mandatory salt iodisation programme introduced in 1995. This was in response to the high prevalence of vitamin A deficiency at that time among South African children. The government also introduced routine vitamin A supplementation for children younger than five years in 2003 (NDOH, 2010). South Africa undertook landscape analysis (Nishida, 2009) assessments in order to assess the country's readiness to accelerate action in nutrition, and also to review gaps and bottlenecks and identify opportunities for integrating new and existing effective nutrition actions (NDOH, 2010). The analysis revealed several weaknesses and challenges, including lack of multi-stakeholder response, lack of effective monitoring and evaluation, lack of political commitment (budget allocation), shortage of qualified personnel and lack of policy direction.

The nutrition actions for South Africa are highlighted in the INP of the NDOH. The INP has provided a broad framework to initiate the reorientation of nutrition services in South Africa since 1994, and significant gains have been made in this period, particularly in respect of the development of specific policies and the implementation of micronutrient strategies (NDOH, 1995). The Strategic Plan of the NDOH and the 10-Point Plan of the health sector for 2009-2014 now requires a more focused and targeted nutrition strategy, which aims to contribute directly to the achievement of the sector's key outcomes, while supporting effective nutrition action by other stakeholders (NDOH, 2009).

2.9 Fortified complementary foods and complementary food supplements

Complementary feeding interventions are usually targeted at the age range of six to 23 months (WHO & UNICEF, 2003; Bhutta *et al.*, 2013). This is the period of a high incidence of growth faltering, micronutrient deficiencies and infectious illnesses in developing countries. Plant-based (cereal and legume) traditional or local unfortified complementary foods do not provide an adequate supply of key nutrients (particularly iron, zinc and vitamin B6) to support optimum growth and

development (WHO & UNICEF, 2003; WHO, 2013a), during the complementary feeding period (six to 23 months). Normally, the addition of animal-source foods as an early complementary food for exclusively breastfed infants is feasible and may be associated with improved zinc status in some settings (Krebs *et al.*, 2006). However, in poor resource settings this option may not be feasible because of increased cost. Moreover, the limited amounts of animal-based foods that can feasibly be consumed by infants makes it difficult to meet the daily requirements for key nutrients. Therefore, additional investigations into alternative low-cost complementary feeding practices for breastfed infants in developing countries are warranted.

Particularly in Sub-Saharan Africa the most frequently consumed cereal- and legume-based complementary diets contain anti-nutritional factors: phytates, polyphenols, oxalates, proteinase inhibitors lectin, raffinose, oligosaccharides and saponins (Sandberg, 2002). These anti-nutritional factors reduce the bioavailability of minerals (Fe, Zn, Ca, P and K) (Dewey, 2013) and this limits mineral absorption and utilisation by the children. Traditionally the phytates can be reduced via fermentation, soaking, germination or sprouting and pounding, but these techniques are probably not sufficient to compensate for the low nutrient density of cereal-based complementary foods. The addition of *phytase* to complementary foods may be a more effective option to deal with the problem of phytates (Mamiro *et al.*, 2004; Gibson *et al.*, 2010; Troesch *et al.*, 2011). However, further research is needed to demonstrate the efficacy of this approach in children six to 23 months of age.

In settings where the diets are mostly cereal- and legume-based with little or no supply of animal-based foods, FCF and CFS can be used to address this nutrient gap (WHO, 2013a; WHO & FAO, 2014). Based on the guidelines of the Maternal, Infant, and Young Child Nutrition (MIYCN) Working Group - Subgroup on Formulation Guidelines, Formulations for Fortified Complementary Foods and Supplements (MIYCN, 2009), there are three types of products that can be used for children in the complementary feeding stage, namely (1) fortified blended foods (FBF), (2) CFS and MNP (MIYCN, 2009). These three will be the focus of this current review and less emphasis will be placed on discussing ready-to use-supplementary (RUSF) or therapeutic foods (RUTF) for treating moderately or severely malnourished children (MIYCN, 2009), because they provide a larger proportion of daily energy needs and are therefore “foods” themselves and not supplementary.

Complementary foods (CF) and CFS help to prevent dietary inadequacies during the complementary feeding period (MIYCN, 2009; Dewey, 2013). A number of products have been developed, which include the following: micronutrient sprinkles that are added to food just before feeding (Nestel & Alnwick, 1996), fortified spreads that can be added to food just before feeding or fed as a snack (Briend, 2001), water-dispersible or crushable micronutrient tablets (Gross, 2000), LNS (Adu-Afarwuah *et al.*, 2007; Adu-Afarwuah *et al.*, 2008; Dewey & Adu-Afarwuah, 2008; Iannotti *et al.*, 2014; Hess *et al.*, 2015), and MNP (Wang *et al.*, 2007). In 2009 the MIYCN working group of the Ten-Year Strategy to Reduce Vitamin and Mineral Deficiencies, Sub-Group on Formulation Guidelines, published a comprehensive review of evidence on CF and CFS (MIYCN, 2009). The paper summarised the characteristics of FBF, CFS and MNP for home fortification that had been used successfully in research studies and national supplementation programs in low- and middle-income countries (LMIC). These types of fortified products that have been developed are summarised in **Table 2.4**.

Table 2. 4: Summary of characteristics of commonly used fortified blended foods and supplements¹

Type	Description/Purpose	Examples
FBF	• FBF, are often used in national feeding programmes, as they provide a replacement for less nutritious cereal-based diets (MIYCN, 2009). • Traditionally FBF contained only cereals, legumes and sugar or oil (as corn soya blends); recently some have been developed to include milk, additional fat sources providing EFAs, high-quality protein, and/or minerals such as calcium and phosphorus (MIYCN, 2009).	Examples include; • Corn Soy Blend (CSB), • Favina (Vietnam), • Mi Papilla (Ecuador), • Koba Aina (Madagascar)
CFS	• CFS, which are fortified food-based products to be added to other foods (as “point of use” or “home” fortificants) or eaten alone to improve the intake of macronutrients, micronutrients and essential fats during the complementary feeding period (MIYCN, 2009; Dewey, 2013)	Examples include; • LNS in form of SQ-LNS and/or MQ-LNS
MNP	• MNP, which are home fortificants containing only vitamins and minerals that are used to fortify the complementary food consumed by the child (MIYCN, 2009).	Examples include; • Sprinkles • Powders

¹Table 2.4 was adapted from (MIYCN, 2009).

FBF are used as a replacement for the traditional local porridge or in addition to traditional porridge. These are normally found as fortified infant cereals made from rice, wheat, corn or millet, soy, peanuts or milk; sugar and oil (MIYCN, 2009). Common examples are Favina (Vietnam) (Bruyeron *et al.*, 2007), Mi Papilla (Ecuador) (Lutter *et al.*, 2008) and Koba Aina (Madagascar) (GRET, 2009). Traditionally FBF contained only cereals, legumes and sugar or oil (as corn soya blends); recently some have been developed to include milk, additional fat sources providing EFAs, high-quality protein and/or minerals such as calcium and phosphorus (MIYCN, 2009).

CFS are fortified food-based products that are developed to be added to other foods (as “point of use” or “home” fortificants) or eaten alone as snacks to improve both macronutrient and micronutrient intake (MIYCN, 2009; Dewey, 2013). They are normally in the form of fortified peanut spread or fortified full-fat soy flour given in smaller amounts than recommended for supplementary feeding. Examples include RUSF (Nutraset, 2014) and *Nutributter*® (Adu-Afarwuah *et al.*, 2007). CFS in the form of RUSF are better suited to meet the nutritional needs of young and moderately malnourished children than FBF and they have improved the treatment of uncomplicated forms of severe acute malnutrition among children (UNICEF, 2013). CFS can also be designed to provide higher fat content than the normal diet, EFAs, milk, micronutrients, macro-minerals and high-quality protein. LNS such as *Plumpynut*® and *Nutributter*® are a subcategory of CFS.

According to (MIYCN, 2009), MNP contain only vitamins and minerals and are designed to be added to traditional infant foods, used as “point-of-use fortificants” or “home fortificants.” MNP are designed with a carrier such as maltodextrin or rice flour included in small amounts (e.g., 1 to 3 g) and are usually available in the form of fortified powders and crushable tablets. Common examples include sprinkles (SGHI, 2014) and Chispitas in Bolivia (Telleria, 2006). Home fortification of foods with MMN powders has been shown to be effective in reducing anaemia and iron deficiency in children six to 23 months of age (De-Regil *et al.*, 2013). Therefore, home fortification of children's foods with MNP or SQ-LNS may help to prevent micronutrient deficiencies at reasonable cost and improve child development (Allen, 2012).

By comparison, FBF are often used in national feeding programmes as they provide a replacement for less nutritious cereal-based diets (MIYCN, 2009). Nonetheless, they are generally more expensive than RUSF or MNP because they provide macronutrients as well as micronutrients. Micronutrient powders have been widely tested and shown to be effective for reducing anaemia and iron deficiency in large-scale programmes (De-Regil *et al.*, 2013). On the other hand, fortified CFS, usually in the form of SQ-LNS, have also been shown to influence growth, perhaps because of the EFAs and milk that they contain (Adu-Afarwuah *et al.*, 2007; Phuka *et al.*, 2008; Mangani *et al.*, 2012), while also improving micronutrient status. In general, the decision on product or type (FFB, CFS or MNP) of product to use depends on the setting and on the ability of manufacturers to produce high-quality products. According to some researchers and based on evidence from recent studies (de Pee & Bloem, 2009; Bhutta *et al.*, 2013; Thakwalakwa *et al.*, 2014), nutrition-specific interventions have the potential to improve growth if they target the 1000-day window of opportunity.

In summary, most nutritious supplementary and fortification products that are provided for children from age six to 23 months or six to 59 months can be grouped into three categories as outlined below in the order of preference; (1) LNS, mainly SQ-LNS (Arimond *et al.*, 2013; Mangani *et al.*, 2013; Thakwalakwa *et al.*, 2014; Abbeddou *et al.*, 2015; Flax *et al.*, 2015; Hess *et al.*, 2015; Ickes *et al.*, 2015; Lesorogol *et al.*, 2015; Segrè *et al.*, 2015), (2) MNP (Barth-Jaeggi *et al.*, 2015; Jefferds & Flores-Ayala, 2015; Jefferds *et al.*, 2015; Osei *et al.*, 2015) and (3) FBF (Irena *et al.*, 2013; Bauserman *et al.*, 2015; Chavasit *et al.*, 2015).

2.10 Small quantity lipid-based nutrient supplements

The predominantly cereal- and legume-based diets in most developing countries do not supply adequate daily nutrients (Maleta *et al.*, 2015) for the optimum growth and development of infants and young children. This results in poor pregnancy outcomes and poor growth and development. Thus, SQ-LNS were designed to fill this gap between typical intakes and needs (Arimond *et al.*, 2013). The daily ration of SQ-LNS provides ~20 g or ~110–120 kcal along with additional protein, EFA and approximately 22 micronutrients, including zinc. SQ-LNS provide low energy to ensure that breast milk intake is not compromised and allow for higher intakes of local foods, including animal-source foods, fruit and vegetables (GAIN, 2014). SQ-LNS also cost less to produce than

high-energy-containing products with similar formulations and are thus more affordable for low-income consumers. There is clearly a need for additional research to understand the potential growth-promoting effect of SQ-LNS and certain ingredients in SQ-LNS, such as milk powder and EFAs (Dewey & Arimond, 2012).

The contribution of SQ-LNS to the prevention of growth faltering and improvement of the micronutrient status of infants is only beginning to be understood. A study in Burkina Faso (Hess *et al.*, 2015) reported that SQ-LNS supplementation results in increased growth and reduced stunting, wasting and anaemia in children. On the contrary, the findings of a trial in Malawi (Maleta *et al.*, 2015) failed to support the hypothesis that SQ-LNS supplementation for infants and children promotes linear growth or prevents growth faltering between six and 18 months of age. In summary, there is still inconclusive evidence on whether SQ-LNS supplementation could address the problem of malnutrition in infants and children.

Therefore, this current study was designed to investigate the hypothesis that infants randomised to receive low-energy, fortified lipid-based nutrient supplements (SQ-LNS A and SQ-LNS B) would have improved linear growth, nutritional and health outcomes compared to children who are randomised to a control/delayed intervention group. The baseline relationships between socio-demographic factors and feeding practices and iron status were also explored.

2.11 Small quantity lipid nutrient supplements and growth of children younger than two years

In developing countries infant and weaning diets are based largely on plant sources with anti-nutritional factors (few animal-based foods). These do not meet the nutritional requirements for children aged six to 23 months (WHO & UNICEF, 2003; de Pee & Bloem, 2009). These plant-based weaning foods have low energy density and this appears to be the major contributor to growth faltering and undernutrition in children six to 23 months (Prentice & Paul, 2000). The commonly used weaning foods and adult foods usually contain low amounts of fat, which causes a sharp transition from adequate fat intake to probably inadequate fat intake when children are older than six months (Prentice & Paul, 2000).

SQ-LNS supplementation has the potential to prevent growth faltering and micronutrient malnutrition in infants and children, but more research needs to be done to produce more conclusive results. Some studies have reported improvements in linear growth in study populations (Phuka *et al.*, 2009; Campoy *et al.*, 2012; Mangani *et al.*, 2013; Iannotti *et al.*, 2014; Thakwalakwa *et al.*, 2014; Hess *et al.*, 2015). However, other studies have found no significant positive effects on linear growth (Wright *et al.*, 2006; Rosenfeld *et al.*, 2009; de Jong *et al.*, 2011; Huffman *et al.*, 2011; Imdad *et al.*, 2011; Makrides *et al.*, 2011; Maleta *et al.*, 2015)

It can be concluded that there is no conclusive evidence on the benefits of SQ-LNS supplementation for improving linear growth in infants and children and more trials are required to provide insight into this area.

2.12 Small quantity lipid nutrient supplements and biochemical markers of micronutrient status (Fe, Zn, vitamin A) in children younger than two

The short- and long-term outcomes of early nutrition have been extensively studied in recent decades. There is now growing acceptance of the importance of the first 1000 days, emphasising that maternal nutrition and weaning diets play an important role in determining health outcomes for infants and young children (SUN, 2012; WHO, 2013a; WHO & FAO, 2014).

Deficiencies of key nutrients, namely protein, energy, iron, zinc and vitamin A are a public health concern among infants and young children in low-income countries (WHO & FAO, 2006; Habicht & Pelto, 2012; Jefferds *et al.*, 2013). This calls for early dietary interventions and novel strategies to prevent nutritional deficiencies and improve the nutritional status of vulnerable children (Agostoni *et al.*, 2008). One promising approach is the use of SQ-LNS, which can be added to complementary food at the time of consumption, a strategy now referred to as home fortification (WHO & FAO, 2006; MIYCN, 2009; De-Regil *et al.*, 2013). The home fortification interventions are increasing and being scaled up in regions with widespread problems of undernutrition and micronutrient deficiencies worldwide (Jefferds *et al.*, 2013).

Supplementation with poly-unsaturated fatty acids (PUFA) promotes positive birth outcomes and notably increased birth size, but it is unclear whether these differences translate into improved

postnatal growth or if postnatal supplementation improves linear growth in infants (Hoffman *et al.*, 2008; Gonzalez-Casanova *et al.*, 2015). However, these PUFA should be formulated to provide infants with the correct amounts and ratio of n-6/n-3 (PUFA) in order to promote optimum growth and development.

Available evidence shows that there is growing interest in the potential benefits of using SQ-LNS for preventing undernutrition and micronutrient deficiencies in children (Jefferds *et al.*, 2013; GAIN, 2014). It remains unclear whether PUFA (DHA, ARA) on their own (Bernardi *et al.*, 2012; Much *et al.*, 2013) or as ingredients of SQ-LNS (Puett *et al.*, 2013) would improve linear growth in infants. Determining this may be interesting, considering that the current trial utilised SQ-LNS that contained DHA and ARA. More questions need to be answered on their effectiveness and/or cost-effectiveness in normal and even emergency settings (Chaparro & Dewey, 2010).

2.13 Small quantity lipid nutrient supplements growth hormone and insulin-like growth factor-1 in children younger than two years

IGF-1, also called somatomedin C, has a molecular structure similar to insulin IGF-1. It is generated in the liver and acts as an anabolic effector for the linear growth-promoting effects of the pituitary GH (Laron, 2004; Hwa *et al.*, 2013). IGF-1 plays an important role in childhood growth and continues to have anabolic effects in adulthood; deficiency of IGF-1 may therefore result in growth faltering (Hwa *et al.*, 2013), hyperglycaemia and increased morbidity (Beardsall *et al.*, 2014) in children.

Insulin regulates the secretion of IGF-I and also acts as a GH (Laron, 2004; Hwa *et al.*, 2013). However, whether the insulin growth effect is direct or mediated by IGF-1 or leptin remains unclear (Laron, 2004). The circulating IGF-I concentrations are regulated by genetic factors, nutrient intake, GH and other hormones such as T4, cortisol and sex steroids (Clemmons, 2006). Maternal and weaning diets have an effect on growth and growth-regulating hormones such as IGF-1 in the early years of life (Michaelsen, 2013) and therefore affect linear growth in children. The GH-IGF axis consists of a cascade of finely tuned molecular mechanisms, which are vulnerable to protein-calorie deficiency (Savage, 2013). IGF-1 deficiency and associated growth deceleration can be caused by abnormal pituitary GH secretion, decreased GH binding to its receptor (GHR), post-GHR signal

transduction, IGF-1 gene transcription, IGF binding protein (IGFBP) deficiency and decreased IGF-1 binding to its own receptor (IGF1R) (Savage, 2013).

Therefore, this GH-IGF-I axis is one of the main actors in the linear growth process and abnormalities in this axis can be responsible for short or tall stature or stunting (Castell *et al.*, 2013). Considering the evidence of differences in inflammatory status between stunted and non-stunted infants and children, it can be hypothesised that low-grade inflammation inhibits hepatic production of IGF-1. This has been observed in children suffering from chronic inflammatory diseases (De Benedetti *et al.*, 1997; Walters & Griffiths, 2009), and high levels of CRP and acid glycoprotein (AGP) during infancy were associated with stunting. Low-grade chronic inflammation associated with gastric enteropathy in infancy may impair infant growth (Prendergast *et al.*, 2014) and result in linear growth retardation by suppressing the IGF-1 production mechanism (De Benedetti *et al.*, 1997; Walters & Griffiths, 2009). This disrupts the GH-IGF-1 growth axis. This mechanism suggests that stunting may be driven by intestinal damage and chronic inflammation during foetal and postnatal life in addition to dietary inadequacy and warrants further investigation.

In short, stunting is influenced by both maternal and infant factors. The pregnancy nutritional and inflammatory status of the mother may result in intrauterine stunting and low birth weight (Prendergast *et al.*, 2014). Likewise, low-grade inflammation early in infancy can also result in stunting. This shows that the cause of higher levels of CRP and AGP in stunted compared to non-stunted infants remains unclear and further studies are required to provide more insight into this area.

2.14 Relationship between demographic, socio-economic factors and anthropometric indicators among children younger than two years

Malnutrition in infants and young children is a problem of public health importance in developing countries (Mostafa, 2011). The commonly used anthropometric indicators of malnutrition, namely stunting and wasting or thinness, define the degree of nutritional insult to the child. Linear growth retardation or stunting indicates chronic malnutrition, which reflects repeated exposure to adverse economic conditions, poor sanitation and the interactive effects of poor nutrient intakes and infection as outlined in the malnutrition conceptual framework (UNICEF, 1990). On the other hand,

wasting or thinness shows acute malnutrition as a result of recent illness and/or nutrient deprivation (WHO, 1995; Caulfield *et al.*, 2006). This shows that the causes of childhood malnutrition are varying, multidimensional, interrelated and complex. Managing malnutrition therefore requires a holistic and multi-stakeholder approach. This is important for development in low-income countries, as the nutritional status of children or child growth is internationally recognised as an important indicator for development (Mushtaq *et al.*, 2011).

Researchers (Owusu *et al.*, 2004; Engebretsen *et al.*, 2008; Abubakar *et al.*, 2012), have indicated that indicators of socio-economic status (SES) are associated with children's nutritional status in Sub-Saharan Africa. These include maternal and paternal educational level, parental income and family assets. In addition, child nutrition outcomes in low-income countries have been shown to be characterised by large rural-urban disparities (Van de Poel *et al.*, 2007). Despite these observations, there is still a need for further investigation into the relationship between SES and growth faltering. This is supported by the fact that the prevalence of stunting and underweight has been reported to show both inter- and intra-country variation in Sub-Saharan Africa (Zere & McIntyre, 2003; Benson & Shekar, 2006; Black *et al.*, 2008). Therefore, there is a need to examine the prevalence, risk factors and determinants of poor growth in a variety of contexts in order to adopt population-specific interventions.

2.15 Relationship between pro-inflammatory cytokines and markers of cardiovascular risk and anthropometric indicators among children younger than two years

Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. They are classified as either *pro-inflammatory*; tumour necrosis factor-alpha (TNF-alpha), IL-6, IL-8, and IL1-beta or *anti-inflammatory*; IL-4, IL-10, and IL-13 (Dinarello, 2000). These compounds may seem analogous to hormones, but they are not hormones (Dinarello, 2000). The markers of cardiovascular risk include CRP, homocysteine, and plasminogen activator inhibitor-1 (PAI-1) (Stentz *et al.*, 2004).

Pro-inflammatory cytokines and markers of future cardiovascular risk have become one of the central themes in the pathogenesis of linear growth faltering (Prendergast *et al.*, 2014). The focus on the role of inflammatory mediators and markers is helping to examine and understand the

pathogenesis of short stature syndrome (stunting) in the earlier stages of life. This will enable adoption of effective interventions within the 1000-day window period of opportunity. Previous studies (Rao *et al.*, 2011) have shown that pro-inflammatory cytokines impair growth in children with inflammatory bowel disease by inhibiting signal transduction from GH to IGF-1. It is therefore hypothesised that a similar mechanism occurs in children experiencing linear growth faltering.

An inflammatory process per se may also directly inhibit linear growth. These effects could be a result of inhibition of the maturation of growth plate chondrocytes by pro-inflammatory cytokines, such as TNF-alpha (Ballinger, 2002). The anthropometric indicators have been shown to be associated with serum markers of low-grade inflammation (Ramirez Alvarado & Sanchez Roitz, 2012) and markers such as CRP and AGP (Prendergast *et al.*, 2014:620.4). Extensive enteropathy during infancy is associated with low-grade chronic inflammation and this may be the mechanism associated with impaired infant growth (Prendergast *et al.*, 2014).

2.16 Conclusions and summary

In this era of SDGS, worldwide there has been some progress in meeting the 2025 targets for nutrition in respect of stunting, wasting and overweight among children under age five, anaemia in women 15-49 years of age and rates of exclusive breastfeeding for infants from birth to six months of age (Haddad *et al.*, 2015). Although the prevalence of stunting is gradually declining, there has been no improvement in the rate of anaemia for women 15-49 years. Moreover, overweight and obesity rates are actually increasing (Haddad *et al.*, 2015).

Among children under five years, 161 million are stunted, 51 million are wasted and 42 million are overweight (Haddad *et al.*, 2015). This emphasises the need to focus interventions within the critical 1000-day window of opportunity. Improving the quality of children's diet is one of the proven effective interventions for preventing stunting during the complementary feeding period and this is associated with improved linear growth. An additional burden is micronutrient deficiencies, specifically of iron, zinc and vitamin A.

The review showed that there is growing interest in the potential benefits of using SQ-LNS for preventing undernutrition and micronutrient deficiencies in children (Puett *et al.*, 2013). However, their efficacy in improving linear growth or preventing growth faltering in infants and young

children remains unclear. Thus, there is still inconclusive evidence on the effectiveness, and no evidence on the cost-effectiveness of SQ-LNS supplementation in community settings (Chaparro & Dewey, 2010; Puett *et al.*, 2013). Future research should focus on controlled and long-term follow-up trials in order to obtain more conclusive results.

On a pragmatic level there is a strong need to critically consider contextual factors and establish the right combination of nutrition-specific and nutrition-sensitive interventions that are most likely to succeed in specific settings. Political support, a multi-sectoral approach, integration of nutrition into other development programmes and community participation in programme activities are the core foundations that contribute to success (Haddad *et al.*, 2015).

“The world faces many seemingly intractable problems. Malnutrition should not be one of them. Ending it is a choice that national leaders must be supported, and sometimes pressured, to make”.

Global Nutrition Report (GNR), 2015

2.17 References

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The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community.



Top Left: Source data verification by the study monitor. **Top right:** Paperwork by field team at the study site

Bottom Left: The Author busy working at the study site. **Bottom right:** Tswaka study team at the farewell party.



This chapter is presented in article format and is written according to the referencing requirements of the *Public Health Nutrition Journal* to which it was submitted. The font and spacing were kept the same throughout the thesis. All figures and tables are presented at the end of the article.

CHAPTER 3: RESEARCH ARTICLE 1

The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community.

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Abstract

Objective: To determine the prevalence and factors associated with stunting in 6 months old South African infants.

Design: This cross-sectional study was part of the baseline of a randomised controlled trial. The weight-for-length (WLZ), length-for-age (LAZ) and weight-for-age z-scores (WAZ) were based on the WHO classification. Blood samples were analysed for haemoglobin (Hb), plasma ferritin (PF), and soluble transferrin receptor (sTfR). Socio-economic, breastfeeding and complementary feeding practices were assessed by questionnaire.

Setting/Subjects: Infants aged 6 months ($n=750$) from a peri-urban area of Matlosana municipality, North West province of South Africa.

Results: Stunting, underweight, wasting and overweight affected 28.5%, 11.1%, 1.7% and 10.1% of infants respectively. Exclusive breastfeeding to age of 6 months was reported in 5.9% of the infants. Multivariable binary logistic regression showed that birth weight (kg) (OR 0.12, 95%CI 0.07 to 0.21, $P<0.001$) and maternal height (cm) (OR 0.94, 95%CI 0.91 to 0.98, $P=0.001$) were inversely associated with stunting; while male sex (OR 1.73, 95%CI 1.10 to 2.70, $P=0.014$) was associated with higher odds for stunting. Stunting was also associated with higher plasma sTfR (>8.3 mg/L) concentrations.

Conclusions: The association between stunting and lower birth weight, shorter maternal height and male sex reflects possibly the intergenerational origins of stunting. Therefore, interventions that focus on improving pre-conceptual and maternal nutritional status combined with strategies to promote appropriate infant feeding practices may be an important strategy to prevent stunting in vulnerable settings.

Keywords: stunting, low birth weight, complementary feeding, breastfeeding, iron deficiency anaemia

Introduction

Stunting affects approximately 159 million children under 5 years worldwide and a greater proportion of these children are in sub-Saharan Africa and south-central Asia⁽¹⁾. Childhood stunting and micronutrient deficiencies are usually associated with poor nutrition and increased exposure to infections and unsanitary environments⁽²⁾. In South Africa, stunting remains the most prevalent form of undernutrition in children under 5 years⁽³⁾. The results of the 2012 South African National Health and Nutrition Examination Survey (SANHANES) showed that stunting in South African children was highest in the age group of 0-3 years, 26.9% and 25.9% for boys and girls respectively⁽⁴⁾.

In South Africa, malnutrition is often associated with socio-demographic factors, income level, weekly expenditure on food, employment status, education level of the mother and food insecurity⁽⁵⁾. Complementary foods commonly consumed are usually cereal based and deficient in key micronutrients^(6; 7). This may result in increased risk of micronutrient deficiencies, resulting in a vicious cycle between malnutrition and infection that may be linked to the moderately high prevalence of stunting among South African children under 5 years of age⁽⁴⁾.

The relationship between stunting and socio-demographic, household and environmental determinants is still not clearly understood. Intergenerational factors such as maternal short stature may increase the risk of poor offspring birth outcomes and growth retardation^(8; 9; 10). Stunting begins in utero and is linked to maternal short stature^(8; 9; 10) and poor maternal nutrition⁽²⁾ resulting in intrauterine growth restriction and low birth weight. This points towards the importance of interventions from pre-conception through to the 1000-day window period in order to prevent the intergenerational cycle of stunting.

There is a need to understand the interplay between several factors associated with stunting in order to develop and scale up population, sex and age specific interventions, and help to encrypt the multifaceted causal matrix. Studies on factors associated with stunting among children under 5 years within an age range^(7,8,31) have been published, but there is a lack of information about the prevalence and factors associated with stunting in specifically 6 months old infants in developing countries. Therefore, the aim of this study was to identify maternal, socioeconomic, feeding practices and child characteristics associated with stunting among 6-month old infants from a peri-urban area in South Africa. The data for this study was collected as the baseline for a randomized

controlled trial assessing the efficacy of lipid based nutrient supplements (LNS) on growth of 6-month old infants. The trial is registered (NTC01845610) at <http://clinicaltrials.gov>.

Materials and methods

Study site, sampling and participants

This article presents data on the factors associated with stunting in 6-month old black infants ($n=750$) using baseline data of a randomised control trial. The data was collected between September 2013 and January 2015. The study was carried out in the peri-urban area of Matlosana municipality, North West province of South Africa. Trained fieldworkers recruited potentially eligible mother/infant pairs through five primary health care clinics and house-to-house visits. A total of 998 mother/caregiver-infant pairs were recruited, of whom 235 failed to come for the final screening visit and 13 were excluded because they were not eligible to be included in the randomized controlled trial. The sample size ($n=750$) was based on sample size calculations for the randomized controlled trial, which had linear growth as the main outcome. The sample size was adequate for the cross-sectional analyses performed for this part of the study with the main aim to determine variables associated with stunting, assuming a probability of stunting of at least 0.2 and a minimum odds ratio of 1.3 (or 0.8 for an inverse association) at a type 1 error of 5% and power of 80%⁽¹¹⁾.

Inclusion and exclusion criteria

Black infants of age 6 to 7 months from a peri-urban area were enrolled in the study. Infants were excluded if they had never received any breast milk previously, had severe obvious congenital abnormalities, haemoglobin (Hb) < 70 g/L, weight-for-height z-score < -3 , other diseases or recent hospitalisation, the caregivers planned to move out of the study area within the next seven months, were receiving special nutritional supplements as part of feeding programmes; were diagnosed with HIV infection (we did not test for HIV status in the study), were known to be allergic/intolerant to peanuts, soy, cow's milk protein, and fish, or they had not been born as a singleton. Infants were enrolled if they came with the parent(s) or caregiver, however, for assessing maternal height, only data from biological mothers was used.

Data collection, measurements and handling

Weight and recumbent length were taken according to WHO standardized techniques⁽¹²⁾. The anthropometry assessors were trained according to the WHO Training Course on Child Growth Assessment for the infants⁽¹³⁾. Infants were undressed and weighed to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG, Hamburg, Germany, maximum weight 20 kg). Recumbent length was measured to the nearest 0.1 cm using an infantometer (Seca model 416, GmbH & Co. KG, Hamburg, Germany). Mid-upper arm circumference (MUAC) was measured using a measuring tape (Seca 201, GmbH & Co. KG., Hamburg, Germany) and head circumference (HC) was measured using a measuring tape (Seca 212, GmbH & Co. KG., Hamburg, Germany). All measurements were done in duplicate and if the first two measurements differed by >0.05kg for weight or by >0.2 cm for length or circumference a third measurement was done and the two closest values were recorded and the means were calculated. The anthropometric indices length-for-age z-scores (LAZ), WLZ, weight-for-age z-scores (WAZ), head circumference-for-age z-scores (HCZ), BMI-for-age z-scores (BAZ) and mid-upper arm circumference-for-age z-scores (MUACZ) were generated using WHO *Anthro* 2005 software. Birth weight was obtained from the infant's Road-to-Health booklet.

The weight of mothers was measured to the nearest 0.01 kg using a digital adult scale (UC-321 Precision A&D Company, Ltd, Tokyo, Japan), while the heights of the mothers were measured using a mechanical stadiometer (Seca, Birmingham, UK) according to standard methods⁽¹⁴⁾. The standard formula, $\text{weight (kg)} / [\text{height (m)}]^2$, was used to calculate body mass index (BMI)⁽¹⁵⁾. The scales were calibrated on a daily basis.

A set of unquantified food frequency questions was used to assess dietary intake of the infants during the past week (7 days). Breastfeeding and complementary feeding practices were assessed based on a WHO questionnaire⁽¹⁶⁾. The questionnaire also had questions on socio-demographics, water and sanitation, size of households, and education, employment status and marital status of mothers/caregivers.

Anaemia and iron status were analysed from blood samples (4 mL) which were collected via antecubital venipuncture into EDTA-coated trace-element free evacuated tubes (Becton Dickinson) by the study nurse. In cases where obtaining a blood sample was not successful a finger prick was performed to assess the haemoglobin (Hb) status. Hb was determined for all infants ($n=750$) using a

Hemocue machine (Ames Mini-Pak haemoglobin test pack and Ames Minilab, Bio Rad Laboratories (Pty) Ltd). A blood sample was successfully obtained from 485 infants. Blood for later analyses was prepared by centrifuging at 500 xg for 15 min at room temperature and aliquoted plasma was stored at -80°C in temperature monitored freezers at North-West University. For analysis, the samples were shipped to Vitmin Lab Willstaett, Germany as per shipment regulations and specifications of the National Department of Health. Plasma ferritin (PF) and soluble transferrin receptor (sTfR) concentrations were determined using a sensitive Sandwich ELISA technique⁽¹⁷⁾. High-sensitivity C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) were measured with an ELISA kit from Human Diagnostics (Wiesbaden, Germany).

Definitions

Anthropometric status was assessed using the WHO Child Growth Standards⁽¹⁸⁾. Wasting was defined as WLZ less than -2SD , stunting as LAZ less than -2SD , underweight as WAZ less than -2SD and overweight as WLZ greater than $+2\text{SD}$ ⁽¹⁹⁾. Low-birth weight (LBW) was defined as birth weight below 2.5 kg regardless of gestational age⁽²⁰⁾. Maternal short stature was defined as height below 150.1 cm, which is the median minus 2 SD of the reference height for 18-year-old girls⁽²¹⁾. Anaemia was defined as $\text{Hb} < 11\text{g/dL}$, iron deficiency (ID-PF) as $\text{PF} < 12\text{ }\mu\text{g/L}$ and iron deficiency anaemia (IDA-PF) as both $\text{PF} < 12\text{ }\mu\text{g/L}$ and $\text{Hb} < 11\text{ g/dL}$ ^(22; 23). ID-sTfR was defined as $\text{sTfR} > 8.3\text{ mg/L}$ and IDA-sTfR was defined as $\text{Hb} < 11\text{ g/dL}$ and $\text{sTfR} > 8.3\text{ mg/L}$ (test-kit reference value). Inflammation was detected by acute phase proteins, $\text{AGP} > 1\text{ g/L}$ and $\text{CRP} > 5\text{ mg/L}$ ⁽²⁴⁾. Individual PF concentrations were adjusted by using correction factors (CFs) specific to each subject's inflammatory status⁽²⁴⁾.

Statistical analysis

Shapiro-Wilk test and Q-Q plots were used to check for normality of the continuous variables. Results are reported as the mean $\pm$ SD for continuous normally distributed data, or as the median and interquartile range (IQR) for continuous non-normally distributed data. The independent t-test was used to test for significance of the difference between two means, the Mann-Whitney test for significance of differences between median values and the Pearson chi-square test for associations between categorical data. Univariate logistic regression analysis was done to explore and understand the relationships between variables and stunting. Factors significantly associated with stunting were

then included in the multivariable binary logistic regression analysis with stunting (stunted vs non-stunted) as the dependent variable and using the backward elimination technique. The factors that were included in the regression models were sex, birth weight (kg), Hb (g/dL), sTfR (mg/L), AGP (g/L), education level of the mother/caregiver, and consumption of jarred commercial infant foods. Maternal height (cm) was included in the final model based on theoretical evidence that the mother's stature influences birth outcomes and stunting^(8; 10). The Nagelkerke R^2 and the Hosmer and Lemeshow tests were used to evaluate the goodness of fit of the model and as basis for selecting the final model. The P value, odds ratio (OR) and 95% CI are reported for the respective regression coefficients. For all analyses, statistical significance was set at $P < 0.05$). In univariate logistic regression $P < 0.10$ was used as a cut-off point to retain variables in the regression model. The data was analysed using SPSS software version 23 (IBM, Corporation USA).

Ethics

Ethical approval was obtained from the Ethics Committees of North West University (NWU) (NWU-00001-11-A1) and the South African Medical Research Council (SAMRC), (EC-01-03/2012). After institutional ethical approval, the project was reviewed by local authorities. The provincial, district and community's approval to conduct the study was sought through an engagement process with relevant stakeholders. Informed written consent was obtained from parents of all the study participants. This trial was registered at <http://clinicaltrials.gov> as NTC01845610.

Results

A total of 750 infants (387 boys, 363 girls) with a mean $\pm$ SD age of 6.2 ± 0.3 months participated in this study. Significantly more boys than girls were stunted (32% vs. 24.8%, $P=0.028$). Low birth weight was recorded in 14.0% of the infants and of these 58.8% were stunted compared to 41.2% who were not stunted. Socio-demographic and household characteristics compared by stunting status are presented in **Table 1**. The majority (91.7%) of the women who participated in the study were the biological mothers of the infants and their mean age $\pm$ SD was 27.1 ± 6.6 years. More than half of the mothers/caregivers (55.3%) were not married and most (81.3%) had at least 10 years of schooling (grade 10). Most households had at least one person employed and the median number of beneficiaries of social grants per household was 2 (IQR 1,3). The median size of households was 5 (IQR 4,7) people.

Breastfeeding and complementary feeding practices

Table 2 shows the summary of the mother/caregiver's feeding practices for their infants at age 6 months. Nine of the caregivers did not respond to the food frequency questions, as they were not the full-time caregivers. At age 6 months, 70.1% of the infants were still being breastfed, with breast milk being either the only milk feed or being given in combination with other milk feeds. Of the 750 infants, 5.9% were exclusively breastfed to the recommended age of 6 months.

Among the infants that were already consuming complementary feeds ($n=741$) the mean $\pm$ SD ages for introducing liquids and semi-solids were 2.5 ± 1.7 months and 3.8 ± 1.5 months respectively. The liquids introduced first ($n=713$) were water (53.6%), formula milk (39.1%), and a variety of other liquids (rooibos tea, sweetened drink, sugar water, and cow's milk) (7.3%). The foods introduced first ($n=701$) were commercial infant cereal (63.8%), jarred commercial infant foods (20.3%), maize meal porridge (8.7%) and other foods including sorghum porridge, oats porridge and vegetables (7.2%). Milk feeds given to the infants at the age of 6 months were breast milk only (52.7%), breast milk and formula milk (14.9%), breast milk and cow's milk (2.4%), formula milk only (27.7%) and cow's milk only (1.1%); while 1.2% received no milk feeds. Foods that were frequently consumed (at least 4 days during the past week) were infant cereal (68.1%), sugar (27.9%), and jarred commercial infant food (22.7%). Other complementary foods eaten at least once during the previous week included vegetables (43.3%), fruits (26.4%), eggs (23.9%), red meat (5.1%), chicken (28.9%), liver (10.5%) and fish (2.7%).

Anthropometric status of the infants and mothers

Results show that 28.5% of the infants were stunted, 1.7% were wasted, 11.1% were underweight and 10.1% were overweight. Mean anthropometric indices of the total group and comparison according to stunting status are presented in **Table 3**. Boys had significantly lower mean LAZ [(-1.57 ± 1.11) vs. (-1.31 ± 1.02)] and HCZ [(-0.05 ± 1.05) vs. 0.12 ± 0.95] than girls. Both sexes had relatively low LAZ and WAZ with reference to WHO growth standards. Stunted infants had significantly lower z-scores for all anthropometric indicators compared to the non-stunted infants.

Maternal weights and heights were obtained from 539/688 (78.3%) biological mothers. The mean height was 156.8 ± 6.05 cm. A total of 70 (13%) of the mothers had a short stature (height <150.1 cm). Based on BMI, 31 (5.8%) of the mothers were underweight, 201 (37.3%) had normal weight, 165 (30.6%) were overweight and 142 (26.3%) were obese. There was no significant difference in

the proportion of stunted infants for short stature versus normal stature mothers (32.9% versus 26.9%; $P=0.296$). Although not statistically significant ($P=0.118$), underweight mothers tended to have a greater proportion of stunted children (38.7%) compared to normal weight (31.3%), overweight (26.1%) and obese (21.8%) mothers.

Anaemia and iron status of the infants

Table 1 also shows the prevalence of anaemia, ID and IDA in the study infants. In this study boys had a higher prevalence of anaemia than girls (41.3% versus 31.4%, $P=0.005$). Stunted infants had a higher prevalence of anaemia than their non-stunted counterparts [45.3% vs. 33%, ($P=0.002$)]. Stunting was also associated with ID-sTfR ($P=0.002$); IDA-sTfR ($P=0.003$), while there was a trend towards an association with iron deficiency, based on ID-PF ($P=0.083$) and IDA-PF ($P=0.055$).

Logistic regression for the factors associated with stunting

The exploratory univariate analysis revealed that the factors significantly associated with stunting were low birth weight ($P<0.001$), male sex ($P=0.028$), education level $<grade10$ of mother/caregivers ($P=0.014$), anaemia ($P=0.001$), ID-sTfR ($P=0.003$), underweight ($P<0.001$), and not consuming commercial jarred infant foods at least once during the preceding week ($P=0.020$) (**Table 4**). These findings guided the development of a multivariable logistic regression analysis model. Maternal height was included in the final model as short mothers had significantly shorter infants [LAZ -1.70 ± 1.03] compared to normal height mothers [LAZ -1.41 ± 1.08 ; ($P=0.033$)].

The summary of the three models for the multivariable logistic regression analysis are presented in **Table 5**. Model 1 includes all infants of whom the data for variables in the model were complete and was limited by the data for mother's height ($n=518$). Models 2 and 3 include sTfR and AGP, resulting in a smaller sample size ($n=334$, 44.5%), due to low success with sampling of venous blood in this age group. The results based on model 1 show that boys were 1.73 times more likely to be stunted compared to girls (95%CI 1.10 to 2.70, $P=0.017$). Stunting showed an inverse relationship with both birth weight (kg) [OR 0.12, 95%CI 0.07 to 0.21, $P<0.001$] and maternal height (cm) [OR 0.94, 95%CI 0.91 to 0.98, $P=0.001$]. There was a tendency for a negative association between consumption of jarred commercial infant foods and stunting (OR 0.69, 95%CI 0.44 to 1.07, $P=0.099$). Hb (g/dL) and education level of the mother/caregiver showed no association with stunting ($P>0.05$) (Table 5, Model 1). Model 3 shows that higher sTfR (mg/L)

concentration was associated with higher odds for stunting and there was an inverse association between consumption of jarred commercial infant foods and stunting (Table 5).

Discussion

The results of this study show that stunting affected almost a third (28.5%) of the study population. This is of public health concern as there is evidence that stunting may result in poor cognitive and physical development, reduced productivity and increased risk of chronic diseases in adulthood⁽²⁵⁾. Stunting was associated with lower birth weight ($P<0.001$), shorter maternal height ($P=0.001$), and male sex ($P=0.017$) and with higher sTfR concentrations (mg/L) (Table 5). These results support the notion that stunting is associated with poor maternal nutritional status and highlights the need for interventions to prevent the intergenerational origins of stunting.

Compared to the WHO cut-off values for public health significance⁽¹²⁾, the observed prevalence of stunting (28.5%), underweight (11.1%) and wasting (1.7%), indicate that both chronic malnutrition and acute malnutrition were problems of public health significance in this community. In a review paper, Du Plessis and co-workers ⁽²⁶⁾ concluded that high levels of stunting in South Africa is a consequence, in part, of poor breastfeeding and complementary feeding practices, and the poor quality of complementary diets. The observed 28.5% stunting prevalence agrees with findings from the 2012 SANHANES, that found that for children 0-3 years old stunting for boys and girls was 26.9% and 25.9% respectively⁽⁴⁾. However, stunting at age 6 months and stunting over an age range of 0-3 years may not be directly comparable, because the prevalence of stunting has been shown to double within the first two years of life⁽²⁷⁾.

On the contrary, regional studies involving 6–12 months old South African infants reported lower prevalence of stunting at 11%⁽²⁸⁾, 16%⁽²⁹⁾ and 13%⁽²⁶⁾ in KwaZulu-Natal province, and 12% in Eastern Cape province⁽²⁷⁾. Although these differences may be attributed partly to non-representativeness of the regional data, the observed stunting prevalence supports the view that the epidemiology of stunting varies within a country and between boys and girls. This was also reflected in the 2012 SANHANES data for children under 15 years of age, which showed that overall boys were more stunted than girls, and that the boys from North West (23.7%) for example had higher prevalence of stunting compared to those from KwaZulu-Natal (13.5%) and Gauteng (11.9%) provinces⁽⁴⁾.

Logistic regression showed that birth weight (kg) was inversely associated with stunting ($P<0.001$, Table 5). This is in line with previous findings and points towards the association between maternal undernutrition, low birth weight and stunting in children⁽²⁾. Maternal short stature, combined with poor nutrition during pre-conception and pregnancy, may result in small birth size^(30; 31; 32) and subsequent stunting in children^(8; 9; 10). Maternal short stature may therefore explain to some extent the observed 14% low birth weight and 28.5% stunting in our study. Although there is a need to address postnatal factors associated with stunting, evidence shows that, to prevent stunting, a stronger focus is needed on improving the prenatal environment in order to prevent intrauterine growth restriction and the occurrence of low birth weight^(33; 34). This highlights the importance of focusing on women of child bearing age to prevent growth faltering in children.

Multivariable logistic regression analysis showed that boys were 1.73 times ($P=0.017$) more likely to be stunted than girls (Table 5), which concurs with the findings from 16 demographic and health surveys from 10 sub-Saharan countries⁽³⁵⁾. The most probable hypothesis explaining why boys are more vulnerable to stunting than girls is that it occurs already during pregnancy, with sex differences in foetal growth^(36; 37). According to Di Renzo *et al.* (2007) females have a selective advantage over males *in utero* which is associated with subsequent improved outcomes in the perinatal period; male sex is therefore an independent risk factor for small birth size and other adverse pregnancy outcomes⁽³⁸⁾. One other plausible explanation for the higher odds of stunting in boys is that they are selectively more vulnerable to environmental infections resulting in them having increased likelihood for neonatal morbidity compared to female infants⁽³⁹⁾. Although these hypotheses may partly explain our findings, the underlying mechanisms that predispose boys to higher odds of stunting compared to girls are still poorly understood and speculative.

Infants with higher sTfR (mg/L) concentrations were 1.12 times more likely ($P=0.021$) to be stunted than those with lower sTfR (mg/L) concentrations in logistic regression analysis (Model 3, Table 5). The 30.1% prevalence of ID based on sTfR observed in this study may be a more accurate reflection of true ID, compared to the 16% ID based on PF^(24; 40). The co-existence of stunting and iron deficiency in the study infants may reflect underlying poor maternal nutrition⁽⁴¹⁾. Interventions aimed at preventing stunting should therefore also focus on preventing anaemia in young and pregnant women, coupled with promotion of breastfeeding and appropriate complementary feeding from age 6-23 months to maintain the infants' iron stores^(2; 42).

A significant number of infants were stunted (28.5%), anaemic (36.4%) and/or iron deficient (30.1% based on ID-sTfR), despite the fact that fortified infant foods were consumed by the majority of infants. Receiving commercial jarred infant foods at the time of the survey was the only dietary factor that differed between stunted and non-stunted infants. However, in the multivariable binary logistic regression analysis, the negative association between consumption of commercial jarred infant foods and stunting was significant only in model 3 (Table 5), which was based on the smaller sample size ($n=334$). We acknowledge that consumption of specific complementary foods during the past week does not reflect early feeding practices. It should however be noted that that duration of exclusive breastfeeding ($P= 0.361$); and age of introducing milk feeds ($P= 0.186$), other liquids ($P= 0.644$) and semi/solid foods ($P= 0.464$) did not differ between stunted and non-stunted infants (Table 2). At the age of six months, most infants have consumed a relatively small total amount of complementary foods and over a relatively short period. It is therefore unlikely that differences in complementary foods consumed could have affected linear growth in our study population.

The early use of commercial infant foods as observed in this study, has been previously reported for South African infants^(4; 5). Early introduction of complementary foods explains the low exclusive breastfeeding rates (5.9%) observed in this study, which is similar to the 2012 SANHANES findings of 7.4% exclusive breastfeeding⁽⁴⁾. Siziba et al reported 12% exclusive breastfeeding in four of the nine provinces of South Africa⁽⁴³⁾. The risk of mixed feeding over exclusive breastfeeding for infants younger than 6 months is an increased risk of infections, which in the long-term may lead to stunting via the enteropathy mechanism⁽⁴⁴⁾. In addition, stopping breastfeeding and introducing solids before 4 months increases the risk of obesity later in childhood⁽⁴⁵⁾. Within the South African context of high HIV/AIDS prevalence and poverty, and high prevalence of obesity, efforts should be made to counter the strong cultural beliefs and other barriers to exclusive breastfeeding⁽⁴⁶⁾ as part of stunting prevention strategy.

The cross-sectional nature of this study limits ability to make inferences on causation. Other limitations include that the gestational age of infants could not be recorded accurately due to lack of information on health records. Gestational age is important in the interpretation of low birth weight^(47; 48), therefore the interpretation of low birth weight could have been compromised. Models 2 and 3 of the multivariate analysis are based only on 44.5% of the total study sample for infants

who had all variables included in the models. It is possible that the analyses for these models for the multivariate analysis could be underpowered. Due to difficulty in obtaining blood samples, sTfR and PF values were available for only 485 (64.7%) of the 750 infants. Therefore, all iron indicators except Hb presented in this paper can be considered exploratory. However, when comparing those with a blood sample ($n=485$) to those without a blood sample ($n=266$), no significant differences were observed for mother's height ($P=0.678$), and sex distribution ($P=0.619$), but there was a significant difference between the two groups for low birthweight (52.9% vs. 47.1%, $P=0.012$).

Nevertheless, the study contributed to the body of knowledge that shows the link between socio-economic factors, maternal factors, feeding practices and stunting in 6-month old infants from vulnerable populations. Furthermore, the 10.1% prevalence of obesity and 28.5% prevalence of stunting indicate the presence of the double burden of malnutrition already during infancy and reflect the nutrition transition in South Africa. There is therefore a need for co-ordinated efforts and effective implementation of existing plans and strategies that focus on the 1000-day window of opportunity to prevent long-term consequences of stunting without exacerbating the problem of overweight and obesity.

Conclusions

The study showed that the prevalence of stunting (28.5%) was of public health significance and was significantly associated with lower birth weight, shorter maternal height, male sex and being iron deficient (sTfR). Interventions that focus on improving pre-conceptual and maternal nutritional status, as well as early feeding practices may be an important strategy to prevent stunting in infants in vulnerable populations to prevent the long-term consequences of stunting on cognitive, motor and physical development.

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Conflicts of interest

The authors declare no conflict of interest except CM. Smuts who received speaking honoraria from Unilever.

Author contributions

TM was involved in supervising field data collection and data quality control, data analysis and interpretation of results, and drafted the paper. MR contributed to supervising field data collection and quality control for feeding practices data, and review of the paper. MS, MF and SK initiated the study and contributed training, guidance on data collection, quality control and analysis, academic input and review of the paper. All authors read and approved the final manuscript.

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Tables

Table 3. 1: Baseline socio-demographic, household characteristics and iron status and comparison according to stunting¹

	Total (n=750)	Not stunted (n=536)	Stunted (n=214)	P²
Caregiver's relation with infant				
Biological mother	688 (91.7)	488 (91)	200 (93.5)	0.749
Grandmother	32 (4.3)	26 (4.9)	6 (2.8)	
Aunt	21 (2.8)	16 (3)	5 (2.3)	
Father	6 (0.8)	4 (0.7)	2 (0.9)	
Not related caregiver	3 (0.4)	2 (0.4)	1 (0.5)	
Marital status of mother/caregivers				
Not married	415 (55.3)	298 (55.6)	117 (54.5)	0.328
Living together	212 (28.3)	143 (26.7)	69 (32.2)	
Married	80 (10.7)	61 (11.4)	19 (8.9)	
Common-law husband/wife	26 (3.5)	19 (3.5)	7 (3.3)	
Widowed/widow	10 (1.3)	8 (1.5)	2 (0.9)	
Separated/divorced	7 (0.9)	7 (1.3)	0 (0.0)	
Level of education				
Higher than grade 10 (FET) ³	601 (81.3)	442 (83.6)	159(75.7)	0.002*
Less than grade 10	138 (18.7)	87 (16.4)	51 (24.3)	
Tap water at home	719 (95.8)	513 (95.7)	206 (96.3)	0.626
Flush toilet at home	713 (95.1)	511 (95.3)	202 (94.4)	0.703
Electricity at home	692 (92.3)	502 (93.7)	190 (88.8)	0.024*
Iron and Inflammatory status				
Anaemic (n=750)	274 (36.5)	177 (33)	97 (45.3)	0.002*
CRP >5 mg/L (n=485).	72 (14.8)	50 (14.3)	22 (16.3)	0.577
AGP >1 g/L	156 (32.2)	104 (29.7)	52 (38.5)	0.063
ID-PF ⁴	78 (16.1)	50 (14.3)	28 (20.7)	0.083
ID-sTfR ⁵	146 (30.1)	92 (26.3)	54 (40)	0.002*
IDA-PF ⁶	51 (10.5)	31 (8.9)	20 (14.8)	0.055
IDA-sTfR ⁷	71 (14.6)	41 (11.7)	30 (22.2)	0.003*

CRP, C-reactive protein; AGP, Alpha-1 glycoprotein; ID, iron deficiency; sTfR, soluble transferrin receptor; Hb, haemoglobin; PF, plasma ferritin. ¹All values are number (n), (%), ²Pvalue for Pearson's Chi-Square. ³FET, further education and training corresponds to more than 10 years of schooling in South Africa.; Anaemic(n=750) as Hb<11 g/dL; ⁴ID-PF as PF <12 µg/L, ⁵ID-sTfR as sTfR>8.3 mg/L, ⁶IDA-PF as Hb<11 g/dL + PF <12 µg/L, ⁷IDA-STfR as Hb<11 g/dL + sTfR>8.3 mg/L (n=485). The cut-offs for anaemia, ID and IDA were based on WHO standards ⁽⁴⁸⁾.

*Significant at $P<0.05$ for Chi-Square.

Table 3. 2: The feeding practices at age 6 months for the infants¹

Characteristic	Total (n=750)	Not stunted (n=536)	Stunted (n=214)	P ²
Age, cessation of exclusive breastfeeding				
0–2 months	367 (48.9)	268 (50.0)	99 (46.3)	0.361
3–4 months	271 (36.1)	194 (36.2)	77 (36)	
5–6 months	112 (14.9)	74 (13.8)	38 (17.8)	
Age, milk feeds introduced				
0–2 months	159 (21.4)	118 (22.3)	41 (19.2)	0.186
3–4 months	113 (15.2)	88 (16.7)	25 (11.7)	
5–6 months	67 (9.0)	46 (8.7)	21 (9.8)	
Not Started	403 (54.3)	276 (52.3)	127 (59.3)	
Age, other liquids introduced				
0–2 months	342 (45.6)	248 (46.3)	94 (43.9)	0.644
3–4 months	253 (33.7)	183 (34.1)	70 (32.7)	
5–6 months	116 (15.5)	80 (14.9)	36 (16.8)	
Not Started	39 (5.2)	25 (4.7)	14 (6.5)	
Age, semi-solid/solid foods introduced				
0–2 months	123 (16.6)	93 (17.5)	30 (14.2)	0.464
3–4 months	326 (43.9)	227 (42.7)	99 (46.9)	
5–6 months	249 (33.6)	182 (34.3)	67 (31.8)	
Not started	44 (5.9)	29 (5.5)	15 (7.1)	
<i>Foods that infants were consumed (at least once in the previous week):</i>				
Formula milk	352 (47.5)	262 (49.5)	90 (42.5)	0.081
Dairy foods ³	412 (55.6)	299 (56.5)	113 (53.3)	0.425
Jarred commercial infant foods	410 (55.3)	307 (58.0)	103 (48.6)	0.019*
Infant cereals	596 (80.4)	419 (79.2)	177 (83.5)	0.184
Maize meal porridge ⁴	289 (39.0)	205 (38.8)	84 (39.6)	0.826
Fruits and vegetables ⁵	462 (62.3)	338 (63.9)	124 (58.5)	0.170
Animal source foods ⁶	223 (30.1)	163 (30.8)	60 (28.3)	0.501
Sweetened cold drinks ⁷	173 (23.3)	122 (23.1)	51 (24.1)	0.772
Fats ⁸	398 (53.7)	281 (53.1)	117 (55.2)	0.610

¹All values are number (n), (%), ²P value for Pearson's Chi-Square test. ³Dairy foods, cow's milk and yoghurt. ⁴Maize meal porridge, home prepared maize porridge and instant maize porridge. ⁵Fruits and vegetables, fresh fruits and vegetables and fruit juice. ⁶Animal source foods, red meats, chicken, liver and fish. ⁷Sweetened cold drinks, fizzy drinks and dilutable drinks. ⁸Fats, cooking oil and margarine used in preparing infant foods. *Significant at $P < 0.05$ for Chi-Square.

Table 3. 3: Mean anthropometric indices and comparison according to stunting¹

(Mean ± SD)	All Infants (n=750)	Not stunted (n=536)	Stunted (n=214)	P ²
LAZ	-1.44 ± 1.07	-0.94 ± 0.75	-2.72 ± 0.60	<0.001*
WLZ	0.54 ± 1.15	0.62 ± 1.15	0.33 ± 1.13	0.002*
WAZ	-0.57 ± 1.21	-0.16 ± 1.06	-1.60 ± 0.93	<0.001*
BAZ	0.37 ± 1.19	0.52 ± 1.18	0.01 ± 1.15	<0.001*
MUACZ	0.25 ± 1.09	0.51 ± 1.03	-0.39 ± 0.97	<0.001*
HCZ	0.03 ± 1.00	0.28 ± 0.92	-0.57 ± 0.97	<0.001*

LAZ, length for age z-score; WLZ, weight for length z-score; WAZ, weight for age z-score; BAZ, BMI for age z-score; MUACZ, mid-upper arm circumference z-score; HCZ, head circumference z-score. ¹Anthropometric values are mean, (95% CI). ²P value for t-test. *Significant at $P < 0.05$ for Chi-Square.

Table 3. 4: Factors associated with stunting at 6 months of age from univariate logistic regression analysis ($P<0.1$)

	B	S.E.	P¹	Odds Ratio	95% CI: Odds Ratio	
					Lower	Upper
Male sex (boys)	0.36	0.16	0.028*	1.43	1.04	1.97
Wasted (WLZ <-2SD)	0.78	0.56	0.166	2.18	0.72	6.56
Underweight (WAZ <-2SD)	2.53	0.28	<0.001*	12.55	7.22	21.82
Low birth weight (<2.5 kg)	1.51	0.22	<0.001*	4.53	2.93	7.00
ID-sTfR (sTfR >8.3mg/L)	0.63	0.21	0.003*	1.87	1.23	2.84
Anaemia (Hb <11g/dL)	-0.20	0.06	0.001*	0.82	0.73	0.93
ID-PF (PF < 12 µg/L)	0.45	0.26	0.085	1.57	0.94	2.62
Raised AGP >1 g/L	0.39	0.21	0.064	1.48	0.98	2.25
Mother/caregiver education <10y ²	0.49	0.20	0.014*	1.63	1.10	2.41
Maternal height <150.1 cm	0.29	0.28	0.297	1.33	0.78	2.28
<i>Consumption of foods at least once during the previous week:</i>						
Fruits and vegetables	-0.23	0.17	0.170	0.80	0.58	1.10
Infant cereals	0.28	0.21	0.185	1.33	0.87	2.02
Jarred commercial infant foods	-0.38	0.16	0.020*	0.68	0.50	0.94
Formula milk	-0.29	0.16	0.082	0.75	0.55	1.04

Hb, haemoglobin; ID, iron deficiency; sTfR, soluble transferrin receptor. ¹P value from univariate binary logistic regression analysis. ²Education <grade 10 for mother/caregiver. *Significant at $P<0.05$ for Chi-Square.

Table 3. 5: Summary of three multivariable binary logistic regression analysis models on odds for stunting

	¹ Model 1 [Full & final model 1 (n=518)]			² Model 2 [Full model (n=334)]			² Model 3 [Final model 2 (n=334)]		
Variable	B (SE)	P ³	OR (95%CI)	B (SE)	P ³	OR (95%CI)	B (SE)	P ³	OR (95%CI)
Sex	0.55 (0.23)	0.014*	1.73	0.34 (0.28)	0.230	1.40	0.36 (0.28)	0.197	1.43
(0=girls, 1=boys)			(1.10, 2.70)			(0.81, 2.43)			(0.83, 2.48)
Birth weight (kg)	-2.09 (0.26)	<0.001*	0.12	-1.71 (0.34)	<0.001*	0.18	-1.72 (0.34)	<0.001*	0.18
			(0.07, 0.21)			(0.09, 0.35)			(0.09, 0.35)
Hb (g/dL)	-0.09 (0.08)	0.305	0.92	-	-	-	-	-	-
			(0.78, 1.08)						
Maternal height (cm)	-0.06 (0.02)	0.001*	0.94	-0.05 (0.02)	0.024*	0.95	-0.05 (0.02)	0.024*	0.95
			(0.91, 0.98)			(0.91, 0.99)			(0.91, 0.99)
Education level of mother/caregiver	0.22 (0.30)	0.472	1.24	0.38 (0.36)	0.284	1.47	-	-	-
(0 = >grade 10, 1 = < grade 10)			(0.69, 2.23)			(0.73, 2.95)			
Consumption of jarred commercial infant foods ⁴	-0.38 (0.23)	0.099	0.69	-0.55 (0.28)	0.051	0.58	-0.61 (0.27)	0.025*	0.54
(0 = No, 1 = Yes)			(0.44, 1.07)			(0.33, 1.00)			(0.32, 0.93)
sTfR (mg/L)	-	-	-	0.11 (0.05)	0.017*	1.12	0.11 (0.05)	0.021*	1.12
						(1.02, 1.23)			(1.02, 1.22)
AGP (g/L)	-	-	-	-	-	-	-	-	-
Model goodness of fit	⁵ R Square (0.293), P ⁶ =0.652 (Model 1)			⁵ R Square (0.243), P ⁶ =0.051 (Model 2)			⁵ R Square (0.239), P ⁶ =0.212 (Model 3)		

¹Model 1: only 518/750 of the participants had complete data for all the variables included in the model. ²Model 2 and 3 had a smaller sample size (n=334/750) with complete data for all the variables included in the model due to difficulty in obtaining blood samples for ID-sTfR. Conditional backward elimination was used to select variables. ³Pvalue from multivariable binary logistic regression analysis. ⁴Consumption of jarred infant foods at least once in the previous week. Dependent variable was stunting vs. non-stunted. ⁵The Nagelkerke R Square, ⁶P-value for Hosmer and Lemeshow test. *Significant at P<0.05 for Chi-Square.

Effects of small-quantity lipid-based nutrient-supplements on growth in 6- to 12-month-old South African infants: a randomised control trial



Top Left the exit visit of the last infant from the study and **Top Right** the Author (front left) with field team at the study site.

Bottom Left SK handling anthropometry training for assessors and **Bottom Right** the Anthropometry assessors.

This chapter is presented in article format and is written according to the referencing requirements of the *American Journal of Clinical Nutrition* to which it will be submitted. The font and spacing were kept the same throughout the thesis. All figures and tables are presented within the text rather than at the end of the article for examination purposes.

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CHAPTER 4: RESEARCH ARTICLE 2

Effects of small-quantity lipid-based nutrient-supplements on growth in 6- to 12-month-old South African infants: a randomised control trial.

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Abstract

Background: There is inconclusive evidence on the efficacy of small-quantity lipid-based nutrient supplements (SQ-LNS) as home fortificants on prevention of malnutrition in children aged 6-23 months. **Objective:** To investigate the hypothesis that provision of SQ-LNS from 6 months of age would result in improved growth in infants compared to a control group. **Design:** This was a randomised controlled trial of 750 infants randomised to SQ-LNS A with essential fatty acids (EFAs), SQ-LNS B with EFAs, docosahexaenoic acid (DHA) and arachidonic acid (ARA), *phytase*, powder milk and lysine and a control group. At baseline and age 8, 10 and 12 months, length-for-age, weight-for-length and weight-for-age z-scores were assessed. There were weekly home visits to monitor adherence, morbidity, and developmental outcomes over a 6-month intervention period. The linear splines model was used to examine intervention effects longitudinally. **Results:** The results showed an overall positive effect on length for age z-scores (LAZ) ($P = 0.036$) in the SQ-LNS B group compared to the control, this was driven by significantly better linear growth at age 8 and 10 months., [LAZ ($\pm$ SE) increased by 0.13 ± 0.07]. The prevalence of stunting was 28.5% at baseline and 38.3% at end of the study. The end point prevalence of stunting was 41.1%, 38.3%, 36.3% among infants from the SQ-LNS A, SQ-LNS B and control group respectively ($P=0.664$). **Conclusions:** Both SQ-LNS A and SQ-LNS B did not show an effect on growth at 12 months of age. However, SQ-LNS B showed better linear growth at 8 and 10 months of age compared to control among infants from a peri-urban setting in South Africa. This trial was registered at <http://clinicaltrials.gov> as NTC01845610.

Key Words: stunting, lipid-based nutrient supplements, motor development, linear growth, South Africa

Introduction

Growth faltering and associated stunting mainly occurs within the 1000 day window period (1) and is a strong predictor of poor child development (2). In 2014, stunting was the most prevalent form of undernutrition affecting 159 million children less than five years old globally. Most of the stunted children lived in South Asia and Sub-Saharan Africa (3). Although, the pathogenesis of linear growth faltering is still poorly understood and unclear (4), stunted children usually experience delayed cognitive development, increased morbidity and mortality (5).

There is evidence that for children the complementary feeding period, age 6-23 months is the most critical (1), due to increased energy and nutrient needs for growth. This is important for settings in which the complementary diets are limited in quality and quantity (6, 7). In Asia and Africa where stunting is most prevalent, the complementary diets are based largely on plant sources with high levels of anti-nutritional factors i.e. phytates and have low energy and nutrient content (6). This appears to be the major contributor to growth faltering and associated high levels of stunting and undernutrition in infants and children in these regions.

In efforts to address this problem of poor complementary diets, small-quantity lipid-based nutrient supplements (SQ-LNS) have been designed for home fortification of local foods, and not to replace them (6). SQ-LNS have been evaluated for efficacy and effectiveness in several trials (8-11). These supplements are unique, in that they provide a low energy dose (~20 g or ~110–120 kcal/day) with reference to the estimated energy requirements (EER) of 743 kcal/day for children aged 6-12 months, but they still contain the full complement of vitamins, minerals, and essential fatty acids (12, 13), this ensures that breast milk intake is not compromised.

These SQ-LNS have been shown to prevent linear growth faltering from 6 to 11 months in studies from Ghana (9), Malawi (14), Democratic Republic of Congo (15) Haiti (8), and Burkina Faso (16). However, some trials have failed to find significant positive effects on linear growth in 6 months old infants followed for 6 six months (10, 17). The failure to show intervention effects can be attributed to differences in the respective study designs. Overall the evidence indicates that SQ-LNS have the potential to prevent growth faltering in infants and children. There is limited evidence to support the proposition that SQ-LNS are effective

in preventing growth faltering in infants aged 6-12 months. Therefore, more research needs to be done to ascertain their efficacy and effectiveness in low-income settings.

The impact of SQ-LNS in the context of a maize-based diet has not been well investigated, particularly in South Africa and other countries where maize is staple. Compared to rice or wheat, maize has higher levels of phytates, which bind trace elements such as iron and zinc, and inhibit their absorption (18). Testing the efficacy of a newly developed high quality product that contains both docosahexaenoic acid (DHA) to improve vision and cognition (reviewed by Hoffman et al. 2009) and *phytase* (150 FTU) to improve iron and zinc bioavailability (Troesch et al. 2011), is important in the context of a maize-based weaning diets.

Therefore, the aim of this study was to investigate the effects of providing soy based SQ-LNS from 6 months of age on linear growth, weight gain infants over a 6-month period in a peri-urban setting in South Africa. The SQ-LNS used in this study are cost effective home fortificants compared with previous SQ-LNS formulations like *NutriButter*® (8, 9, 16) considering that soy is cheaper than peanuts.

Subjects and methods

Study site

The intervention study was carried out in the peri-urban area of the Matlosana municipality, North West province of South Africa, involving 750 approximately 6-month-old infants (5.85–7.01 months) from September 2013 to July 2015. Infants born as a singleton resident in the study area and not known to be HIV positive at the screening visit were enrolled in the study at age 6 months but less than 7 months. The inclusion criteria included infants who were apparently healthy, planning to stay in the study area for at least the next 7 months, no known allergy to peanut, soy, milk and/or fish, not lactose intolerant, no congenital abnormalities, previously breastfed, did not received any special nutritional supplements. Infants were excluded if they had weight for length z-score (WLZ) <-3SD, and haemoglobin <7.0 g/dL.

The mother-infant pairs were recruited from the study area by fieldworkers speaking the local language who explained the expectations of the study to the mothers and if they were willing to participate they signed an informed consent form.

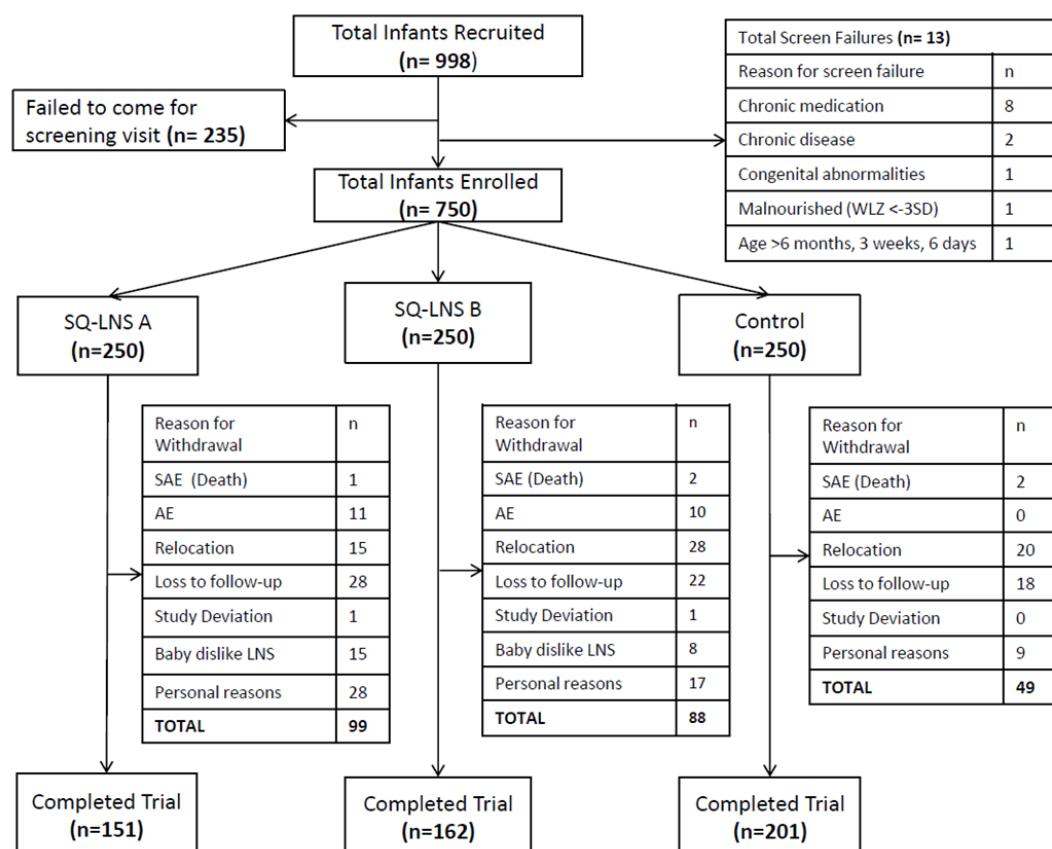


Figure 4. 1: Flow diagram of participant progression through the intervention study¹.

¹Total of 998 infants were recruited and 750 were randomly assigned to one of the 3 groups: SQ-LNS, small quantity lipid based supplements (SQ-LNS A, and SQ-LNS B) and control. In total 514 (68.5%) infants completed the trial. The infants were followed weekly by field workers to monitor morbidity, adherence and achievement of motor milestones. WLZ, weight for length z scores; AE, adverse events; SAE, serious adverse events.

Study design and subjects

The study was a 6 month follow up, randomised controlled trial, with the blinding done for the statistician, anthropometry assessors, and laboratory staff. Infants were recruited when they were 4–6 months old and at the age of 6 months, block randomisation of sizes 3, 6 and 9 was used to randomly allocate the infants to one of three groups SQ-LNS A ($n = 250$), SQ-LNS B ($n = 250$) and control group ($n = 250$). Group randomisation lists were prepared using

NQuery version 7 (Statistical Solutions Ltd, Cork, Ireland) and at the baseline visit the next sequential baby and group code was allocated to the next infant enrolled.

The study was approved by the ethics committees of North-West University (NWU) (NWU-00001-11-A1) and the South African Medical Research Council (SAMRC) (EC-01-03/2012) and designed in accordance with the Helsinki Declaration (19). After institutional ethical approval, the project was reviewed by the provincial Department of Health and Social Development for registration with the Directorate for Policy, Planning and Research. The community's approval to conduct the study was sought through an engagement process with relevant community-based stakeholders. Infants who were found to be severely anaemic or malnourished during screening were referred to the primary health care clinic for medical attention and excluded from the study. The Data Safety Monitoring Board comprised of a paediatrician/allergy expert, nutrition researcher, biostatistician and they carried out quarterly review of blinded adverse events (AE) and serious adverse events (SAE) data. The randomized controlled trial was registered at <https://clinicaltrials.gov/> registry as NTC01845610.

Sample size calculation

The sample size for the trial was based on the expected difference in growth achieved during the 6 months of active intervention. Using linear growth as main outcome sample size was computed based on an expected difference of 0.15 length-for-age z-scores (LAZ scores) at 12 months of age with a pooled standard deviation of 0.54 (i.e. effect size of 0.27) between intervention groups and control group with 80 % power and a type-1 error of 5 %. The required sample size of 186 infants per group was adapted to allow for an expected drop-out rate of 25%. Therefore 250 infants per group (750 in total) were enrolled [A total of 998 caretaker-infant pairs were recruited, 235 failed to come for a baseline visit and 13 were excluded because they were not eligible] (**Figure 4.1**).

Study Foods

The infants were allocated to one of three groups SQ-LNS A received a fortified fat-based paste containing essential fatty acids (without DHA and ARA). SQ-LNS B received a fortified fat-based paste containing essential fatty acids and added docosahexaenoic acid (DHA), arachidonic acid (ARA) and *phytase*. Both products were soy-based. The control group did not receive SQ-LNS for the duration of the trial (age 6-12 months) and only

received SQ-LNS after the end of the trial (age 12-18 months). The two SQ-LNS had different packaging and as such it was not possible to blind the participants and study staff involved in distribution of the SQ-LNS. The nutritional profiles of SQ-LNS A and SQ-LNS B are presented in **Table 4.1**.

Table 4. 1: SQ-LNS nutritional profile per portion and dietary reference intakes for infants 6-12 month old¹

Nutrient	SQ-LNS A	SQ-LNS B	AI/RDA ² (6-12 mo of age)
Amount (g) (1 portion)	20	20	-
Energy (kcal)	114	113	-
Energy density (kcal/g)	5.7	5.7	-
Protein (g)	3.0	3.7	11
% calories from protein	10%	13%	-
Fat (g)	8.0	8.8	30
% calories from fat	63%	70%	-
Essential fatty acids			
Linoleic acid (LA), (g)	1.5	1.8	4.6
α -linolenic acid (ALA), (mg)	265	348	500
n-6/n-3 ratio	5.7	5.0	-
Long-chain polyunsaturated fatty acids			
Docosahexaenoic acid (DHA), (mg)	-	75	-
Arachidonic acid (ARA), (mg)	-	75	-
Micronutrients			
Vitamin A (μ g)	200	200	500
Vitamin D (μ g)	2.5	2.5	10
Vitamin E (mg)	2.5	2.5	5
Vitamin K (μ g)	7.5	7.5	2.5
Thiamine (mg)	0.25	0.25	0.3
Riboflavin (mg)	0.25	0.25	0.4
Niacin (mg)	3	3	4
Pantothenate (mg)	1.0	1.0	1.8
Vitamin B6 (mg)	0.25	0.25	0.3
Biotin (μ g)	4.0	4.0	-
Folate (B9) (μ g)	80	80	80
Vitamin B12 (μ g)	0.45	0.45	0.5
Vitamin C (mg)	23.3	23.3	50
Calcium (mg)	250	396	260
Iodine (μ g)	45	45	130
Iron (mg)	5.8	5.8	11
Zinc (mg)	6.2	6.2	2.5
Copper (mg)	0.28	0.28	0.22
Selenium (μ g)	8.5	8.5	20
Magnesium (mg)	-	30	75
Manganese (mg)	-	0.6	0.6
Phosphorus (mg)	-	230	275
Potassium (mg)	-	257	700
Other			
L-Lysine (mg)	-	160	-
Phytase (FTU)	-	200	-

AI, adequate intake; SQ-LNS, small-quantity lipid-based nutrient supplement; RDA, Recommended Dietary Allowance. ¹The SQ-LNS supplements had specified storage temperature 22-35°C and packed in 20g sachets by Unilever R&D (Vlaardingen, Netherlands) and DSM nutritional products Ltd (Basel, Switzerland). The AI/RDA² are based on the Food and Nutrition Board, Institute of Medicine, National Academies (20-25).

The efficacy trial was preceded by acceptability trials of the two SQ-LNS products for infants 6-12 months old in the same community. Based on the mother's own acceptance and the perception of her infant's acceptance the products were considered acceptable by more than 80% of the mothers (26).

Intervention and follow-up

At baseline (infant age 6 months) and end (infant age 12 months) the infant's data on anthropometry, dietary, morbidity and blood samples were collected. The fieldworkers performed weekly home visits to deliver supplements and monitor adherence and morbidity surveillance. Every week the consumption of the supplements was recorded on a standard adherence form to account for supplements used, not used and collect the empty sachets as part of the adherence assessment. Weekly the fieldworkers left the mothers with 2 weeks' supply to cater for missed visits. The two treatment groups and the control group were treated and monitored using the same criteria except that the control group did not receive supplements. Infants consumed 1 sachet (20g) of the SQ-LNS daily for 6 months (26 weeks). Mothers were advised to give SQ-LNS paste as part of the first meal and mixing it with usual complementary foods.

Adherence was calculated based on the formula: *Adherence = Total intake = (sum of weekly intake [g]) / (days in study*20)*. The weights and lengths were measured at bi-monthly intervals (8 months and 10 months old). Monitoring visits were conducted bi-weekly by an independent clinical research organisation (OnQ Research PTY LTD, Johannesburg, South Africa) and monthly refresher trainings were conducted for the fieldworkers and other study personnel. All data collection tools and procedures were conducted following the principles of good clinical practice (GCP) and interviews were conducted in the mother's preferred language.

Measurement of outcome variables

Structured questionnaires that were developed based on WHO guidelines (27), were used to assess the breastfeeding and complementary feeding practices of study infants retrospectively. The indicators used included duration of exclusive breastfeeding, age of introduction of liquids, milk feeds and solid foods and meal frequency per day. A structured questionnaire was used and it covered questions on socio-demographics, water and sanitation, education of mothers and fathers, size of households, employment status, marital status, and

primary health care usage for the infants and morbidity. Anthropometric status was assessed using the WHO Child Growth Standards (28). Anthropometric indexes for infants (WAZ, LAZ, and WLZ) were calculated based on the WHO classification (28). Wasting was defined as WLZ less than -2SD, stunting as LAZ less than -2SD and underweight as WAZ less than -2SD (29).

Weight and recumbent length were taken according to WHO standardized techniques (30). The anthropometry assessors were trained according to the WHO Training Course on Child Growth Assessment for the infants (31). Infants were undressed and weighed to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG., Hamburg, Germany, maximum weight 20 kg). Recumbent length was measured to the nearest 0.1 cm using infantometer (Seca model 416, GmbH & Co. KG., Hamburg, Germany). Mid-upper arm circumference (MUAC) and head circumference were measured using a measuring tape (Seca 201, GmbH & Co. KG., Hamburg, Germany) and a measuring tape for head circumference (Seca 212, GmbH & Co. KG., Hamburg, Germany) respectively. All measurements were done in duplicate and if the first two measurements differed by >0.05 kg for weight or by >0.3 cm for length a third measurement was done and the two closest values were recorded. Calibration of instruments and validation of measurements and random auditing were done on a daily basis. Anthropometric indices LAZ, WAZ and WLZ were generated using WHO *Anthro* 2005 software.

Statistics

The socio-economic, anthropometric and blood results data were captured by an independent clinical research organisation (CRO) (ClinTec, International Pvt. Ltd, Bangalore, India). The feeding practices data was entered into EpiData version 3.1 (EpiData Association, Denmark). Quality control and source data verification was done bi-weekly during monitoring visits by the CRO.

The basis of the analysis of the trial was intention to treat, to investigate the effectiveness of SQ-LNS with respect to linear growth and secondary outcomes. A mixed effects regression (piecewise) model was fitted via maximum likelihood to all participants in the trial ($n = 750$) which is needed for the intention to treat analysis (32). The splines were created by using the *mkspline* program in STATA version 14 (StataCorp Ltd, USA). Two knots were used and they were placed at 252 days (36 weeks) and 317 days (45 weeks) which were the median

time points for the visits at ages 8 and 10 months old. The baseline value of the measurement (at age 6 months) was used as a covariate in the model to improve precision. The mixed effect model was implemented in STATA 14 using the mixed command. Including sex as a random effect made very little difference to the fixed effects estimates and thus the intention to treat model for this study did not include an adjustment for sex.

Data analysis was performed using SAS V9.4 (SAS Institute, Inc, Cary, NC), STATA V14 (StataCorp Ltd, USA) and SPSS software version 23 (IBM, Corporation USA). The normality of the data was tested using Shapiro-Wilk test. Descriptive data is reported using frequencies and cross-tabulations. Continuous data is reported as mean and 95% confidence intervals (CI) for normally distributed data, whereas data not normally distributed is presented as the median and interquartile range (IQR). To test for significant differences between means of groups the ANOVA for all three groups and independent t-test for comparison of each intervention with control were used, while associations between categorical data were determined using the Pearson chi-square test. Descriptive statistics were used to describe socio-economic status and feeding practices. For all analysis, statistical significance was set at $P < 0.05$ and 95% confidence interval was used for treatment effects.

Results

A total of 750 infants were randomly assigned to one of the three groups and 514 (68.5%) infants completed the trial. The reasons for lost to follow up were mothers relocating out of the study area or changing address without notifying study staff, infants refusing the SQ-LNS, AE/SAE related concerns and due to personal reasons (Figure 4.1). In addition, five babies died due to causes unrelated to the study. Baseline characteristics did not differ by trial arm with the exception of infant sex ($P = 0.026$) and employment status of the mothers and/or caregivers ($P = 0.027$) in the SQ-LNS A group compared with SQ-LNS B and control groups (**Table 4.2**). The mean age in months (95% CI) was 6.22 (6.21 to 6.24) and 51.6% were boys. Low birth weight (birth weight < 2500 g for full-term babies) was prevalent in 14% and 70.1% of infants were still being breastfed at age 6 months.

Table 4. 2: Baseline characteristics of the participants at enrolment, for intervention groups and control

Characteristics	SQ-LNS A (n = 250)	SQ-LNS B (n = 250)	Control (n = 250)	P ¹
Infant's sex, male [n (%)]	113 (45.2)	143 (57.2)	131 (52.4)	0.026*
Female [n (%)]	137 (54.8)	107 (42.8)	119 (47.6)	
Age, months mean (SD)	6.22 (0.26)	6.22 (0.24)	6.22 (0.25)	0.949
Birth weight, kg, mean (SD)	2.97 (0.54)	2.94 (0.52)	3.03 (0.47)	0.118
Low birth weight** [n (%)]	38 (15.6)	40 (16.7)	24 (9.8)	0.061
<i>Anthropometric status</i>				
LAZ, mean (SD)	-1.45 (1.06)	-1.53 (1.06)	-1.36 (1.09)	0.883
Stunted, <-2 LAZ, [n (%)]	69 (27.6)	74 (29.6)	71 (28.4)	
WAZ, mean (SD)	-0.62 (1.19)	-0.54 (1.18)	-0.55(1.27)	0.839
Underweight, <-2 WAZ, [n (%)]	26 (10.4)	27 (10.8)	30 (12)	
WLZ, mean (SD)	0.47 (1.14)	0.65 (1.15)	0.49 (1.12)	0.176
Wasted, <-2 WLZ, [n (%)]	4 (1.6)	3 (1.2)	6 (2.4)	0.578
Overweight >+2 WLZ, [n (%)]	18 (7.2)	30 (12.0)	28 (11.2)	0.163
Breastfeeding at 6 months [n (%)]	182 (72.8)	178 (71.2)	165 (66.3)	0.252
Age EBF stopped, [n (%)]				
0-2 months	117 (46.8)	116 (46.4)	134 (53.6)	0.254
3-4 months	100 (40.0)	93 (37.2)	78 (31.2)	
5-6 months	33 (13.2)	41 (16.4)	38 (15.2)	
<i>Caregiver characteristics</i>				
Caregiver age (years), mean (SD)	28.1 (8.40)	27.9 (8.03)	29.3 (9.28)	0.125
Education, >=Grade 10, [n (%)]	209 (85.0)	197 (79.4)	195 (79.6)	0.201
Married [n (%)]	29 (11.6)	20 (8.0)	31 (12.4)	0.774
<i>Household characteristics</i>				
Electricity at home [n (%)]	229 (91.6)	230 (92.0)	233 (93.2)	0.784
Total number of people, median (IQR)	6 (4, 7)	5 (4, 7)	6 (4, 7)	0.867
Children <5years old, median (IQR)	1 (1, 2)	1 (1, 2)	1 (1, 2)	0.923
People earning income, median (IQR)	1 (1, 1)	1 (0, 1)	1 (0, 1)	0.285
Child grants, median (IQR)	2 (2, 3)	2 (1, 3)	2 (1, 3)	0.157

Data presented as mean standard deviation (SD) or number (n) (%). **22 (2.9%) infants had missing information on birth weight. ¹P-value for Pearson Chi-square and ANOVA. Values with in the same row with (*) are significantly different, $P < 0.05$. Exclusive breastfeeding; EBF, IQR; interquartile range, LAZ, Length for age z-score; WAZ, weight for age z-score; WLZ, weight for length z-score. Anthropometry indicators (LAZ, WLZ and WAZ) were calculated based on WHO classifications (28).

The prevalence of stunting, wasting, underweight and overweight were 28.5%, 1.7%, 11.1%, 10.1% at baseline (age 6 months) and 38.3%, 1.6%, 1.4%, 5.1% at the end of the study (age 12 months) respectively. At all-time points, there was no significant differences across groups for all anthropometric indicators ($P > 0.05$). The intervention groups had significantly lower LAZ when compared to control group at baseline. This imbalance in LAZ at baseline was adjusted for in the estimation of intervention effects. The regression model results show that there was a significant interaction with intervention in SQ-LNS B ($P = 0.036$) over time but not for SQ-LNS A ($P = 0.457$). Therefore, taking into account the difference in mean LAZ at baseline between control and SQ-LNS B (-0.17 units) the participants in SQ-LNS B had better growth over the study period than what was experienced in the control group (**Table 4.3**).

Table 4. 3: Mean length for age (LAZ) and weight for age (WAZ) over study periods by group and predicted linear spline LAZ and WAZ over study period and mean differences between active arms and control¹

Month	SQ-LNS A		SQ-LNS B		control		SQ-LNS A vs. control				SQ-LNS B vs. control			
	Mean	SE	Mean	SE	mean	SE	mean difference	lower	upper	P ²	mean difference	lower	upper	P ²
Month 0 (n = 250)														
LAZ	-1.47 ^a	0.07	-1.54 ^b	0.07	-1.37 ^c	0.07	-0.10	-0.29	0.09	0.313	-0.17	-0.11	0.26	0.436
WAZ	-0.63	0.08	-0.54	0.08	-0.55	0.08	-0.08	-0.29	0.14	0.486	0.01	-0.21	0.22	0.941
Month 2 (n = 165)														
LAZ	-1.32	0.07	-1.34	0.07	-1.28	0.07	-0.04	-0.24	0.16	0.690	-0.06	-0.26	0.13	0.541
WAZ	-0.63	0.08	-0.48	0.08	-0.56	0.08	-0.07	-0.29	0.16	0.574	0.08	-0.14	0.31	0.481
Month 4 (n = 134)														
LAZ	-1.42	0.08	-1.40	0.08	-1.39	0.07	-0.03	-0.24	0.18	0.784	-0.02	-0.23	0.19	0.860
WAZ	-0.60	0.09	-0.51	0.09	-0.59	0.09	-0.01	-0.25	0.23	0.947	0.08	-0.16	0.32	0.497
Month 6 (n = 151)														
LAZ	-1.67	0.07	-1.67	0.07	-1.59	0.07	-0.08	-0.29	0.12	0.433	-0.08	-0.29	0.12	0.421
WAZ	-0.69	0.09	-0.57	0.09	-0.63	0.09	-0.06	-0.3	0.18	0.625	0.05	-0.19	0.29	0.667

¹All values are means $\pm$ SE unless otherwise stated. ²P-value for t-test. Values with in the same row with different superscript are significantly different. Infant anthropometric measurements of length and weights were taken at bi-monthly visits to the study site during the intervention period (6–12 months old). LAZ, length-for-age z score; SQ-LNS, small quantity lipid-based nutrient supplement; WAZ, weight-for-age z score.

The observed trend from a mixed effects regression model using linear splines showed an increase in LAZ with a peak at month 4 (age 10 months old) of the trial and gradually decreasing towards month 6 (age 12 months old) (**Figure 4.2**). There were significant differences at month 2 ($P = 0.032$) and month 4 ($P = 0.008$) between LAZ of the SQ-LNS B group compared to the control group (**Table 4.4**). SQ-LNS A group did not have any significant effects on LAZ for the duration of trial.

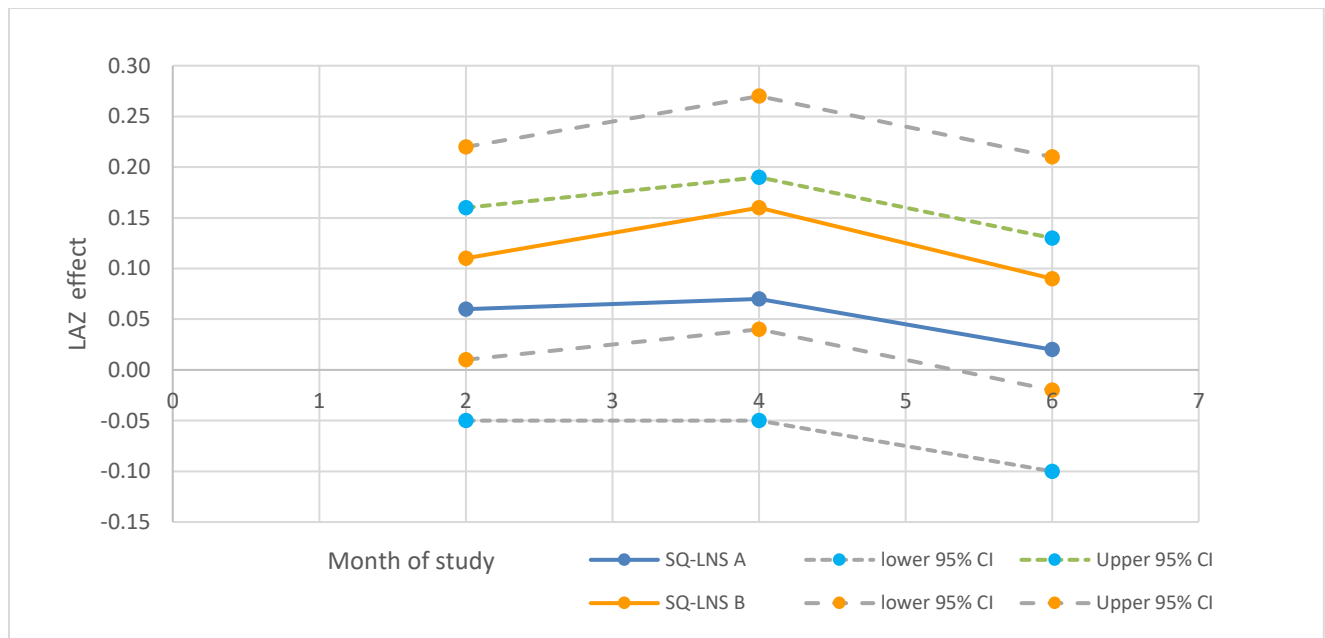


Figure 4. 2: Mixed effects linear splines for length for-age z score (LAZ) intervention effect through the study period¹.

¹This illustrates trajectories of growth for the SQ-LNS A (blue line), and SQ-LNS B group (orange line) for LAZ for children assessed bi-monthly (visits 2, 4 and 6). This descriptive data that was not adjusted for sex, the SQ-LNS B group showed a length-for-age z score that was maintained at higher levels than in the control and SQ-LNS A groups until the end of the intervention period. The difference was significant at month 2 and 4 of the trial and was not significant at month 6 (end of study)

Table 4. 4: Estimated intervention effects for LAZ and WAZ taking baseline differences into account

month	SQ-LNS A vs. control				SQ-LNS B vs. control			
	mean difference	lower	upper	P^1	mean difference	lower	upper	P^1
month 2								
LAZ	0.06	-0.05	0.16	0.279	0.11	0.01	0.22	0.032*
WAZ	0.01	-0.07	0.09	0.783	0.08	-0.001	0.15	0.054
month 4								
LAZ	0.07	-0.05	0.19	0.246	0.16	0.04	0.27	0.008*
WAZ	0.07	-0.04	0.18	0.228	0.08	-0.03	0.19	0.161
month 6								
LAZ	0.02	-0.10	0.13	0.774	0.09	-0.02	0.21	0.115
WAZ	0.02	-0.09	0.12	0.764	0.05	-0.06	0.15	0.388
Overall								
LAZ				0.457				0.036*
WAZ				0.559				0.186

¹Chi-Squared test. Values with in the same row with (*) are significantly different, $P < 0.05$. Infant anthropometric measures of length and weight were taken at bi-monthly visits to the study site during the intervention period (6–12 months old). LAZ, length-for-age z score; SQ-LNS, small quantity lipid-based nutrient supplement; WAZ, weight-for-age z score.

The same model was used for WAZ and the same cut-points and splines were used. An independent covariance structure for the random effects was specified to secure proper convergence of the model. Tests for arm by time interaction effects overall showed that there was no statistically significant intervention effect on the WAZ profiles of SQ-LNS A and SQ-LNS B vs. control, p -value= 0.560 and 0.186 respectively. In this study, we observed a low prevalence (1.7%) of wasting at end of trial and therefore WLZ modelling results are not presented. SQ-LNS A underweight profile showed significant interaction with extensive underweight at end of the trial (age 12 months) ($P=0.011$). While, SQ-LNS B showed no difference in underweight over time compared to the control ($P=0.675$). There was no significant change in overweight in the intervention groups compared to control [SQ-LNS A ($P=0.278$) and SQ-LNS B ($P=0.226$)]. There was a trend showing that overweight decreased at 12 months in all groups. To test for an intervention effect for MUAC and HC, linear regression models were fitted with baseline values as covariate and adjusted for time differences at 6 months and sex. The results show that for SQ-LNS A and SQ-LNS B there was no effect on MUAC and HC ($P > 0.05$). Girls had marginally smaller HC compared to boys in this study.

Intake and adherence

The maximum (per protocol analysis) intake for both SQ-LNS A and SQ-LNS B was higher at months 4 and 6 (age 10 and 12 months old). The SQ-LNS B group had the best per protocol intake analysis in the period 2-4 months (age 8-10 months old). Based on the duration of the trial (182 days) and the numbers of supplements issued and empty sachets collected during the weekly home visits for accountability monitoring, the estimated mean adherence to the intervention (proportion of days when the supplements were consumed) was 94.1% and 94.4% ($P > 0.05$) in the SQ-LNS A and SQ-LNS B respectively. The reported average weekly consumption showed that the majority of the infants consumed all of the weekly supply of the supplements in 78.8% and 78.2% of infants from the SQ-LNS A and SQ-LNS B groups respectively ($P > 0.05$). The analysis of the association of SQ-LNS intake per week and LAZ using linear splines showed that there was no association between total intake and LAZ at any time point for SQ-LNS A ($P = 0.134$) or SQ-LNS B ($P = 0.115$) in this study.

Morbidity and adverse events

The two products SQ-LNS A and B had a positive effect on the overall days of fever, suggesting that the supplements may have impact by reducing in the total days of fever related infectious morbidity compared to the control group. SQ-LNS A improved respiratory-related illness, as it reduced the total incidences for wheezing. However, SQ-LNS A and SQ-LNS B increased the overall days and the number of incidences with diarrhoea ($P < 0.001$) and vomiting ($P < 0.001$). Both SQ-LNS A and SQ-LNS B increased the overall days and number of incidences of rash/sores. In this study, we did not observe any incidences of supplement related serious adverse reactions and/or allergies.

Discussion

In this study, an overall positive effect on LAZ ($P = 0.036$) was observed for SQ-LNS B compared to the control, this was mainly driven by intervention effects at age 8 and 10 months, as at age 12 months (after 6 months of receiving the SQ-LNS) the intervention effect disappeared. The observed positive effects of SQ-LNS B on LAZ are consistent with findings from Ghana (9), Malawi (14), Chad (33), Haiti (8), and Burkina Faso (16). On the contrary two studies from Malawi (10, 17), did not find significant positive effects on linear growth. However, the previous studies measured the change in linear growth less frequently (1–2 times) compared to the current study that measured it 3

times during the intervention. This is very informative to indicate changes over time in different directions.

Therefore, the results from this study may be used as basis to design further studies to understand the efficacy of SQ-LNS supplements on preventing stunting and improving linear growth in infants in the complementary feeding period. This is supported by findings from the Malawi study where they reported that the positive effects of LNS supplementation on stunting were sustained even after 2 years of a non-intervention period (34). Elsewhere in Bangladesh, one year daily supplementation with fortified complementary foods combined with nutrition counselling resulted in increased linear growth and reduced the prevalence of stunting for children at age 18 months (35).

There was a non-significant increase in the prevalence of stunting in all three groups, from 28.4% to 41.1% and 31.2% to 38.3% in the SQ-LNS A and SQ-LNS B groups respectively and from 28.4% to 36.3% in the control group. This indicate that both SQ-LNS A and SQ-LNS B did not have an effect on the prevalence of stunting in infants by the end of the 6-month intervention period. The stunting prevalence at baseline and end of study were higher than other anthropometric indicators indicating that chronic malnutrition was the predominant form of malnutrition. The 2012 SANHANES data showed that for children 0-3 years old, stunting for boys and girls was 26.9% and 25.9% respectively (36). Previous studies involving 6-12 months old South African infants have shown lower prevalence of stunting between 6 and 24 months of age (37-39). Therefore, the observed 28.5% and 38.3% stunting at ages 6 and 12 months old in this current study was higher than expected. This could have contributed to the limited observed effect of SQ-LNS on linear growth. SQ-LNS are developed to be used for prevention of undernutrition in more food secure situations to fill certain nutrient gaps in the diet (12). It may therefore be that the earlier intervention is needed (e.g. preconception, during pregnancy) to affect linear growth.

The trend of improved linear growth was not sustained over the last two months of the intervention (Figure 2). The effects on LAZ and WAZ increased from baseline and peaked at month 4 (age 10 months old) and gradually declined at month 6 (age 12 months). The same trend was observed in the Haiti (8) and Malawi studies (10) where they also observed that by month 4 (age 10 months old), all groups showed declining LAZ trajectories. In the current study, there was no association between SQ-LNS intake and LAZ for both intervention groups. Still the current study finding that the intervention effect was not sustained to the age of 12 months is difficult to explain. Contributing

factors may be that the dose of the supplement not being sufficient at 10 to 12 months of age to sustain growth. The fact that fortified infant foods were used less as the children became older indicates that SQ-LNS B combined with poor nutrient rich complementary foods was not sufficient to sustain improved growth beyond age of 10 months. This may suggest that fortified commercial infant foods may play an important role on the micronutrient status and growth of infants.

Another possible reason may be that eating patterns changed, with fewer infants being breastfed and the complementary diet relying less on fortified commercial infant products. Nevertheless, the observed intervention effect may be the result of the difference in the nutrient content between the two intervention products. The SQ-LNS B contained additional components such as DHA, ARA, *phytase* and milk powder. These additional components may influence linear growth in a multi-factorial way as supplementation with DHA and ARA during pregnancy and postnatally to infants showed improved birth outcomes and promoted growth in infants postnatally (40-42). The addition of *phytase* to SQ-LNS has potential to promote growth via the promotion of the bioavailability of iron and zinc that is essential in promoting linear growth (43-45). However, evidence for linear growth promotion from zinc supplementation studies have been inconclusive (46). The added milk powder provides extra bioavailable protein that could have had a positive effect on linear growth (10, 13), this could be mediated via the effects of milk protein on the IGF-1 axis as milk intake was positively associated with IGF-I concentrations and linear growth (47, 48). The mechanisms of how milk promotes growth are not clear.

The results of the intervention effects of SQ-LNS A and SQ-LNS B on the infectious morbidity show that both supplements increased the overall days and the number of incidences with diarrhoea ($P < 0.001$) and vomiting ($P < 0.001$), and the overall days and number of incidences of rash/sores. The current results seem to support current concerns that in malaria prone areas the provision of supplements with high iron content may increase risk of adverse effects (49). This may have likely limited the SQ-LNS effect on the LAZ. Although some studies investigated malaria as potential contributory factor this particular study area is not classified as an endemic malaria area (50). In response to the WHO 2007 guidance on the iron composition of complementary food supplements (49) the International Lipid-Based Nutrient Supplements (iLiNS) project decreased the iron content of SQ-LNS (Nutributter®) used in iLiNS trials from the 9 mg in to 6 mg (13). This dosage was

evaluated in a iLiNS trial in Malawi and they found that the provision of SQ-LNS containing 6 mg Fe/day did not increase the incidence and duration of illnesses in 6-18 months infants (51). Considering that both SQ-LNS A and SQ-LNS B that were used in this study provided lower amount of iron (5.8mg Fe/day) then the current findings of increased adverse events [These adverse events were mostly classified as mild severity on scale of mild, moderate and severe] in a non-malaria endemic study area highlights the need for further investigation to ascertain the safety of SQ-LNS supplements in this and similar settings. One approach would be to divide the daily dose of SQ-LNS (Fe/day~6 mg) into two meals (Fe/meal ~3 mg) (13) , this level will prevent enteropathy and associated adverse effects of a higher dose of iron given once in one meal (Fe/meal ≥ 6 mg) (49).

A limitation of this study might be that intervention effects could have been influenced by the higher rate of withdrawals 236/750, (31.5%) recorded in this study compared to the 25% that was used for sample size calculation. Mothers' relocating from the study area was given as the main reason given for dropping out across all groups. This suggests that a peri-urban area is prone to high population mobility.

In conclusion, SQ-LNS B showed better linear growth at 8 and 10 months of age compared to control group. However, both products did not show an effect on linear growth at 12 months of age. Therefore, we conclude that SQ-LNS are not efficacious in improving linear growth in infants from age at age 12 months old. This may suggest a need to integrate the use of home fortification strategies with existing stunting reduction interventions, or to increase dosage of the SQ-LNS from the age of 8 months to get more conclusive results on their effectiveness to promote optimum growth in infants during the complementary feeding period.

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Conflict of interest

The authors declare no conflict of interests. CMS received speaking honoraria from Unilever. SJMO, MB and LF work for GAIN, Unilever R&D and DSM respectively.

Author contribution statement

The responsibilities of the authors were as follows: TMM was involved in supervising field data collection and quality control and study product management, data analysis and interpretation of results, writing of the paper. MR contributed to supervising field data collection and data quality control of feeding practices, revision of paper. CMS, MF, and HSK were involved in training, guidance on data collection, quality control, academic input and revision of paper. CL provided guidance on statistical analysis and interpretation. SJMO, MB and LF contributed to the conceptualisation process, study design and implementation. All authors read and approved the final manuscript for submission.

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Effect of small-quantity lipid-based nutrient supplements on anaemia and iron status in 6-month-old infants from a peri-urban South African community: a randomised controlled trial.



Top Left the Author conducting training of fieldworkers and **Top Right** the visit by sponsors at the study site.

Bottom Left Working in the source and CRF File room and **Bottom Right** the Two PIs and Study Mangers of the trial

This chapter is presented in article format and is written according to the referencing requirements of the *American Journal of Clinical Nutrition* to which it will be submitted. The font and spacing were kept the same throughout the thesis. All figures and tables are presented within the text rather than at the end of the article for examination purposes.

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CHAPTER 5: RESEARCH ARTICLE 3

Effect of small-quantity lipid-based nutrient supplements on anaemia and iron status in 6-month-old infants from a peri-urban South African community: a randomised controlled trial

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Abstract

Background: Small-quantity lipid-based nutrient supplements (SQ-LNS) have the potential to prevent micronutrient deficiencies in infants aged 6-23 months. **Objective:** To evaluate the efficacy of two SQ-LNS's on improving haemoglobin (Hb) and plasma ferritin (PF) concentrations and reducing the prevalence of anaemia and iron deficiency (ID) in infants. **Design:** Infants aged 6 months were randomly assigned to SQ-LNS A with essential fatty acids (EFAs), SQ-LNS B with EFAs, docosahexaenoic acid (DHA) and arachidonic acid (ARA), *phytase*, powder milk, lysine and control group for a 6 months' follow-up trial. Anthropometry and dietary intake were assessed bimonthly. Hb, PF, soluble transferrin receptor (sTfR), C-reactive protein (CRP), alpha-2 acid glycoprotein (AGP), were measured at baseline and end. Generalised linear, quantile and logistic regression models were used in data analysis. **Results:** The Hb concentrations increased in SQ-LNS A ($P = 0.027$) and SQ-LNS B ($P = 0.005$) groups. The risk of anaemia reduced in SQ-LNS A (OR 0.56, 95% CI 0.34, 0.92, $P = 0.021$) and SQ-LNS B, (OR 0.61, 95% CI 0.38, 0.99, $P = 0.044$). The SQ-LNS B group had higher PF ($P = 0.013$), whereas SQ-LNS A did not differ significantly ($P = 0.149$) from control group. The odds of ID based on PF reduced in SQ-LNS A, ($P = 0.004$) and SQ-LNS B, ($P < 0.001$) groups. The odds of IDA decreased significantly in SQ-LNS A ($P < 0.0001$) and SQ-LNS B ($P = 0.001$) groups. **Conclusion:** SQ-LNS A and SQ-LNS B had a positive intervention effect on ID and IDA. These results show that the use of SQ-LNS as home fortificants may be an effective option for reducing the prevalence of iron deficiency and anaemia in this and similar settings. This trial was registered at <http://clinicaltrials.gov> as NTC01845610.

Key words: anaemia, iron deficiency, complementary feeding, ferritin, sTfR, South Africa

Introduction

Anaemia and iron deficiency remain problems of public health importance worldwide. Evidence collected from 1990 to 2010 for 187 countries, both sexes revealed that the most vulnerable age group affected by anaemia and its associated complications was in children under age 5 years (1). Almost half of this burden was as a result of iron deficiency anaemia (IDA) and the remainder was due to other risk factors of anaemia that differ by geography, age, and sex. Children and women of reproductive ages are mostly at high risk (1). In children, vulnerability increases during the complementary feeding period when prenatal iron stores get depleted due to increased iron requirements. The breastmilk iron and overall nutrient content will no longer be sufficient to sustain optimum growth and development for infants from age 6-23 months (2).

The causal link between iron deficiency (ID) and suboptimal growth and development in children is still unclear (3, 4). The focus should be on promoting nutrition sensitive interventions to improve ID and anaemia in low income countries (5), within the first 1000-day window period (6, 7). Nevertheless, iron supplementation or fortification should be conducted with caution as increased levels of unabsorbed iron in the lower gastro intestinal tract (GIT) may increase risk of infection in children (8). This risk of adverse effects is higher for children in malaria-endemic areas (9).

Nutrition specific interventions have been shown to be effective (7). One promising approach is the use of small-quantity lipid-based nutrient supplements (SQ-LNS) (~20 g or ~110-120 kcal day⁻¹), used as home fortificants (10, 11). SQ-LNS have the unique advantage over other multiple micronutrient supplements as they provide moderate amounts of micronutrients including iron, energy, protein, and essential fatty acids (EFA) (12). SQ-LNS have potential to improve growth and micronutrient status of infants in the complementary feeding period (13-16). They contain essential fatty acids (EFA) omega-3 and omega-6 which have been shown to be promote growth and development in infants (17) and may be important in preventing growth faltering. The use of home fortificants is being scaled up in regions with widespread prevalence of stunting and micronutrient deficiencies (11).

Trials investigating the potential of SQ-LNS and medium-quantity LNS (MQ-LNS at ~50 g or ~250–280 kcal per day) to prevent malnutrition and promote growth have yielded mixed results (13, 14, 16, 19-21). Although the results are inconclusive they highlight that there is

growing interest on the potential benefits of using SQ-LNS for preventing undernutrition and micronutrient deficiencies in children (22).

Micronutrient deficiencies mainly affects children, pregnant and lactating women and SQ-LNS are designed to fill gaps in essential nutrients for these vulnerable groups. Therefore, the aim of this study was to investigate the effect of providing SQ-LNS from 6 months to 12 months of age on Hb and PF levels and the prevalence of anaemia, ID and IDA in 6-month-old infants from a peri-urban setting in South Africa.

Subjects and methods

Study site and eligibility

The intervention study was carried out in the peri-urban area of the Matlosana municipality in South Africa, from September 2013 to July 2015 involving apparently healthy infants ($n = 750$). Infants born as singletons who were resident in the study area and not known to be HIV positive at the screening visit were enrolled in the study at age 6 months but less than 7 months. The inclusion criteria included infants who were apparently healthy, planning to stay in the study area for at least the next 7 months, no known allergy to peanut, soy, milk and/or fish, not lactose intolerant, no congenital abnormalities, previously breastfed, did not received any special nutritional supplements. Infants were excluded if they had weight for length z-score (WLZ) $<-3SD$, and haemoglobin <7.0 g/dL.

Study design and subjects

The study was a randomised controlled trial with 6 months of follow-up. The statistician, laboratory staff and fieldworkers who collected source data were blinded. Infants were recruited when they were 4–6 months old and at the age of 6 months they were randomly assigned to (1) SQ-LNS A ($n = 250$), (2) SQ-LNS B ($n = 250$) and (3) control group ($n = 250$). Group randomisation lists were prepared using *NQuery* version 7 (Statistical Solutions Ltd, Cork, Ireland). At the baseline visit, the next sequential baby and group code was allocated to the next infant enrolled. The mothers who agreed to participate signed an informed consent form. Infants who were found to be severely anaemic and/or wasted during screening were excluded from the study and referred to local clinics and/or hospital.

The Ethics Committees of North West University (NWU) (NWU-00001-11-A1) and the South African Medical Research Council (SAMRC) (EC-01-03/2012) approved the study. After institutional ethical approval, the project protocol was reviewed by local authorities

from the health department and local municipality. Permission was also sought from the community. The Data Safety Monitoring Board comprised of a paediatrician, nutrition researcher, and a biostatistician and they carried out quarterly reviews of blinded AE and SAE data. The randomized controlled trial was registered at <https://clinicaltrials.gov/> registry as NTC01845610.

Sample size and power

The current study was part of a randomized trial to determine the efficacy of SQ-LNS on linear growth and micronutrient status. Sample size was calculated based 80% power and a type-1 error of 5% in order to detect differences between the intervention groups vs. the control group that is equal or higher than a “medium” effect size of 0.25, (23). The required sample size for linear growth as primary outcome was 186 infants per group was adapted to allow for an expected drop-out rate of 25%, to enrol 250 infants per group (750 in total). This was based on an expected difference of 0.15 length-for-age z-scores (LAZ scores) at 12 months of age with a pooled standard deviation of 0.54 (i.e. effect size of 0.27). The exclusion criteria used was weight-for-length z-scores (WLZ) less than -3SD. The G*Power version 3.1 (Düsseldorf, Germany) was used for iron status indicators (Hb, PF and sTfR) and the expected sample size using effect size of 0.27, $\alpha < 0.05$, 80% power and expected drop-out rate of 25% was 58 infants per group. A total sample size of 174 is sufficient to detect differences between intervention groups and control group.

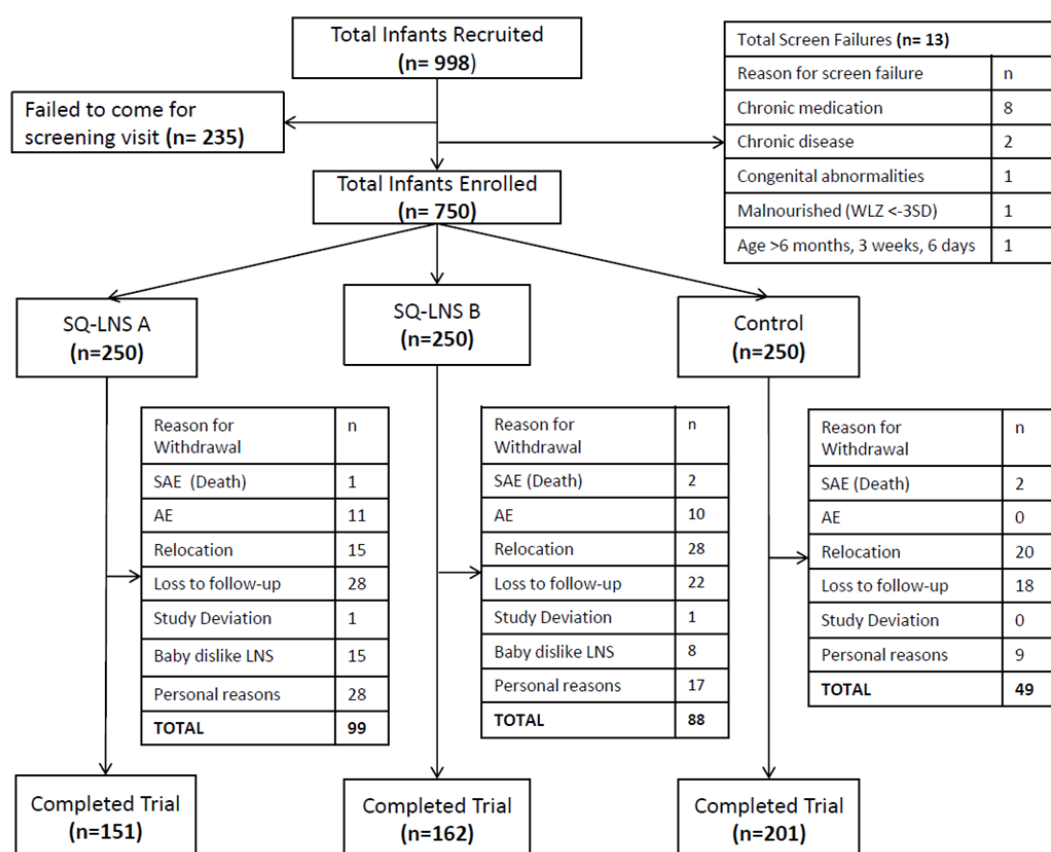


Figure 5. 1: Flow diagram of infant numbers from recruitment to data analysis¹.

¹A total of 998 infants were recruited and 750 were randomly assigned to one of the three groups: SQ-LNS, small quantity lipid based supplements (SQ-LNS A, and SQ-LNS B) and control. In total 514 (68.53%) infants completed the trial. WLZ, weight for length z scores; AE, adverse events; SAE, serious adverse events.

Study Foods

The investigational soy-based lipid based nutrient supplements were (1) SQ-LNS A, a fortified fat-based paste containing essential fatty acids, (2) SQ-LNS B a fortified fat-based paste with added docosahexaenoic acid (DHA), arachidonic acid (ARA) and *phytase*. The control group did not receive anything for the duration of the trial (age 6-12 months), but were given SQ-LNS A, from age 12 to 18 months. The nutritional profiles of SQ-LNS A and SQ-LNS B are presented in **Table 5.1**, indicating also other differences in micronutrient contents.

Table 5. 1:Nutritional profiles of the SQ-LNS products used in the RCT

	SQ-LNS A	SQ-LNS B	WHO/FAO RNI ³
Amount (g) (1 portion)	20	20	-
Energy (kcal)	114	113	-
Energy density (kcal/g)	5.7	5.7	-
Protein (g)	3.0	3.7	10.5g
% calories from protein	10%	13%	-
Fat (g)	8.0	8.8	40-60% energy ¹
% calories from fat	63%	70%	-
Essential fatty acids			
Linoleic acid (LA), (g)	1.5	1.8	3.0-4.5% energy
α -linolenic acid (ALA), (mg)	265	348	0.4-0.6% energy
n-6/n-3 ratio	5.7	5.0	-
Long-chain polyunsaturated fatty acids			
Docosahexaenoic acid (DHA), (mg)	-	75	-
Arachidonic acid (ARA), (mg)	-	75	-
Micronutrients			
Vitamin A, (μ g)	200	200	400 mg
Vitamin D, (μ g)	2.5	2.5	5 mg
Vitamin E, (mg)	2.5	2.5	2.7 mg
Vitamin K, (μ g)	7.5	7.5	10 mg
Thiamine, (mg)	0.25	0.25	0.3 mg
Riboflavin, (mg)	0.25	0.25	0.4 mg
Niacin, (mg)	3	3	4 mg
Pantothenate, (mg)	1.0	1.0	1.8 mg
Vitamin B6, (mg)	0.25	0.25	0.3 mg
Biotin, (μ g)	4.0	4.0	
Folate (B9), (μ g)	80	80	80 mg
Vitamin B12, (μ g)	0.45	0.45	0.7 mg
Vitamin C, (mg)	23.3	23.3	30 mg
Calcium, (mg)	250	396	400 mg
Iodine, (μ g)	45	45	90 mg
Iron, (mg)	5.8	5.8	18.6 mg ²
Zinc, (mg)	6.2	6.2	8.4 mg ³
Copper, (mg)	0.28	0.28	-
Selenium, (μ g)	8.5	8.5	
Magnesium, (mg)	-	30	54 mg
Manganese, (mg)	-	0.6	-
Phosphorus, (mg)	-	230	-
Potassium, (mg)	-	257	-
Other			
L-Lysine, (mg)	-	160	-
Phytase, (FTU)	-	200	-

¹Acceptable macronutrient distribution range, 40–60% at 6 months (24).²RNI for an assumed bioavailability of 5% for infants 7-12 months old and ³RNI for low bioavailability. SQ-LNS A & B are small-quantity lipid based nutrient supplements. The supplements were packed in 20g sachets by Unilever R&D (Vlaardingen, Netherlands) and DSM nutritional products Ltd (Basel, Switzerland) for SQ-LNS A and B respectively.

Intervention and follow-up

The trial design consisted of repeated measurements which were carried out at baseline visit (infant age 6 months) and exit visit (infant age 12 months) as well as bimonthly visits (at 8 months and 10 months) for anthropometric follow-ups. In addition, there were weekly home visits performed by fieldworkers to monitor adherence and to administer the morbidity (AE/SAE) questionnaire. The fieldworkers always re-stocked the mothers with two weeks' supply of the SQ-LNS on their weekly monitoring visits. The two treatment groups and the control group were treated and monitored using the same criteria except that the control group did not receive supplements. Infants consumed 1 sachet (20 g) of the SQ-LNS daily for 6 months (26 weeks). Adherence was calculated based on the formula: *Adherence = Total intake = (sum of weekly intake [g]) / (days in study * 20)*. The SQ-LNS were mixed with usual complementary foods given to the infants. At baseline, breastfeeding and complementary feeding practices were evaluated retrospectively based on WHO guidelines for assessing infant and young child feeding practices (25).

Measurement of outcome variables

A structured questionnaire that covered questions on socio-demographic variables, such as water and sanitation, education of mothers and fathers, size of households, employment status, marital status, use of health facilities and recent morbidities was used to obtain source data. Breastfeeding and complementary feeding practices were assessed retrospectively using a structured questionnaire that was developed based on World Health Organization (WHO) guidelines for assessing infant and young child feeding practices. A set of unquantified food frequency questions was used to obtain descriptive qualitative information on the usual consumption of foods by the infants over the past 7 days.

Iron status was determined by blood sample analysis. A 4-ml blood sample was taken into Ethylenediaminetetraacetic acid (EDTA) coated trace-element free evacuated tubes (Becton Dickinson) via antecubital venipuncture at the age of 6 and 12 months by a professional nurse. If blood drawing failed, a finger prick blood sample was taken. Haemoglobin (Hb) concentration was measured on all samples ($n = 750$) using the Hemocue that uses the direct cyanmethaemoglobin method [Ames Mini-Pak haemoglobin test pack and Ames Minilab, Bio Rad Laboratories (PTY) Ltd]. Blood was successfully collected from 485/750 (64.7%) of the infants at baseline and 357/750 (47.6%) at end point. At the field laboratory, the blood was processed to obtain plasma aliquots and stored at 4°C. The samples were carried daily

from the study site in cooler bags with refrigerant gel ice packs to the North-West University for storage at -80°C . The samples were later shipped for analysis to Vitmin Lab Willstaett, Germany in accordance with the standard shipment procedures of Department of Health in South Africa. No screening for HIV was conducted for this study.

Plasma ferritin (PF) and soluble transferrin receptor (sTfR) concentrations were measured using a sensitive Sandwich ELISA (Ramco Laboratories) technique (26). The acute and chronic inflammation markers, high-sensitivity C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) were measured using ELISA kits from Human Diagnostics (Wiesbaden, Germany).

In this study anaemia was defined as Hb <11 g/dL, iron deficiency (ID) as PF <12 $\mu\text{g/L}$ and iron deficiency anaemia (IDA) as both SF <12 $\mu\text{g/L}$ and Hb <11 g/dL (25). Based on sTfR analysis ID was defined as sTfR >8.3 mg/L and IDA as Hb <11 g/dL and sTfR > 8.3 mg/L (test-kit reference value). Inflammatory markers AGP and CRP were used to detect presence of inflammation as AGP >1 g/L and CRP >5 mg/L (27). Individual's PF concentrations were adjusted by using correction factors (CFs) specific to each subject's inflammatory status (27).

Anthropometric status was assessed using the WHO Child Growth Standards (28). Anthropometric indexes for infants (WAZ, LAZ, and WLZ) were calculated based on the WHO classification (28). Wasting was defined as WLZ less than -2SD, stunting as LAZ less than -2SD and underweight as WAZ less than -2SD (29).

Weight and recumbent length were taken according to WHO standardized techniques (30). The anthropometry assessors were trained according to the WHO Training Course on Child Growth Assessment for the infants (31). Infants were undressed and weighed to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG., Hamburg, Germany, maximum weight 20 kg). Recumbent length was measured to the nearest 0.1 cm using an infantometer (Seca model 416, GmbH & Co. KG., Hamburg, Germany). All measurements were done in duplicate and if the first two measurements differed by >0.05 kg for weight or by > 0.3 cm for length a third measurement was done and the two closest values were recorded. Calibration of instruments and validation of measurements and random auditing were done on a daily basis. Anthropometric indices were generated using WHO *Anthro* 2005 software.

Statistics

Source data verification was done bi-weekly by an independent clinical research organisation (OnQ Research, Johannesburg, South Africa). Data were entered using *EpiData* 3.1 (EpiData Association, Denmark) and quality checks were done to verify missing data and eliminate probable errors in data entry. The basis of the analysis of the trial was intention to treat. A mixed effects linear regression model was used to model the outcome with participants as the random effects to account for the dependence between the repeated measurements. Imputed quantile regression was used to assess the intervention effects of the SQ-LNS on haemoglobin concentrations. The formal inference analysis was used to test effect of the SQ-LNS on the likelihood of anaemia. The baseline value of the measurement (at 6 months) was used as a covariate in the model to improve precision. Tables of baseline demographic (**Table 5.2**) and anthropometric and iron status data (**Table 5.3**) for the infants were compiled.

The Shapiro-Wilk test was used to check for normality of the data. Descriptive data were reported by frequencies and/or cross-tabulations. Results are reported as mean with 95% confidence intervals (CI) for continuous normally distributed data and data not normally distributed is reported as the median and interquartile range (IQR). The Pearson Chi-Square test was used to test for associations between categorical data. ANOVA and Kruskal-Wallis tests were used to test for differences across groups for data with a normal and non-normal distribution, respectively. Likelihood ratio test was used to compare odds ratios and z test was used for log odds. ID-PF, ID-sTfR formal quantification of reduced risk was conducted using generalized linear regression models. Formal inference adjusted odds ratios (AOR) for anaemia, ID and IDA were done using logistic regression adjusting for baseline Hb. While the quantifying difference using an adjusted logistic regression model PF. For all analysis, statistical significance was set at 0.05 ($P < 0.05$). The data was analysed using SAS V9.4 (SAS Institute, Inc, Cary, NC), STATA V14 (StataCorp Ltd, USA) and SPSS software version 23 (IBM, Corporation USA).

Results

Recruitment and withdrawals

A total of 998 infants were recruited and at age 6 months 750 who met the inclusion criteria were randomly assigned to the SQ-LNS A, SQ-LNS B and control groups (Figure 5.1). A total of 514 (68.5%) infants completed the trial. This shows a drop-out rate of 31.5% (236/750), which is higher than the expected 25%, mostly due to relocation, loss to follow-up with change in contact information and personal reasons. There was no significant difference in the distribution of withdrawals across the study groups ($P = 0.368$). However, it is important to note that the control group had fewer withdrawals compared to the SQ-LNS groups. The demographic, socio economic and feeding characteristics of the participants across groups at baseline are presented in Table 5.2. The baseline anthropometric and iron status across the three groups are presented in Table 5.3. In this study, 48.9% of the infants had stopped exclusive breastfeeding (EBF) at age 0-2 months compared to 14.9% stopping EBF at age 5-6 months and 70.1% were still being breastfed at age 6 months.

Table 5. 2: Baseline demographic, socio economic and feeding characteristics of the participants at enrolment across groups¹

Characteristics	SQ-LNS A (n = 250)	SQ-LNS B (n = 250)	Control (n = 250)	P ²
Infant sex, male [n (%)]	113 (45.2)	143 (57.2)	131 (52.4)	0.026*
Age, months mean (SD)	6.22 (0.26)	6.22 (0.24)	6.22 (0.25)	0.949
Birth weight** mean (SD)	2.97 (0.54)	2.94 (0.52)	3.03 (0.47)	0.118
<i>Breastfeeding and feeding practices</i>				
Infants breastfeeding at 6 months [n (%)]	182 (72.8)	178 (71.2)	165 (66.3)	0.252
Age exclusive breastfeeding stopped, [n (%)]				
0-2 months	117 (46.8)	116 (46.4)	134 (53.6)	0.254
3-4 months	100 (40.0)	93 (37.2)	78 (31.2)	
5-6 months	33 (13.2)	41 (16.4)	38 (15.2)	
Age that liquids were introduced to infants, [n (%)]				
0-2 months	111 (44.4)	110 (44.0)	121 (48.4)	0.384
3-4 months	91 (36.4)	84 (33.6)	78 (31.2)	
5-6 months	34 (13.6)	47 (18.8)	35 (14.0)	
Not started	14 (5.6)	9 (3.6)	16 (6.4)	
Age that solids were introduced to infants, [n (%)]				
0-2 months	34 (13.7)	36 (14.5)	53 (21.6)	0.230
3-4 months	111 (44.6)	110 (44.4)	105 (42.9)	
5-6 months	91 (36.5)	85 (34.3)	73 (29.8)	
Not started	13 (5.2)	17 (6.9)	14 (5.7)	
<i>Caregiver characteristics</i>				
Age in years, mean (SD)	28.1 (8.40)	27.9 (8.03)	29.3 (9.28)	0.125
Education, ≥Grade 10, [n (%)]	209 (85.0)	197 (79.4)	195 (79.6)	0.201
Married [n (%)]	29 (11.6)	20 (8.0)	31 (12.4)	0.774
Total people employed in household, median (IQR)	1.0 (0.0)	1.0 (1.0)	1.0 (1.0)	0.027*
<i>Household Characteristics</i>				
Electricity at home, [n (%)]	229 (91.6)	230 (92.0)	233 (93.2)	0.784
Tap water at the household, [n (%)]	237 (94.8)	241 (96.4)	241 (96.4)	0.426
Flush toilet at home, [n (%)]	237 (94.8)	239 (95.6)	237 (94.8)	0.139

¹Data presented as mean standard deviation (SD) or number (n) (%). ²Pearson Chi-square or ANOVA. SQ-LNS A & B are small-quantity lipid based nutrient supplements. Values across same row with (*) are significantly different, $P < 0.05$. **22 (2.93%) infants had missing information on birth weight (<2500 g).

Table 5. 3: Baseline (age 6-month-old) anthropometric and iron status across the three groups at enrolment¹

Characteristics	SQ-LNS A (n = 250)	SQ-LNS B (n = 250)	Control (n = 250)	P ²
Low birth weight** [n (%)]	38 (15.6)	40 (16.7)	24 (9.8)	0.061
<i>Anthropometric status</i>				
Length-for-age z score (LAZ), mean (SD)	-1.43 (1.06)	-1.53 (1.06)	-1.36 (1.09)	0.883
Stunted, <-2 LAZ [n (%)]	69 (27.6)	74 (29.6)	71 (28.4)	
Weight-for-age z score, mean (SD)	-0.62 (1.19)	-0.54 (1.18)	-0.55(1.27)	0.839
Underweight, <-2 WAZ, [n (%)]	26 (10.4)	27 (10.8)	30 (12)	
Weight-for-length z score, mean (SD)	0.47 (1.14)	0.65 (1.15)	0.49 (1.12)	0.578
Wasted, <-2 WLZ, [n (%)]	4 (1.6)	3 (1.2)	6 (2.4)	
Overweight >+2 WLZ, [n (%)]	18 (7.2)	30 (12.0)	28 (11.2)	0.163
<i>Iron Status</i>				
Haemoglobin (Hb), g/dL, mean (SD)	11.32 (1.31)	11.37 (1.43)	11.34 (1.37)	0.525
Anaemic (Hb <11g/dL), [n (%)]	98 (35.8)	86 (31.4)	90 (32.8)	
Adjusted ³ Plasma ferritin (PF), median (IQR)	24.48 (25.16)	25.08 (23.70)	25.71 (25.80)	0.614
Iron deficiency (ID) (PF<12 µg/L), [n (%)]	23 (13.7)	32 (19.8)	23 (14.8)	0.285
IDA = Hb <11 g/dL + PF<12 µg/L, [n (%)]	18 (10.7)	14 (8.6)	13 (8.4)	0.728
Soluble transferrin receptor (sTfR), mean (SD)	7.64 (2.98)	7.72 (2.80)	7.26 (2.36)	0.283
Raised C-reactive protein (CRP) >5 mg/L, [n (%)]	29 (17.3)	23 (14.2)	20 (12.9)	0.524
Raised Alpha-1 glycoprotein (AGP) >1 g/L [n (%)]	51 (30.4)	53 (32.7)	52 (33.5)	0.815

¹Data presented as mean standard deviation (SD) or number (n) (%). **22 (2.93%) infants had missing information on birth weight (<2500 g). ²Chi-Square or ANOVA. ³PF adjusted for inflammation considering both AGP and CRP, at baseline and end (27). SQ-LNS A & B are small-quantity lipid based nutrient supplements. Values across same row with (*) are significantly different, *P* <0.05. IDA, Iron deficiency anaemia.

Effect of SQ-LNS supplements on haemoglobin (Hb) and anaemia

There was significant (SQ-LNS A, $P = 0.027$; SQ-LNS B, $P = 0.005$) increases in Hb observed in the intervention groups compared to the control group. (**Table 5.4**). Imputed quantile regression for Hb adjusted for baseline revealed that both treatments had a significant (SQ-LNS A, $P = 0.018$; SQ-LNS B, $P = 0.006$) positive impact on Hb concentration in comparison to the control group. The relative improvement in Hb was 3.4% for SQ-LNS A and 4.2% for SQ-LNS B, which reflects a ‘modest’ intervention effect. There was a significant difference in anaemia (Hb <11 g/dL) across groups at end of trial (age 12 months) ($P = 0.044$).

The formal inference reporting odds ratios, adjusted for baseline Hb (**Table 5.5**) indicated that the likelihood of anaemia decreased significantly in both treatment groups, OR = 0.56 (95% CI, 0.34, 0.92) $P = 0.021$ for SQ-LNS A and OR = 0.61 (95% CI, 0.38, 0.99) $P = 0.044$ for SQ-LNS B respectively. Therefore, both SQ-LNS A and SQ-LNS B groups had a significant positive impact on Hb in comparison to the control group.

Effect of SQ-LNS supplements on plasma ferritin (PF) and iron deficiency (ID)

Table 5.4 show the intervention effect on PF concentration, adjusted for CRP and AGP. The SQ-LNS B group had significantly higher PF ($P = 0.013$) than the control group. Adjusting for baseline PF showed that SQ-LNS B remained significantly different from the control group, although with smaller effect. Table 5.4 also indicates the complete versus imputed effect estimates of SQ-LNS supplements on haemoglobin concentration compared to control at age 12 month. The adjusted (CRP, AGP) vs. adjusted (CRP, AGP and baseline value) effect estimates of SQ-LNS supplements on PF (PF <12 µg/L) concentration are also presented in Table 5.4. Logistic regression analysis (Table 5.5) showed that the likelihood of iron deficiency decreased significantly in both treatment groups compared to control group SQ-LNS A OR = 0.41 (95% CI, 0.22, 0.76, $P = 0.004$) and SQ-LNS B OR = 0.26. (95% CI, 0.14, 0.50, $P < 0.001$), respectively.

Table 5. 4: Complete versus imputed effect estimates for the intervention effect of SQ-LNS supplements on Hb and PF concentration compared to control at age 12 months¹

	SQ-LNS A vs. control	P ²	SQ-LNS B vs. Control	P ²
<i>Haemoglobin (g/dL)</i>				
Completed (<i>n</i> = 514) ³	3.93 (0.44, 7.42)	0.027*	4.93 (1.50, 8.35)	0.005*
Imputed (<i>n</i> = 750) ^{3,4}	3.94 (0.68, 7.19)	0.018*	4.81 (1.40, 8.22)	0.006*
<i>Plasma ferritin, (µg/L)</i>				
Unadjusted ⁵	3.66 (-0.93, 8.25)	0.117	6.36 (1.93, 10.80)	0.005*
Adjusted ⁴	3.23 (-1.16, 7.61)	0.149	5.37 (1.13, 9.61)	0.013*

¹All values are median difference (95% CI). SQ-LNS A & B are small-quantity lipid based nutrient supplements. sTfR, soluble transferrin receptor; IDA, iron deficiency anaemia [defined as Hb < 11 g/dL and sTfR >8.3 mg/l (32). Values in the same row with (*) are significantly different, *P* < 0.05. ²p-value based on ANOVA. ³Adjusted for baseline value. ⁴Adjusted for child sex and baseline value. ⁵Haemoglobin concentration was imputed at end (age 12 months) and done within each group based on intention to treat analysis. ⁵All plasma ferritin values for subjects that had raised CRP >5 mg/L were adjusted based on standard methods (27).

Effect of SQ-LNS supplements on Iron deficiency anaemia (IDA) and soluble transferrin receptor (sTfR)

Tables 5.5 show the prevalence and likelihood of anaemia, ID and IDA at age 12 months. At baseline, there was no difference between the three groups (*P* = 0.941), but at the end of the trial there was substantial differences in the proportion of children with iron deficiency between groups (*P* <0.001). Logistic regression analysis (Table 5.5), adjusting for baseline Hb, showed that the likelihood of IDA decreased significantly in both treatment groups SQ-LNS A [OR 0.11, 95% CI 0.03, 0.37, (*P* <0.001)] and SQ-LNS B [OR 0.19, 95% CI, 0.07, 0.48, (*P* = 0.001)] respectively. There was no difference between groups at baseline or endpoint for sTfR. There was a large increase in the control group, but not in two treatment groups. Logistic regression analysis showed that there was no intervention effect (Table 5.5).

Table 5. 5: Prevalence and likelihood of anaemia, ID and IDA at 12 months¹

	SQ-LNS A	SQ-LNS B	Control	P ²
<i>Anaemia Status (n = 750)</i>	151	162	201	
Hb= Anaemic (Hb <11 g/dL), [n (%)]	36 (23.8)	41 (24.5)	70 (34.8)	0.044*
AOR, (95% CI)	0.56 (0.34, 0.92)	0.61 (0.38, 0.99)	reference	
<i>Iron deficiency (n = 485)</i>				
ID=(PF) <12 µg/L, [n (%)]	19 (19.6)	15 (13.8)	53 (36.8)	<0.001*
AOR, (95% CI)	0.41 (0.22, 0.76)	0.26 (0.14, 0.50)	reference	
IDA= Hb <11 g/dL + PF<12 µg/L, [n (%)]	4 (3.1)	6 (5.5)	30 (20.8)	<0.001*
AOR (95% CI)	0.11 (0.03, 0.37)	0.19 (0.07, 0.48)	reference	
ID-sTfR=sTfR >8.3 mg/L, [n (%)]	32 (33.0)	37 (33.9)	59(41.0)	0.356
AOR, (95% CI)	0.71 (0.41, 1.21)	0.74 (0.44, 1.24)	reference	

¹All values are n (%). SQ-LNS A & B are small-quantity lipid based nutrient supplements. PF; plasma ferritin, sTfR, soluble transferrin receptor; ID; iron deficiency, IDA, iron deficiency anaemia [defined as Hb < 11 g/dL and PF<12 µg/L (32). Values in the same row with (*) are significantly different, $P < 0.05$.

²Differences across arms based on logistic regression. COR; crude odds ratio, AOR; adjusted odds ratio adjusted for baseline differences in sex, using control group as reference. ³significantly changed from 6 months' prevalence (see Table 3), based on McNemar's Chi-square, $P < 0.05$. ⁴The plasma ferritin values for subjects that had raised CRP >5mg/L were adjusted based on standard methods (27).

Intake and adherence

The maximum (per protocol analysis) intake for both SQ-LNS A and SQ-LNS B was higher at months 4 and 6 (age 10 and 12 months old). The SQ-LNS B group had the best per protocol intake analysis in the period 2-4 months (age 8-10 months old). Based on the duration of the trial (182 days) and the numbers of supplements issued and empty sachets collected during the weekly home visits for accountability monitoring, the estimated mean adherence to the intervention (proportion of days when the supplements were consumed) was 94.1% and 94.4% ($P > 0.05$) in the SQ-LNS A and SQ-LNS B respectively. The reported average weekly consumption showed that the majority of the infants consumed all of the weekly supply of the supplements in 78.8% and 78.2% of infants from the SQ-LNS A and SQ-LNS B groups respectively ($P > 0.05$). The analysis of the association of SQ-LNS intake per week and LAZ using linear splines showed that there was no association between total intake and LAZ at any time point for SQ-LNS A ($P = 0.134$) or SQ-LNS B ($P = 0.115$) in this study.

Morbidity and adverse events

The two products SQ-LNS A and B had a positive effect on the overall days of fever, suggesting that the supplements may have impact by reducing in the total days of fever related infectious morbidity compared to the control group. SQ-LNS A improved respiratory-related illness, as it reduced the total incidences for wheezing. However, SQ-LNS A and SQ-LNS B increased the overall days and the number of incidences with diarrhoea ($P < 0.001$) and vomiting ($P < 0.001$). Both SQ-LNS A and SQ-LNS B increased the overall days and number of incidences of rash/sores. In this study we did not observe any incidences of supplement related serious adverse reactions and/or allergies.

Discussion

The results of this study showed that there were significant intervention effects of SQ-LNS on anaemia (Hb < 11 g/dL), ID, (PF < 12 μ g/L) and IDA, (Hb < 11 g/dL and PF < 12 μ g/L) in both treatment groups when compared to the control group (Table 5.4 & 5.5). Infants in the SQ-LNS groups were less likely to be anaemic, iron deficient, and having iron deficiency anaemia (Table 5.5) when compared to the control group. These results add to the growing body of evidence (33, 34) that shows the importance of supplementation to the reduction of anaemia and iron deficiency in infants. The effect of the SQ-LNS supplements on reducing anaemia and iron deficiency can be attributed to the fact that they contain multiple micronutrients (Table 5.1). Particularly, riboflavin, B-12, and folic acid, which have modulating effects on the metabolism of iron or Hb synthesis (35). The results of the current study indicate that SQ-LNS supplements may have protective effects on anaemia and iron deficiency. This is consistent with findings from an earlier study in Ghana investigating Sprinkles (SP), crushable *Nutritabs* (NT), and fat-based *Nutributter*, all of these home fortificants were effective in reducing the prevalence of iron deficiency in infants (36).

Complementary foods supplemented with Sprinkles were found to have intervention effects on plasma ferritin concentrations and on anaemia for 6 months old infants followed until 18 months old in Cambodia (37). In Democratic Republic of Congo caterpillar cereal was shown to reduce the risk of anaemia and improve Hb levels in 6 month old infants followed to 18 months old when compared to the control group (38). In Malawi, provision of a fat-based spread to 6–17-months old undernourished infants, also showed a significant effect on haemoglobin concentration (20). Supplementation with *Foodlet* containing multiple micronutrients in a multicentre study (39),

showed significant positive effects on haemoglobin and iron status in infants from South Africa (33), Vietnam (40), Peru (41), and Indonesia (42).

Recent studies, in Peru (43) and Brazil (4) reported positive intervention effects of LNS on anaemia and iron deficiency. In the Peru study, LNS supplementation achieved improved haemoglobin levels and reduced the prevalence of anaemia in children under twelve months (43). Therefore, the findings of this current study support the evidence that complementary food fortificants like SQ-LNS are effective in preventing anaemia and iron deficiency in infants during the complementary feeding period.

Although the supplements reduced the risk of anaemia and iron deficiency there was still deficiency reported at the end of the trial in these intervention groups. This agrees with what has been reported in earlier studies (33, 37). This can be attributed to presence of other underlying factors like sickle cell disease (SCD) and other underlying infections and malaria. However, South Africa is not a malarial endemic area (44), in addition, there is low prevalence (<1%) of SCD, (45). It can also be postulated in areas where complimentary diets low in iron and other micronutrients the SQ-LNS containing a dose of 5.8 mg/d of iron used in the current study may not be sufficient to improve iron status based on sTfR. Nevertheless, reasons for anaemia and iron deficiency after six months of supplementation is not clear and will warrant further investigation.

A Kenyan double-blind 1-year trial utilizing 2.5 mg of iron as NaFeEDTA reported that supplementation failed to reduce risk of anaemia in 6-month-old infants. This was attributed to the poor dosage used and to poor absorption as a result of high prevalence of infections (46). In the same study in Kenyan infants it was observed that iron fortification was associated with negative effects on the gut microbiota by promoting proliferation of pathogenic microbes resulting in enteropathy in Kenyan infants (47). This raises questions on the safety of iron containing SQ-LNS supplements in areas where infections are prevalent as diarrhoea has been linked to decreased linear growth in children (48). However, a study in Malawi reported that SQ-LNS containing 6mg Fe/d (10 and 20 g /day sachets) was not associated with increased morbidity in children from 6-18 months old (49). Therefore, it may be speculated that in areas where children are exposed to infections, provision of safe drinking water, sanitation and hygiene (WASH) interventions may modulate the effects of SQ-LNS on micronutrient status and growth of infants and young children.

The results (Table 5.5) showed that the two SQ-LNS supplements did not have any effect on iron deficiency based on sTfR >8.3 mg/L. The observed results of SQ-LNS having an effect on Hb and PF but not sTfR are difficult to explain as these indicators cannot be compared (50). The first technical factor is that cut-offs for sTfR are not well established compared to those for PF (51). In addition, the PF and sTfR concentrations are indicators of different aspects of iron metabolism as sTfR increases in period of ID and decreases in times of iron repletion (52). In addition, PF is insensitive to iron changes in severe ID (53), while sTfR concentrations are only affected after iron stores have been depleted (53, 54). The sTfR concentrations are not influenced by associated chronic disease, infections and/or inflammation (52), except in cases where subjects have thalassemia and sickle cell disease. However, in South Africa there is a very low prevalence of sickle cell disease (<1%) (45).

The observed lack of effect of SQ-LNS on sTfR may be as a result of poor complementary feeding coupled with a very low breastfeeding rate in South Africa (55). This may result in a gradual depletion of iron stores as the children grow older. In 2012 only 7.4% of children were exclusively breastfed (EBF), 75.1% were breastfed though not exclusively and almost 63.5 % were given semi-solid or solid food before 6 months of age in South Africa (56). This may indicate that for infants with such low iron intakes, the dose of 5.8 mg/d of iron used in the current SQ-LNS products may not be sufficient to improve iron status based on sTfR independently.

The first limitation was that it was not a blinded study. However, the statistician and laboratory staff were blinded to group assignment. The Kruskal-Wallis test to assess the distribution of the withdrawals across groups showed that there was no significant difference in the distribution of withdrawals across the study groups ($P = 0.368$). However, there was a trend showing that there were fewer withdrawals in the control group compared to the SQ-LNS groups. Another limitation was that the Hb results were based on either finger prick or venous samples, as finger prick method was used in cases where venous blood could not be obtained. Some researchers have reported that the finger prick method results in higher Hb than venous blood tests, which may result in excess false negative diagnoses among individuals (57, 58). However, this can be justified as in the absence of a venous sample, a capillary sample via finger prick can be used as a reliable alternative in field setting (59). In this study, the results of the comparison of Hb values obtained from capillary versus venous samples showed that there was no significant difference ($P = 0.657$) across groups.

Although, caution should be exercised, the Hb values and anaemia prevalence reported in this study still remain useful at pragmatic level.

Conclusions

Both SQ-LNS A and SQ-LNS B had a positive effect on iron deficiency and iron deficiency anaemia which was associated with the observed lower prevalence of anaemia and iron deficiency in the SQ-LNS groups compared to the control. These findings are important and provide further evidence that the use of SQ-LNS as home fortificants may be an effective option for reducing the prevalence of iron deficiency and anaemia in areas where there are high rates of anaemia, iron deficiency and the complementary diets are deficient in key micronutrients.

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Conflict of Interest

The authors declare no conflict of interests except CMS who received speaking honoraria from Unilever.

Author contribution statement

The responsibilities of the authors were as follows: TMM was involved in supervising field data collection and quality control and study product management, data analysis and interpretation of results, writing of the paper. MR contributed to supervising field data collection and data quality control of feeding practices, revision of paper. CMS, MF, and HSK were involved in training, guidance on data collection, quality control, academic input and revision of paper. CL provided guidance on statistical analysis and interpretation. All authors read and approved the final manuscript for submission.

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Summary, limitations, conclusions and
recommendations



Top Left the study nurse collecting blood sample in the field clinic **Top Right** the good clinical practices (GCP) administrator conducting a training session at the study site.

Bottom Left the fieldworkers discussions at the study site and **Bottom Right** the Home visit fieldworkers collecting weekly supply of the supplements from the study site.

CHAPTER 6: GENERAL SUMMARY

Summary, limitations, conclusions and recommendations

6.1. Introduction

This chapter presents a summary of the main findings from the literature review and the research articles associated with this thesis. The results of this study have been compiled into three research articles/manuscripts that have been presented and discussed separately in Chapters 3, 4 and 5. The public health significance of the results from each of these articles are discussed in this final chapter. This will lead to the summary of the recommendations made based on the main findings from chapter 3, 4 and 5. The current literature in the area of LNS supplements and effects on linear growth and micronutrient deficiencies was explored in Chapter 2.

The literature survey revealed that there is conflicting and inconclusive evidence on the effects of using SQ-LNS supplements as home fortificants on improvement of growth and micronutrient status of infants from low income countries. The use of home fortificants in the form of SQ-LNS is important in areas where the diets are predominantly cereal/legume based and deficient in key nutrients for infants aged 6-23 months (de Pee & Bloem, 2009; Allen, 2012). SQ-LNS were designed to fill this gap between typical intakes and needs (Arimond *et al.*, 2013).

There are studies (Cercamondi *et al.*, 2013; Mangani *et al.*, 2013; Iannotti *et al.*, 2014; Hess *et al.*, 2015) that have reported positive effects SQ-LNS on growth and micronutrient status of children aged 6-12 months old. In the contrary other evidence (Ashorn *et al.*, 2014; Mangani *et al.*, 2014; Ashorn *et al.*, 2015; Maleta *et al.*, 2015) did not observe significant positive effects of SQ-LNS supplementation on linear growth and micronutrient deficiencies in children in the complementary feeding period. There is inconclusive evidence on the efficacy and/or effectiveness of SQ-LNS interventions to prevent stunting and micronutrient deficiencies in infants during the complementary feeding period.

Therefore, this study was designed to investigate the specific objectives as outlined and presented as follows:

1. Investigate the prevalence and factors associated with stunting in infants at age 6 months,
2. Investigate the effect of SQ-LNS A and SQ-LNS B on growth in children from age 6-12 months old when compared to controls,
3. Evaluate the effect of SQ-LNS A and SQ-LNS B on anaemia and iron status from age 6-12 months old when compared to controls.

6.2. The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community

The aim of this study was to identify the predictors of stunting in infants 6 months old participating in a randomised control trial. The results of this cross-sectional study showed stunting prevalence (28.5%) was of public health importance and was associated with lower birth weight ($P<0.001$), shorter maternal height ($P=0.001$) and male sex ($P=0.014$) were significantly associated with higher odds for stunting. Maternal height had significant inverse association with stunting ($P=0.001$), indicating that maternal short stature was associated with higher odds for stunting. The other factors associated with stunting were higher sTfR (mg/L) concentrations, lower Hb (g/dL) and low education level of the mother/caregiver, while the consumption of jarred infant foods at least once in the previous week ($P=0.086$) showed a trend to be associated with lower odds for stunting.

These results support the view that stunting is associated with poor maternal nutrition, caring capacity and feeding practices. The provision of lipid based nutrient supplements during pregnancy has been shown to result in improved birth outcomes and prevent stunting especially in settings where there is an increased risk of IUGR (Mridha *et al.*, 2016). These findings suggest that that interventions aimed at improving nutrition for pre-conceptual, pregnant and lactating women, breastfeeding and complementary feeding practices may help prevent stunting in this and similar settings. The provision of SQ-LNS to pregnant and lactating mothers and to the children from 6-23 months may be one such important strategy to prevent low birth weight and subsequent growth faltering in children.

6.3. Effects of small-quantity lipid-based nutrient-supplements on growth in 6- to 12-month-old South African infants: a randomised control trial

This study investigated the hypothesis that provision of SQ-LNS from 6 months of age would result in improved growth and cognitive development in infants. The findings revealed that the provision of SQ-LNS B as home fortificants for complementary foods improved linear growth in infants from age 6 to 10 months old in infants from a peri-urban setting in South Africa. These positive effects of SQ-LNS B were not sustained beyond age of 10 months, while SQ-LNS A did not have impact on linear growth. The same trend was observed in the Haiti (Iannotti *et al.*, 2014) and Malawi studies (Mangani *et al.*, 2013) where they also observed that by month 4 (age 10 months old), all groups showed declining LAZ trajectories. The fact that fortified infant foods were used less as the children became older indicates that SQ-LNS B combined with poor nutrient rich complementary foods was not sufficient to sustain improved growth beyond age of 10 months.

However, the current findings build on to the growing body of evidence showing the potential of SQ-LNS supplements to promoting healthy growth and development in infants during the complementary feeding period. Future studies can be designed to assess the efficacy and/or effectiveness of providing SQ-LNS to pregnant and lactating mothers and to children from age 6-23 months. This scaling up of SQ-LNS as home fortificants if integrated with existing stunting reduction interventions may yield more meaningful outcomes

6.4. Effect of small-quantity lipid-based nutrient supplements on anaemia and iron status in 6-month-old infants from a peri-urban South African community: a randomised controlled trial

The aim of this study was to evaluate the efficacy of two SQ-LNS supplements on improving haemoglobin (Hb) and plasma ferritin (PF) concentrations and reducing the prevalence of anaemia and iron deficiency in infants. The results of this study showed that there were significant intervention effects of SQ-LNS on anaemia (Hb <11 g/dL), ID, (PF <12 µg/L) and IDA, (Hb <11 g/dL and PF <12 µg/L) in both treatment groups when compared to the control group. Infants in the SQ-LNS groups were less likely to be anaemic, iron deficient, and having iron deficiency anaemia when compared to the control group. These results add to the growing body of evidence that shows the importance of iron containing supplements in preventing anaemia and iron deficiency in infants. The observed effect of the SQ-LNS A and B on reducing anaemia and iron deficiency can be

attributed to the fact that they contain multiple micronutrients. Particularly, riboflavin, B-12 and folic acid, which have modulating effects on the metabolism of iron or Hb synthesis (Domellöf, 2007).

The results of the current study are consistent with findings from an earlier study in Ghana that showed that Sprinkles (SP), crushable *Nutritabs* (NT), and fat-based *Nutributter* were effective in reducing the prevalence of iron deficiency in study infants (Adu-Afarwuah *et al.*, 2008). These current findings provide further evidence that the use of SQ-LNS as home fortificants may be an effective option for reducing the prevalence of iron deficiency and anaemia in areas where there are high rates of anaemia, iron deficiency and where the complementary diets are deficient in key micronutrients.

6.5. Limitations, confounding and bias

This study was a randomised controlled trial conducted based on the 2008 guidelines for good practice in the conduct of clinical trials in human participants in South Africa (GCP) and the 2010 Consort guidelines (Schulz *et al.*, 2010), and with overall guidance from the Helsinki Declaration (WMA, 2012). These measures helped to eliminate bias and strengthen the internal and external validity of the trial findings. The block randomisation of sizes 3, 6 and 9 that was used to randomly allocate the infants to one of three groups eliminates selection bias. This means that any confounders and other potential sources of bias were randomly distributed across the three study groups. Despite having RCT design and adequate sampling the study still had some limitations that are outlined as below:

- Blinding of mother/caregivers was not possible due to the visible differences in the packaging of the two SQ-LNS supplements used in this study. This should not be a limitation as the study was designed to compare the two SQ-LNS to the control group. In addition, laboratory staff and fieldworkers conducting questionnaires and doing assessments were blinded to group assignment to the intervention groups as SQ-LNS A or SQ-LNS B.
- Hb finger prick was used to obtain Hb in cases where blood withdrawal was not successful. Some researchers have reported that finger prick method results in higher Hb concentrations compared to venous blood samples (Neufeld *et al.*, 2002; Alwan *et al.*, 2013). On the other hand, there is evidence to support the use of a capillary blood sample (finger prick) as a reliable alternative to

venous blood sample in field setting (Simmonds et al., 2011). In this study, the results of the comparison of Hb values obtained from capillary versus venous samples showed that there was no significant difference ($P=0.657$) across groups. Although, caution should be exercised, the Hb values and anaemia prevalence reported in this study still remain useful at pragmatic level.

- The study experienced high attrition and total withdrawals at the end of the study were 236/750 (31.5%) which was higher than the expected 25% set in sample size calculations. This suggests that this peri-urban area is prone to high population mobility. However, this study used the intention to treat statistical analysis plan and this meant that all infants enrolled were included in analysis of primary outcomes; linear growth, anaemia and iron deficiency. There were no significant differences in the distribution of withdrawals between control and the SQ-LNS groups (Kruskal-Wallis test, $P=0.368$).

6.6. Conclusions

The cross-sectional baseline results show that stunting prevalence was of public health relevance and was associated with lower birth weight, shorter maternal height and male sex. This may suggest that interventions aimed at improving nutritional status of all women of child bearing age coupled with promotion optimum breastfeeding and complementary feeding may help prevent stunting in this and similar settings. The intervention findings show that both products did not show an effect on growth at 12 months of age, however SQ-LNS B showed better linear growth at 8 and 10 months of age compared to the control group. In addition, both SQ-LNS A and SQ-LNS B had a positive effect on anaemia, iron deficiency and iron deficiency anaemia.

6.7. Public health perspective

Intra uterine growth restriction (IUGR) and poor nutrition early in infancy are now recognized as important determinants of neonatal and infant mortality and growth faltering (Bhutta *et al.*, 2013). Most importantly is the effect of maternal undernutrition which causes IUGR and poor birth outcomes which will lead to stunting in children (Black *et al.*, 2013b). The current findings that maternal height had an inverse relationship ($P=0.001$) with stunting supports this notion. This indicates that maternal short stature combined with poor nutrition during pre-conception and pregnancy can result in poor birth size (Catov *et al.*, 2011; Gernand *et al.*, 2012; Owens *et al.*, 2015) and subsequent stunting in children (Subramanian *et al.*, 2009; Özaltin *et al.*, 2010; Addo *et al.*, 2013). Nutrition specific and nutrition sensitive interventions have greatest impact if they are

targeted within the important 1000-day period (Black *et al.*, 2013a). These findings that low birth weight and maternal short stature were associated with stunting in children may indicate intergenerational nutritional and other related problems that may be difficult to address at 6-months of age (Black *et al.*, 2013a). Therefore, interventions that focus on improving pre-conceptual and maternal nutrition, as well as caring capacity of mothers may be an important strategy to prevent stunting in children in this and similar settings.

The cross-sectional findings also revealed that there is a link between socio-economic factors, feeding practices and maternal characteristics and stunting. In this study, a 10.1% prevalence of obesity and 28.5% prevalence of stunting indicate the presence of the double burden of malnutrition (nutrition transition) in South Africa. Therefore, there is need to ensure co-ordinated efforts and effective implementation of existing plans and strategies that focus on the 1000-day window of opportunity to prevent long-term consequences of stunting.

The proven nutrition specific interventions, to address infant and child under-nutrition include; those aimed at improving maternal nutrition, breastfeeding and complementary feeding practices. One promising approach is the use of SQ-LNS as home fortificants particularly in areas where the staple diets are plant based and likely to be deficient in multiple micronutrients and essential fatty acids (Arimond *et al.*, 2013). The findings from the current intervention study from peri-urban area in South Africa reveals that SQ-LNS B has the potential to improve linear growth (age six-10 months old). In addition, both supplements showed significant positive effects on haemoglobin and plasma ferritin concentrations for infants from aged six-12 months old. These findings agree with findings from earlier studies that reported potential of SQ-LNS to prevent stunting (Adu-Afarwuah *et al.*, 2007; Phuka *et al.*, 2008; Iannotti *et al.*, 2014; Hess *et al.*, 2015).

This suggests that SQ-LNS products have the potential to prevent stunting and micronutrient deficiencies in low income countries if integrated with other interventions that address infection control, provision of safe water and sanitary environment (WASH) and support for breastfeeding. However, it is important to point out that the concept of “prevention” is complicated in the presence of widespread and chronic under-nutrition in low income settings. Therefore, future research should include consideration of operational and implementation issues, as well as cost and comparative cost-effectiveness (Dewey, 2013).

6.8. Recommendations for future research

The results of and recommendations made in this thesis will be disseminated to health professionals, policy makers and wider community through publication in peer-reviewed research journals specifically *The American Journal of Clinical Nutrition* and *Maternal & Child Nutrition Journal*. The findings of this current study were/will presented at key nutrition conferences: (1) *Nutrition Society of South Africa (NSSA) Congress*; 3-5 September 2016, Somerset-West, South Africa, (2) *The 7th African Nutrition Epidemiological Conference (ANEC VII)*; 9-14 October 2016, Marrakech, Morocco, and (3) *Micronutrient Forum (MNF) Conference*; 24-28 October 2016, Cancun, Mexico. Future studies that aim to evaluate use of SQ-LNS as home fortificants to prevent stunting and micronutrient deficiencies should address the following:

1. The cross-sectional results have shown the need for interventions that focus on improving maternal nutrition and caring capacity of mothers/caregivers in the study area and similar settings,
2. The use of SQ-LNS supplements as home fortificants should be promoted and/or scaled up in areas where children are prone to stunting, ID and/or IDA particularly in areas where complementary foods are mainly plant based and lack the key micronutrients,
3. Studies to assess the efficacy and/or effectiveness of providing SQ-LNS to pregnant and lactating mothers and to children from age 6-23 months and integrated with existing stunting reduction interventions may yield more meaningful outcomes,
4. Future studies can investigate the probable linear growth-promoting effects of specific ingredients of SQ-LNS B (DHA, ARA, milk powder and *phytase*),
5. Finally, attention should also be given to the design of explanatory and pragmatic trials on effectiveness of SQ-LNS on linear growth and micronutrient status of infants in low-socio economic settings.

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APPENDICES



APPENDICES

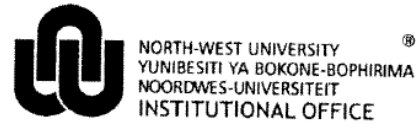
- A** Ethics Approvals from North-West University (NWU) for the Tswaka Trial
- B** Ethics Approvals from NWU for PhD Protocol
- C** Ethics Approval from South African Medical Research council (SA-MRC) for the Tswaka Trial
- D** Informed Consent Form (ICF)
- E** Information Sheet
- F** Author Guidelines: *Public Health Nutrition Journal*
- G** Author Guidelines: *Maternal and Child Nutrition Journal*

APPENDICES (continued)

- H** Anthropometry Sheet
- H** Author Guidelines: *American Journal of Clinical Nutrition*
- I** Anthropometry Sheet
- I** 24hr Dietary Recall
- K** Food Frequency Questionnaire (FFQ)
- L** Morbidity Questionnaire
- M** Baseline Questionnaire
- N** Publication Related to this Thesis
- O** Doctoral Citation for Tonde Matsungu



APPENDIX A: Ethics Approvals from North-West University (NWU) for the Tswaka Trial



Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-4852
Web: <http://www.nwu.ac.za>

ETHICS APPROVAL OF PROJECT

This is to certify that the next project was approved by the NWU Ethics Committee:

Project title :

GAIN
Randomized controlled trial in South Africa comparing the
impact of complementary food products on child growth

Projectleader : Prof M. Smuts

Ethics number: NWU-00011-11-A1

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

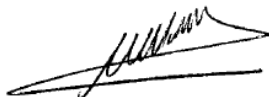
Expiry date: 2017/01/11

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project.

Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

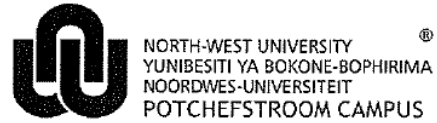
The formal ethics approval certificate will follow shortly.

Yours sincerely



HM Halgryn
NWU Research Ethics Secretariate

APPENDIX B: Ethics Approvals from NWU for PhD Protocol



Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

To whom it may concern

Faculty of Health Sciences
Tel: 018-299 2092
Fax: 018-299 2088
Email: Minrie.Greeff@nwu.ac.za

3 December 2013

Dear Prof. Smuts

Ethics Application: NWU-00001-11-A1 "Randomized controlled trial in South Africa comparing the impact of complementary food products on child growth"

Your application to include the sub-study, entitled "Efficacy of lipid nutrient supplements on improving growth and micronutrient status of infants" under the above mentioned umbrella project has been approved by the Ethics Sub-committee of the Faculty of Health Sciences.

Yours sincerely

A handwritten signature in black ink, appearing to be 'Minrie Greeff', with a long horizontal stroke extending to the right.

Prof. Minrie Greeff
Ethics Sub-committee Vice Chairperson

Original details: Prof. Minrie Greeff(10187308) C:\Users\13210572\Documents\ETHICS\2011 ETHICS\NWU-00001-11-A1 Addisionele versoek 2.docm
3 December 2013

File reference: NWU-00001-11-A1

APPENDIX C: Ethics Approval from South African Medical Research council (SA-MRC)



ETHICS COMMITTEE

PO Box 19070, Tygerberg 7505, Cape Town, South Africa
Francie van Zijl Drive, Parow Valley 7500
Tel: +27 (0)21 938 0341; Fax: +27 (0)21 938 0201
E-mail: adri.labuschagne@mrc.ac.za
<http://www.sahhealthinfo.org/ethics/ethics.htm>

13 September 2013

Prof M Faber
Nutritional Intervention Research Unit
MRC Cape Town

Dear Prof Faber

Protocol ID: EC011-03/2012
Protocol title: Randomised controlled trial in South Africa comparing the impact of complementary food products on child growth
Meeting date: 26 August 2013

Thank you for your application for an amendment, dated 31 July 2013, and your responses dated 8 and 10 September 2013. I am pleased to inform you that ethics approval is now granted for the amendment.

Wishing you well with your research.

Yours sincerely

A handwritten signature in black ink, appearing to read "D. du Toit", is located below the "Yours sincerely" text.

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

MRC Ethics Committee: Prof D du Toit (chairperson), Prof A Dhali, Prof DM Kayongo, Dr NE Khomo, Ms N Morar, Prof N Morojele, Ms L Mpahlwa, Prof H Oosthuizen, Mr D Rebombo, Dr L Schoeman, Dr Y Sikweyiya, Prof A van Niekerk, Ms A Labuschagne

APPENDIX D: Informed Consent Form (ICF)



CONSENT FORM

STUDY: Randomized controlled trial in South Africa comparing the impact of complementary food products on child growth

I voluntarily agree to take part in the study.

I have been informed about and understand the purpose of the study.

I have been informed about and understand the advantages and possible side-effects that may result from procedures.

I understand that I can withdraw my consent at any time without being penalised as far as my routine health care is concerned.

I have been informed that all information will be treated as confidential.

I understand that my baby can be in any of the three groups. If my baby is in either group 1 or group 2, I will receive the nutritional supplement free of charge for six months (starting when my baby is 6 months old) and must mix one portion of the supplement with my baby's normal food every day. If my baby is in group 3, I will receive a 6-month supply of the supplement when my baby is 12 months old.

I understand that, regardless of in which group my baby is, my baby and I need to go to the research site when my baby is 6 and 12 months old for the following:

- I will be asked questions about my household (at the start of the project), the foods that my baby eats and drinks, any illnesses that my baby had during the previous weeks, and development milestones that my baby has achieved.
- I will be asked to recall the foods and drinks that my baby consumed the day before.
- My baby's weight, length, head circumference and upper arm circumference will be measured.
- My weight and height will be measured (at the start of the project).
- I will be asked to express a small amount of breast milk (approximately ¼ cup).
- I will be asked to provide a small amount of urine (at the start and end of the project).
- A small amount of urine will be collected from my baby by using a special nappy.
- A nursing sister will take 4 mL (less than one teaspoon) blood from the vein in my baby's arm. If she cannot get blood from the vein in my baby's arm, she will draw blood from the vein at the top of my baby's hand or do a finger prick.
- The blood will be used to measure the amount of nutrients in my baby's blood. The blood will also be used to measure the body's response to the measles immunization that my baby will get at 9 months at the clinic.

I understand that my baby and I also need to go to the research site when my baby is 8 and 10 months old. During these two visits I will be asked questions about the foods that my baby eats and drinks, and my baby's weight and length will be measured.

My taxi fare to travel from my home in Jouberton to the research site at the Baptists church will be refunded.

I understand that a field worker will visit me at home once a week. During these visits the fieldworker will ask me questions on the usage of the supplement, illnesses that my baby had and developmental milestones that my baby has achieved.

Any abnormal findings will be attended to and referred, where necessary.

If I have any queries or problems regarding the study, I can contact either Prof Amanda Lourens the chairperson of the NWU Ethics Committee at (018) 2992606 or Prof Danie du Toit who is the chairperson of the MRC Ethics Committee at (021) 9380341 or I can send an e-mail to adri.labuschagne@mrc.ac.za.

Baby's code:

--	--	--	--

Recruitment number:

Mother/guardian's name & surname:

Baby's name & surname:

Address:

.....

.....

1. I hereby give consent that my baby may participate in the study.

Signature: Date:

Signed at:

Witness:

2. I hereby give consent that my weight and height can be measured, and some urine can be collected.

Signature: Date:

Signed at:

Witness:

3. I hereby give consent that some expressed breast milk can be collected.

Signature: Date:

Signed at:

Witness:

Fieldworker who informed the mother / guardian:

Researcher's signature:

APPENDIX E: Information Sheet



INFORMATION SHEET

STUDY: Randomized controlled trial in South Africa comparing the impact of complementary food products on child growth

Project leader: Prof Marius Smuts

Co-project leader: Prof Mieke Faber

Dear Parent / Legal Guardian

Who are we?

We are from the North-West University (PUKKE), Potchefstroom and the Medical Research Council, Cape Town. We are studying the effect of newly-developed nutritional supplements on the growth and development of babies. The supplements are in the form of a paste and can be mixed with the food babies normally eat. We invite you and your baby to participate in this important study.

Why are we doing this?

Iron and fatty acids in foods are important for the growth and development of babies. For optimal growth and development of babies, the different types of fatty acids in food need to be present in the right amounts. The aim of this study is to test two types of nutritional supplements in order to see if these supplements can improve the growth and development of babies. This will be done by measuring nutritional status, growth and development of babies after they have eaten the supplement mixed with their usual food every day for six months.

What do we expect from participants during the study?

Seven hundred and fifty (750) babies and their mothers (or primary caregiver) will be recruited through the local clinics. All babies will be 6 months old at the start of the study and will be followed up until they are 12 months old. Only mothers who planned to stay in the Jouberton area for at least the next 7 months can take part in the study. Only apparently healthy babies with no known allergy to soy, peanut, milk/lactose and fish, who are currently breastfeeding or have previously breastfed, will be included in the study. The products contain allergens from soy, milk, and fish, and may also contain traces of peanuts. Twins cannot participate in the study.

Participants will be divided into three groups. The first group will receive a fortified fat-based paste that contains essential fatty acids with other added fatty acids as well as a substance that may improve the absorption of iron. The second group will receive a paste that contains essential fatty acids. Both products contain soy. The third group will not receive any supplement during the 6-month study period, but they will receive a 6-month supply of the fat-based paste when the baby is 12 months old. Each child has an equal chance to be in any of the three groups. The amount of nutrients (e.g. iron and fatty acids) used in the two supplements is safe and no side effects such as nausea or diarrhoea is expected. The supplement will be provided free of charge to all study participants. Mothers will be asked to mix a certain amount of the supplement with the child's usual food daily for 6 months.

If you agree to participate in the study, we will ask the following from you:

When your baby is 6 and 12 months old, you will be asked to go with your baby to the research site which is at the Baptist Church. During these two visits, we will ask the following from you:

- You will be asked questions about your household (at the start of the project), the foods that your baby eats and drinks, any illnesses that your baby had during the previous weeks, and development that your baby has achieved.
- You will be asked to recall the foods and drinks that your baby consumed the day before.
- Your baby's weight, length, head circumference and upper arm circumference will be measured.
- Your weight and height will be measured (at the start of the project).
- You will be maybe asked to express a small amount of breast milk (approximately ¼ cup). We will measure the fatty acids and iodine in the breast milk. After the fatty acids and iodine have been measured, the remaining breast milk will be discarded according to standard procedures.
- A nursing sister will take a 4 mL (less than one teaspoon) blood sample from the vein in your baby's arm to measure the levels of nutrients in blood. We will also use this blood to measure your

baby's response to the measles immunization routinely given at the clinic to babies when they are 9 months old. If the nursing sister cannot get blood from your baby's arm, she will take blood from the vein in top of your baby's hand or by a finger prick. If your baby is showing too much resistance during this process, blood will not be taken from him/her. The procedure is completely safe.

- You will be maybe asked to provide a small amount of urine (at the start and end of the project). We will measure the iodine in the urine.
- We will collect a small amount of urine from your baby using a special nappy. We will measure the iodine in the urine.

You and your baby will be asked to further visit the research site when the baby is 8 and 10 months old. During these two visits you will be asked questions about the foods that your baby eats and drinks, and your baby's weight and length will be measured.

You will also be asked to record daily how much product your baby consumed and to report illness.

A field worker will visit you at home once a week. During these visits the fieldworker will ask you questions on the usage of the supplement, illnesses that your baby may experience and developmental milestones that your baby has achieved.

Who will have access to my child's information?

All information collected about your baby will be treated as confidential (will not be given to or discussed with anybody) and only the researchers will have access to it. No abnormal finding is expected, but should anything abnormal be found we will refer the baby to the local clinic or a medical doctor for the necessary treatment. You will be kept informed in this regard and are welcome to discuss any concerns that you may have with us.

What will the benefit be for my child who participates?

Your child may not benefit from the study, but children may in future benefit from the results. Your child will be monitored for the 6-month period and, should anything abnormal be found, be referred to the local clinic or a medical doctor for the necessary treatment. You will also gain information on your child's nutritional status and development.

What will the risks be for my child who participates?

The nutritional supplement is safe and should not harm your child or make your child sick. Your child may experience some discomfort when the blood samples are taken or when the weight and length are taken. This discomfort will be minimised as the staff taking these measurements will be experienced. If your child does not want to cooperate, the procedures will be stopped.

Must I participate?

Participation in this study is completely voluntary (your own choice). Whether you do, or do not, give your permission will not influence your baby's access to health care in any way.

May I change my mind?

Certainly, you may do this at any time without having to give a reason. The study is completely voluntary and it will not be kept against you in any way should you decide to withdraw from the study.

Who can you contact if there are any queries?

For more information on the study you may contact Prof Marius Smuts at 018-299 4670 or 082 451 0486 OR Prof Mieke Faber at 021-938 0404 or 0824602946 during office hours.

The study has been approved by the Ethics Committee of North-West University (NWU), the Ethics Committee of the Medical Research Council, as well as the Department of Health. If you have any queries or problems regarding the study, you can contact either Prof Amanda Lourens the chairperson of the NWU Ethics Committee at (018) 2992606 or Prof Danie du Toit who is the chairperson of the MRC Ethics Committee at (021) 9380341 or you can send an e-mail to adri.labuschagne@mrc.ac.za.

If you are happy for you and your child to take part in the study, please read and sign the consent form.

Thank you!

APPENDIX F: Author Guidelines: Public Health Nutrition Journal

Directions to Contributors

Public Health Nutrition

(Revised August 2014)

Public Health Nutrition (PHN) provides an international, peer-reviewed forum for the publication and dissemination of research with a specific focus on nutrition-related public health. The Journal publishes original and commissioned articles, high quality meta-analyses and reviews, commentaries and discussion papers for debate, as well as special issues. It also seeks to identify and publish special supplements on major topics of interest to readers.

SCOPE

The scope of *Public Health Nutrition* includes multi-level determinants of dietary intake and patterns, anthropometry, food systems, and their effects on health-related outcomes. We welcome papers that:

- Address **monitoring and surveillance** of nutritional status and nutritional environments in communities or populations at risk
- Identify and analyse behavioral, sociocultural, economic, political, and environmental **determinants of nutrition-related public health**
- Develop **methodology** needed for assessment and monitoring
- Inform efforts to improve **communication of nutrition-related information**
- **Build workforce capacity** for effective public health nutrition action
- Evaluate or discuss the effectiveness of **food and nutrition policies**
- Describe the development, implementation, and evaluation of **innovative interventions and programs** to address nutrition-related problems
- Relate diet and nutrition to **sustainability** of the environment and food systems

Papers that do not fall within the scope as described above may be directed to more appropriate journals. We prefer papers that are innovative (do not repeat research already undertaken elsewhere) and relevant to an international readership.

ARTICLE TYPES

PHN publishes Research Articles, Short Communications, Review Articles, Letters to the Editors, Commentaries and Editorials. Research Articles, Short Communications and Review Articles should be submitted to <http://mc.manuscriptcentral.com/phnutr>. Please contact the Editorial Office on phn.edoffice@cambridge.org regarding any other types of submission.

A typical paper should be no more than 5000 words long, not including the abstract, references, tables, figures and acknowledgements. Papers submitted as Short Communications should consist of no more than 2000 words, plus a maximum of 3 tables OR figures.

For systematic reviews and meta-analyses, the journal endorses the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (see British Medical Journal (2009) 339, b2535). Such submissions should follow the PRISMA guidelines.

Letters or commentaries are welcome that discuss, criticise or develop themes put forward in papers published in PHN or that deal with matters relevant to it. They should not be used as a means of publishing new work. Acceptance will be at the discretion of the Editorial Board, and editorial changes may be required. Wherever possible, letters from responding authors will be included in the same issue.

SUBMISSION AND REVIEW PROCESS

PHN uses ScholarOne Manuscripts for online submission and peer review. As part of the online submission process, authors are asked to affirm that the submission represents original work that has not been

published previously; that it is not currently being considered by another journal; and that each author has seen and approved the contents of the submitted manuscript.

At submission, authors must nominate at least four potential referees who may be asked by the Editorial Board to help review the work. PHN uses a double-blind review process, and manuscripts are normally reviewed by two external peer reviewers and a member of the Editorial Board.

Revisions must be resubmitted within 2 months or they will be deemed a new paper. When substantial revisions are required after review, authors are normally given the opportunity to do this once only; the need for any further changes should reflect only minor issues.

PUBLISHING ETHICS

PHN adheres to the Committee on Publication Ethics (COPE) guidelines on research and publications ethics. The Journal considers all manuscripts on the strict condition that:

- 1) The manuscript is your own original work, and does not duplicate any previously published work;
- 2) The manuscript has been submitted only to the journal - it is not under consideration or peer review or accepted for publication or in press or published elsewhere;
- 3) All listed authors know of and agree to the manuscript being submitted to the journal; and
- 4) The manuscript contains nothing abusive, defamatory, fraudulent, illegal, libellous, or obscene.

Text taken directly or closely paraphrased from earlier published work that has not been acknowledged or referenced will be considered plagiarism. Submitted manuscripts in which such text is identified will be withdrawn from the editorial process. Any concerns raised about possible plagiarism or other violations of ethical guidelines in an article submitted to or published in PHN will be investigated fully and dealt with in accordance with the COPE guidelines.

DETAILED MANUSCRIPT PREPARATION INSTRUCTIONS

Language

Papers submitted for publication must be written in English and should be as concise as possible. We recommend that authors have their manuscript checked by an English language native speaker before submission, to ensure that submissions are judged at peer review exclusively on academic merit.

We list a number of third-party services specialising in language editing and / or translation, and suggest that authors contact as appropriate. Use of any of these services is voluntary, and at the author's own expense.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Authors are advised to consult a current issue in order to make themselves familiar with PHN as to typographical and other conventions, layout of tables etc.

Authorship

The Journal conforms to the International Committee of Medical Journal Editors (ICMJE) definition of authorship. Authorship credit should be based on:

1. Substantial contributions to conception and design, data acquisition, analysis and/or interpretation;
2. Drafting the article or revising it critically for important intellectual content; and
3. Final approval of the version to be published.

The contribution of individuals who were involved in the study but do not meet these criteria should be described in the Acknowledgments section.

Ethical standards

All submissions must abide by the guidelines in the World Medical Association (2000) Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, with notes of clarification of 2002 and 2004 (<http://www.wma.net/en/30publications/10policies/b3/>), the Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the Ethical Conduct of Medical Research Involving

Children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (Arch Dis Child (2000) 82, 177–182).

Cover Letter

Authors are invited to submit a cover letter including a short explanation of how the article advances the field of public health nutrition in terms of research, practice, or policy, and of its relevance to an international readership. The text for the cover letter should be entered in the appropriate box as part of the online submission process.

Title Page

Authors must submit a title page online **as a separate file to their manuscript**, to enable double-blind reviewing. For the same reason, the information on the title page should **not** be included in the manuscript itself. The title page should include:

1. The title of the article;
2. Authors' names, given without titles or degrees;
3. Name and address of department(s) and institution(s) to which the work should be attributed for each author, with each author's institution(s) identified by a superscript number (e.g. A.B. Smith¹);
4. Name, mailing address, email address, telephone and fax numbers of the author responsible for correspondence about the manuscript;
5. A shortened version of the title, not exceeding 45 characters (including letters and spaces) in length;
6. Disclosure statements, as outlined below. These must be included on the title page and **not in the manuscript file**, to enable double-blind reviewing; if the paper is accepted, they will be inserted into the manuscript during production.

Acknowledgments

Here you may acknowledge individuals or organizations that provided advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

Financial Support

Please provide details of the sources of financial support for all authors, including grant numbers. For example, "This work was supported by the Medical research Council (grant number XXXXXXXX)". Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be separated by a semi-colon, with "and" before the final funder. Grants held by different authors should be identified as belonging to individual authors by the authors' initials. For example, "This work was supported by the Wellcome Trust (A.B., grant numbers XXXX, YYYY), (C.D., grant number ZZZZ); the Natural Environment Research Council (E.F., grant number FFFF); and the National Institutes of Health (A.B., grant number GGGG), (E.F., grant number HHHH)".

This disclosure is particularly important in the case of research supported by industry, including not only direct financial support for the study but also support in kind such as provision of medications, equipment, kits or reagents without charge or at reduced cost and provision of services such as statistical analysis. **All such support**, financial and in kind, should be disclosed here.

Where no specific funding has been provided for research, please provide the following statement: "This research received no specific grant from any funding agency, commercial or not-for-profit sectors."

In addition to the source of financial support, please state whether the funder contributed to the study design, conduct of the study, analysis of samples or data, interpretation of findings or the preparation of the manuscript. If the funder made no such contribution, please provide the following statement: "[Funder's name] had no role in the design, analysis or writing of this article."

Conflict of Interest

Conflict of interest exists when an author has interests that might inappropriately influence his or her judgement, even if that judgement is not influenced. Because of this, authors must disclose potentially conflicting interests so that others can make judgements about such effects. Please provide details of all known financial and non-financial (professional and personal) relationships with the potential to bias the work. Where no known conflicts of interest exist, please include the following statement: "None."

For more information on what constitutes a conflict of interest, please see the ICMJE guidelines.

Authorship

Please provide a very brief description of the contribution of each author to the research. Their roles in formulating the research question(s), designing the study, carrying it out, analysing the data and writing the article should be made plain.

Ethical Standards Disclosure

Manuscripts describing experiments involving human subjects must include the following statement: "This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [name of the ethics committee]. Written [or Verbal] informed consent was obtained from all subjects/patients." Where verbal consent was obtained, this must be followed by a statement such as: "Verbal consent was witnessed and formally recorded."

Manuscript Format

The requirements of PHN are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE, and authors are encouraged to consult the latest guidelines, which contain useful, general information about preparing scientific papers. Authors should also consult the CONSORT guidelines for reporting results of randomised trials.

For detailed instructions regarding **mathematical modelling, statistical analysis and nomenclature requirements**, please refer to the Appendix to these instructions.

Typescripts should be prepared with 1.5 line spacing and wide margins (2 cm), the preferred font being Times New Roman size 12. At the ends of lines, words should not be hyphenated unless hyphens are to be printed. **Line numbering and page numbering are required.**

Manuscripts should be organised as follows:

Abstract

Each paper must open with a structured abstract of **not more than 250 words**. The abstract should consist of the following headings: Objective, Design, Setting, Subjects, Results, Conclusions. All the headings should be used, and there should be a separate paragraph for each one. The abstract should be intelligible without reference to text or figures.

Keywords

Authors should list at least four keywords or phrases (each containing up to three words).

Introduction

It is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it.

Methods

For manuscripts describing experiments involving human subjects, the required ethical standards disclosure statement must be included **on the title page only** as described above. It will then be inserted into this section of the manuscript during production.

Results

These should be given as concisely as possible, using figures or tables as appropriate. Data should not be duplicated in tables and figures.

Discussion

While it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful.

References

References should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted^(1,2-4)'. If a reference is cited more than once, the same number should be used each time.

References cited only in tables and figure legends should be numbered in sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text.

Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References.

At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order using the Vancouver system. When an article has more than three authors only the names of the first three authors should be given followed by 'et al.' The issue number should be omitted if there is continuous pagination throughout a volume. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page. References to books and monographs should include the town of publication and the number of the edition to which reference is made. References to material available on websites should include the full Internet address, and the date of the version cited.

Examples of correct forms of references are given below.

Journal articles

1. Setchell KD, Faughnan MS, Avades T *et al.* (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of ¹³C-labeled tracers in premenopausal women. *Am J Clin Nutr* **77**, 411–419.
2. Barker DJ, Winter PD, Osmond C *et al.* (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* **ii**, 577–580.
3. Forchielli ML & Walker WA (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br J Nutr* **93**, Suppl. 1, S41–S48.
4. Skurk T, Herder C, Kraft I *et al.* (2004) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* (Epublication ahead of print version).

Books and monographs

5. Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.
6. Ailhaud G & Hauner H (2004) Development of white adipose tissue. In *Handbook of Obesity. Etiology and Pathophysiology*, 2nd ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.
7. Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.
8. World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation. WHO Technical Report Series* no. 916. Geneva: WHO.
9. Keiding L (1997) *Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–1991 (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991)*. Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

Sources from the internet

10. Nationmaster (2005) HIV AIDS – Adult prevalence rate. http://www.nationmaster.com/graph-T/hea_hiv_aid_adu_pre_rat (accessed June 2013).

Tables

Tables should be placed in the main manuscript file at the end of the document, not within the main text. Be sure that each table is cited in the text. Tables should carry headings describing their content and should be comprehensible without reference to the text. Tables should not be subdivided by ruled lines.

The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the ± sign should not be used. The number of decimal places used should be standardized; for whole numbers 1.0, 2.0 etc. should be used. Shortened forms of the words weight (wt) and height (ht) may be used to save space in tables.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols.

Abbreviations are given in the format: RS, resistant starch. Abbreviations in tables must be defined in footnotes in the order that they appear in the table (reading from left to right across the table, then down each column). Symbols for footnotes should be used in the sequence: *†‡§||¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. 'a,b,c Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$)'. Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ '. The symbols used for P values in the tables must be consistent.

Figures

Figures should be supplied as separate electronic files. Figure legends should be grouped in a section at the end of the manuscript text. Each figure should be clearly marked with its number and separate panels within figures should be clearly marked (a), (b), (c) etc. so that they are easily identifiable when the article and

figure files are merged for review. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations.

We recommend that only TIFF, EPS or PDF formats are used for electronic artwork. Other formats (e.g., JPG, PPT and GIF files and images created in Microsoft Word) are usable but generally NOT suitable for conversion to print reproduction. For further information about how to prepare your figures, including sizing and resolution requirements, please see our artwork guide.

In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, Δ, ▲, □, ■, ×, +. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure size is reduced to fit the printed page. Colour figures will be published online free of charge, and there is a fee of £300 per figure for colour figures in the printed version. If you request colour figures in the printed version, you will be contacted by CCC-Rightslink who are acting on our behalf to collect colour charges. Please follow their instructions in order to avoid any delay in the publication of your article.

Supplementary material

Additional data (e.g. data sets, large tables) relevant to the paper can be submitted for publication online only, where they are made available via a link from the paper. The paper should stand alone without these data. Supplementary Material must be cited in a relevant place in the text of the paper.

Although Supplementary Material is peer reviewed, it is not checked, copyedited or typeset after acceptance and it is loaded onto the journal's website exactly as supplied. You should check your Supplementary Material carefully to ensure that it adheres to journal styles. Corrections cannot be made to the Supplementary Material after acceptance of the manuscript. Please bear this in mind when deciding what content to include as Supplementary Material.

License to Publish form

Authors or their institutions retain copyright of papers published in PHN. The corresponding author is asked to complete a License to Publish form on behalf of all authors, and upload this with the manuscript files **at the time of submission**. The form includes confirmation that permission for all appropriate uses has been obtained from the copyright holder for any figures or other material not in the authors' copyright, and that the appropriate acknowledgement has been made to the original source. If the manuscript is not accepted, the form will be destroyed.

Accepted Manuscripts

PDF proofs are sent to authors in order to make sure that the paper has been correctly set up in type. Only changes to errors induced by typesetting/copy-editing or typographical errors will be accepted. Corrected proofs should be returned within 2 days by email to Gill Watling at gillwatling@btinternet.com. If corrected proofs are not received from authors within 7 days the paper may be published as it stands.

Offprints

A PDF file of the paper will be supplied free of charge to the corresponding author of each paper, and offprints may be ordered on the order form sent with the proofs.

CONTACT

Prospective authors may contact the Editorial Office directly on +44 (0) 1223 327954 (telephone) or phn.edoffice@cambridge.org (email).

Additionally, more information about the journal, including recent issues, can be found at <http://journals.cambridge.org/phn>.

APPENDIX: MATHEMATICAL MODELLING, STATISTICS AND NOMENCLATURE

Mathematical modelling of nutritional processes

Papers in which mathematical modelling of nutritional processes forms the principal element will be considered for publication provided: (a) they are based on sound biological and mathematical principles; (b) they advance nutritional concepts or identify new avenues likely to lead to such advances; (c) assumptions used in their construction are fully described and supported by appropriate argument; (d) they are described in such a way that the nutritional purpose is clearly apparent; (e) the contribution of the model to the design of future experimentation is clearly defined.

Units

Results should be presented in metric units according to the International System of Units (see Quantities, Units and Symbols in Physical Chemistry, 3rd ed. (2007) Cambridge: RSC Publishing), and Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences (1972) London: The Royal Society – as reproduced in *Proceedings of the Nutrition Society* (1972) **31**, 239–247). SI units should be used throughout the paper. The author will be asked to convert any values that are given in any other form. The only exception is where there is a unique way of expressing a particular variable that is in widespread use. Energy values must be given in Joules (MJ or kJ) using the conversion factor 1 kcal = 4.184 kJ. If required by the author, the value in kcal can be given afterwards in parentheses. Temperature is given in degrees Celsius (°C). Vitamins should be given as mg or µg, not as IU.

For substances of known molecular mass (Da) or relative molecular mass, e.g. glucose, urea, Ca, Na, Fe, K, P, values should be expressed as mol/l; for substances of indeterminate molecular mass (Da) or relative molecular mass, e.g. phospholipids, proteins, and for trace elements, e.g. Cu, Zn, then g/l should be used. The 24 h clock should be used, e.g. 15.00 hours.

Units are: year, month, week, d, h, min, s, kg, g, mg, µg, litre, ml, µl, fl. To avoid misunderstandings, the word litre should be used in full, except in terms like g/l. Radioactivity should be given in becquerels (Bq or GBq) not in Ci. 1 MBq = 27.03 µCi (1Bq = 1 disintegration/s).

Statistical treatment of results

Data from individual replicates should not be given for large experiments, but may be given for small studies. The methods of statistical analysis used should be described, and references to statistical analysis packages included in the text, thus: Statistical Analysis Systems statistical software package version 6.11 (SAS Institute, Cary, NC, USA). Information such as analysis of variance tables should be given in the paper only if they are relevant to the discussion. A statement of the number of replicates, their average value and some appropriate measure of variability is usually sufficient.

Comparisons between means can be made by using either confidence intervals (CI) or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED), or the standard errors of the means (SE or SEM) when these vary between means. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values. The degrees of freedom (df) associated with SED, SEM or SD should also be stated. The number of decimal places quoted should be sufficient but not excessive. Note that pH is an exponential number, as are the log(10) values often quoted for microbial numbers. Statistics should be carried out on the scalar rather than the exponential values.

If comparisons between means are made using CI, the format for presentation is, e.g. 'difference between means 0.73 (95 % CI 0.314, 1.36) g'. If significance tests are used, a statement that the difference between the means for two groups of values is (or is not) statistically significant should include the level of significance attained, preferably as an explicit P value (e.g. P=0.016 or P=0.32) rather than as a range (e.g. P<0.05 or P>0.05). It should be stated whether the significance levels quoted are one-sided or two-sided. Where a multiple comparison procedure is used, a description or explicit reference should be given. Where appropriate, a superscript notation may be used in tables to denote levels of significance; similar superscripts should denote lack of a significant difference.

Where the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g. experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included.

Chemical formulas

These should be written as far as possible on a single horizontal line. With inorganic substances, formulas may be used from first mention. With salts, it must be stated whether or not the anhydrous material is used, e.g. anhydrous CuSO₄, or which of the different crystalline forms is meant, e.g. CuSO₄.5H₂O, CuSO₄.H₂O.

Descriptions of solutions, compositions and concentrations

Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0.1 M-NaH₂PO₄. Compositions expressed as mass per unit mass (w/w) should have values expressed as ng, µg, mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. If concentrations or compositions are expressed as a percentage, the basis for the composition should be specified (e.g. % (w/w) or % (w/v) etc.). The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

Gene nomenclature and symbols

The use of symbols and nomenclature recommended by the HUGO Gene Nomenclature Committee is encouraged. Information on human genes is also available from Entrez Gene, on mouse genes from the Mouse Genome Database and on rat genes from the Rat Genome Database.

Nomenclature of vitamins

Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* (1978) 48A, 831–835. **Acceptable name**

Vitamin A

Retinol

Retinaldehyde, retinal

Retinoic acid (all-trans or 13-cis)

3-Dehydroretinol

Vitamin D

Ergocalciferol, ercalciol

Cholecalciferol, calciol

Vitamin E

α-, β- and γ-tocopherols plus tocotrienols

Vitamin K

Phylloquinone

Menaquinone-n (MK-n)†

Menadione

Vitamin B1

Thiamin

Vitamin B2

Riboflavin

Niacin

Nicotinamide

Nicotinic acid

Folic Acid

Pteroyl(mono)glutamic acid

Vitamin B6

Pyridoxine

Pyridoxal

Pyridoxamine

Vitamin B12

Cyanocobalamin

Other names*

Vitamin A1

Retinene

Vitamin A1 acid

Vitamin A2

Vitamin D₂ calciferol

Vitamin D₃

Vitamin K1

Vitamin K2

Vitamin K₃, menaquinone, menaphthone

Aneurin(e), thiamine

Vitamin G, riboflavine, lactoflavin

Vitamin PP

Folacin, vitamin B_c or M

Pyridoxol

APPENDIX G: Author Guidelines: Maternal and Child Nutrition Journal

Maternal & Child Nutrition

© John Wiley & Sons Ltd



Edited by: Victoria Hall Moran and Kathryn Dewey

Impact Factor: 2.973

ISI Journal Citation Reports © Ranking: 2013: 13/118 (Pediatrics); 27/79 (Nutrition & Dietetics)

Online ISSN: 1740-8709

Author Guidelines

1. General

The high profile of maternal and child nutrition has highlighted the need for a focused, well-respected and dedicated forum for the presentation of original research findings in this field. *Maternal & Child Nutrition* keeps the audience fully informed about new initiatives, the latest research findings and innovative ways of responding to changes in public attitudes. Drawing from global sources, the Journal provides an invaluable source of up-to-date information for health professionals, academics and service users with interests in maternal and infant nutrition.

The scope of *Maternal & Child Nutrition* includes pre-conceptual nutrition, antenatal and postnatal maternal nutrition, women's nutrition throughout their reproductive years, and fetal, neonatal, infant and child nutrition, up to and including adolescence.

Topics covered include:

- Nutritional needs of mothers and their children in health and disease
- Physiological, sociocultural, psychological, economic and political aspects of nutrition
- Health improvement
- Health education

- Health policy and assessment in practice
- Inter-agency initiatives
- Food safety and related environmental and regulatory issues
- Nutritional risk assessment
- Evaluation of interventions aimed at improving health
- The role of nutrition in both healthy and vulnerable groups
- Development of research methods, validation of measures

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in [Maternal & Child Nutrition](#). Authors are encouraged to visit Wiley-Blackwell's [Author Services](#) for further information on the preparation and submission of articles and figures.

2. Ethical guidelines

Maternal & Child Nutrition adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authorship: Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the journal. ALL named authors must have made an active contribution to the conception and design and/or analysis and interpretation of the data and/or the drafting of the paper and ALL must have critically reviewed its content and have approved the final version submitted for publication. Participation solely in the acquisition of funding or the collection of data does not justify authorship and, except in the case of complex large-scale or multi-centre research, the number of authors should not exceed six.

Maternal & Child Nutrition adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on

1. substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data,
2. drafting the article or revising it critically for important intellectual content and
3. final approval of the version to be published.

Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Acknowledgements: Under Acknowledgements please specify contributors to the article other than the authors accredited.

2.2. Ethical Approvals

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee, institutional council or review board approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

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Acknowledgments

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 4. analyzed data or performed statistical analysis;
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References

Number references consecutively in the order in which they are first mentioned in the text. For a standard journal article with more than 10 authors, **please list first 10 authors before using "et al."**; list all authors when 10 or fewer. In the text, identify references by Arabic numerals in parentheses (1), not superscript. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Supplemental Material should have a separate reference section.

It is rarely necessary to cite more than 50 references in an original research article. Try to avoid citing published abstracts as references [if a published abstract is cited, include "(abstr)" at the end of the reference]. Abstracts from scientific meetings not published in peer-reviewed journals may not be used as references. Unpublished observations and personal communications (written, not oral) may not be used as references but may be inserted in parentheses with the names of the

responsible researchers and the year of the observation or communication. Authors are responsible for obtaining written permission from everyone so cited and for providing to the Editor a copy of the permission, if requested. Doctoral dissertations may be used as references. Include manuscripts accepted but not yet published; designate journal name followed by "(in press)." Report foreign titles in the original language, identify the language, and provide the English translation in parentheses. The references must be verified by the author against the original documents.

Journals

1. Journal article published electronically ahead of print: Authors may add to a reference, the DOI ("digital object identifier" number unique to the publication) for articles in press. It should be included immediately after the citation in the References.
Bergholdt HKM, Nordestgaard BG, Ellervik C. Milk intake is not associated with low risk of diabetes or overweight-obesity: a Mendelian randomization study in 97,811 Danish individuals. *Am J Clin Nutr* 2015 Jul 8 (Epub ahead of print; DOI: doi:10.3945/ajcn.114.105049).
2. Standard journal article: list all authors when 10 or fewer; when >10, list only the first 10 and add "et al." Abbreviate journal titles according to *Index Medicus* style, which is used in MEDLINE citations.
Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003;78:684–9.
3. Corporate author
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Books and other monographs

1. Personal authors
Shils M, Shike M, Olson J, Ross AC. *Modern nutrition in health and disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.
2. Committee report or corporate author
National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.
Food and Nutrition Board, Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.
3. Chapter in book
Young VR, Tharakan JF. Nutritional essentiality of amino acids and amino acid requirements in healthy adults. 2nd. ed. In: Cynober LA, ed. *Metabolic and therapeutic aspects of amino acids in clinical nutrition*. Boca Raton, FL: CRC Press, 2004:439–70.
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<http://pediatrics.aappublications.org/cgi/content/full/112/4/e303> (accessed 14 October 2003).

Tables

Tables must be included in the text file, and each table should begin on a new page. Double-spacing of tables is preferred but not required. Number tables consecutively with Arabic numerals (do not use 1A, 1B, etc) and supply a brief descriptive title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading or table title. Each table should contain enough detail (including statistics) that the table is intelligible without reference to the text. All nonstandard abbreviations, including group designations, used in a table or table title should be defined in a footnote to the table title, and the abbreviations should be listed in alphabetic order. If the footnote to the table title contains multiple items, the definitions of the abbreviations should be the last item. If a table contains only one abbreviated term in the body of the table, then a separate footnote placed after that abbreviation should be used to define that term. Commonly used approved abbreviations (see [Units and Abbreviations](#)) may be used without explanation. Additionally, explanations are not needed for ANOVA, BMI, F (females), and M (males). For footnotes, use superscript Arabic numerals. For reporting results of statistical analyses, superscript letters can be used if explaining the results in the usual manner would be too complicated (see a recent issue of the *AJCN* for examples). The first appearance in a horizontal row determines the order of the footnotes. Identify statistical measures of variation, such as SD and SE. **Omit internal horizontal and vertical rules** before submitting your tables. Cite each table in the text in consecutive order. Use boldface for the first mention of each table. If you use data from another published source, acknowledge the source fully. Number references in tables according to the location of the first citation of each table in the text. For an illustrated table quality checklist, visit [Illustrated table quality checklist](#).

Figures

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Legends for all figures should be included within the manuscript text file on a separate page and be typed with double-spacing (legends should not be included on the figures themselves). Each legend should contain enough detail, including statistics, to make the figure intelligible without reference to

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Files must conform to the minimum-resolution specifications listed below (see [Image resolution](#)). Figures that are part of the regular manuscript submission and not part of OSM must be uploaded as separate files. Lettering and symbols must be large enough to be readable when the figure is reduced to 1 column width (< 8.5 cm) or, in rare cases, to 2 column widths. Preferred text size is 7 points.

1 column: 18p0 / 3 inches / 7.6 cm

1.5 column: 27p0 / 4.5 inches / 11.5 cm

Maximum width (to span 2 columns): 34p0 / 5.7 inches / 14.4 cm

Maximum height: 53p0 / 8.8 inches / 22.4 cm

The use of color will be evaluated for each figure on an as-needed basis, and authors who are not members of ASN must pay an extra charge if color is used. Reprints of articles with color figures will be billed at a higher charge because of the additional costs of printing color. Do not use 3-dimensional figures unless necessary. When labeling axes, capitalize only the first word and proper nouns; use lowercase letters for the remaining words and put units in parentheses.

Formatting

Microsoft PowerPoint (PPT) and Word (DOC) files can be acceptable if properly prepared and submitted in their native format. When creating print-quality files in MS Office applications, follow these general guidelines:

1. Do not use pattern or texture fills in graphics. Instead use solid fills or percentage screens that will be effectively converted to vector images during file conversion.
2. When inserting pictures or images into files, be sure to select “insert” and not “insert link,” which will not properly embed the hi-res image into the MS Office file.
3. Do not reduce or enlarge the images after placement within the MS Office file. Otherwise the image quality will be affected.
4. A separate file should be submitted for each figure. Make sure that any multi-panel figures (i.e., figures with parts labeled A, B, C, D, etc.) are assembled into one file. Rather than sending four files (Figure 1A, Figure 1B, Figure 1C, Figure 1D), the four parts should be assembled into one piece and supplied as one file.
5. If a figure is very small in the system-generated PDF file, the resolution of the figure file was not high enough. A higher-resolution figure should be uploaded before the PDF is approved.
6. **Authors are requested to create and keep high-resolution print copies of the figures, in the event that they are needed for publication purposes.**

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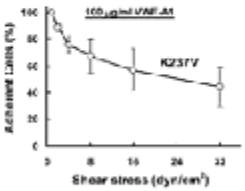
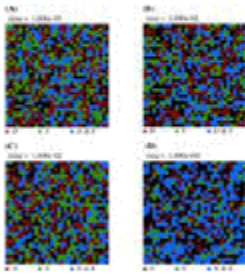
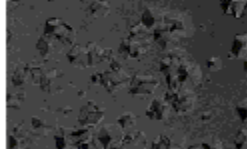
Line art	Combination Halftones (grayscale or color images and type)	Halftones (grayscale or color with no type or lettering)
		
1000 dpi	600 dpi	300 dpi

Figure 1

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Supplemental material may be included with manuscript submissions. Supplemental files for upload may include [required research checklists](#), articles published/in press elsewhere, reports or technical briefs related to manuscript submission, figure source files, questionnaires, permissions, videos, etc. All supplemental data should be clearly labeled either as "Supplemental Data for Reviewers Only" or, as "Online Supplemental Material" (OSM) if it is submitted for online publication only in *The AJCN*. Online-only figures and tables should be labeled "Supplemental Figure 1," Supplemental Table 1," etc. Upload the OSM in the format that will make it most widely accessible to readers. OSM files will not be edited before being posted online; therefore, please be sure that *The American Journal of Clinical Nutrition* format is used and that the files are accurate. Please also upload supplemental files for review only separately from supplemental files for online publication.

APPENDIX I: Anthropometry Sheet



ANTHROPOMETRY

Registration number:

--	--	--	--	--	--

Baby's code (To be filled by the nursing sister):

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Is the respondent the mother or the caregiver of the baby?	1 = Mother	2 = Caregiver	
Mother's / caregiver's age (in years)			
Mother's / caregiver's height (cm)	1 st measurement		
	2 nd measurement		
Mother's / caregiver's weight (kg)	1 st measurement		
	2 nd measurement		

BABY: ANTHROPOMETRIC MEASUREMENTS

Baby's date of birth (day/month/year)			/			/	2	0	1
Baby's sex	1 = Boy	2 = Girl		Baby's birth weight (kg)		.			

DATE	Baseline	Month 2	Month 4	Month 6 (end)
Year	2 0 1	2 0 1	2 0 1	2 0 1
Day / month	d d / m m	d d / m m	d d / m m	d d / m m
Fieldworker code				
Child has edema (*If yes record as AE)	yes* 1 no 2	yes* 1 no 2	yes* 1 no 2	yes* 1 no 2

WEIGHT (kg)	Baseline	Month 2	Month 4	Month 6 (end)
1 st Measurement				
2 nd Measurement				
3 rd (if needed)				

LENGTH (cm)	Baseline	Month 2	Month 4	Month 6 (end)
1 st Measurement				
2 nd Measurement				
3 rd (if needed)				

MUAC (cm)	Baseline	Month 2	Month 4	Month 6 (end)
1 st Measurement				
2 nd Measurement				
3 rd (if needed)				

HC (cm)	Baseline	Month 2	Month 4	Month 6 (end)
1 st Measurement				
2 nd Measurement				
3 rd (if needed)				

Anthropometry (30 Aug 2013)

APPENDIX J: 24hr Dietary Recall



24-hr DIETARY RECALL

Baby's code:

--	--	--	--

Date of the interview (dd/mm/yyyy):

		/			/	2	0	1	
--	--	---	--	--	---	---	---	---	--

Fieldworker's code:

--	--

What day is it today?	1 = Monday	2 = Tuesday	3 = Wednesday	4 = Thursday	5 = Friday	
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Greetings!

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what your baby is eating and drinking. This information is important to know as it will tell us how much and what types of food babies in the area are eating.

There are no right or wrong answers.

Everything you tell me is confidential.

Is there anything you want to ask now? Are you willing to go on with the questions?

I want to find out about everything your baby ate or drank yesterday, including breast milk and water. Please tell me everything your baby ate from the time he/she woke up yesterday, throughout the day and during the night. I will also ask you where your baby ate the food and how much he/she ate.

APPENDIX K: Food Frequency Questionnaire (FFQ)



Baby's code:

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FOOD FREQUENCY

Baby's code:

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Ask for each food item, one at a time, how often the child usually eats the specific food item. The last week (or last seven days) should be taken as guideline, therefore the frequency that the child ate the food item during the last week. Make a cross on the option that describes the mother's answer the best). The options are as follows:

*Every day

*Most days: not every day, but at least 4 times per week

*Once a week: less than 4 times per week, but at least once per week

*Never

Food item	Frequency of intake during the last week			
	Baseline	Month 2	Month 4	Month 6 (end)
Date (dd/mm/yyyy):				
Fieldworker's code:				
Breastmilk	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Formula milk If formula milk was used, please give name of the formula milk:	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Cow's milk / amasi / maas Milk powder e.g. Klim, Nespray	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Yoghurt / danone	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Baby foods in a jar e.g. Purity	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Infant cereals or infant porridge e.g. Nestum, Cerelac, Cream of Maize, Baby Mabele	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never

Baby's code:

--	--	--	--

Food item	Frequency of intake during the last week			
	Baseline	Month 2	Month 4	Month 6 (end)
Porridge made with maize meal (soft, stiff or crumbly)	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Cooked porridge, other than maize meal porridge e.g. oats, mabele	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Instant porridge, e.g. instant Ace	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Bread	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Rice	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Potatoes	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Vegetables, any type (NOT potatoes)	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
<i>If vegetables were eaten, please name the type of vegetables eaten mostly:</i>	1..... 2..... 3.....	1..... 2..... 3.....	1..... 2..... 3.....	1..... 2..... 3.....
Fruit juice (includes juice squeezed from the fruit)	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Fresh fruit (any type)	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
<i>If fruit were eaten, please name the type of fruit eaten mostly:</i>	1..... 2..... 3.....	1..... 2..... 3.....	1..... 2..... 3.....	1..... 2..... 3.....

Baby's code:

--	--	--	--

Food item	Frequency of intake during the last week			
	Baseline	Month 2	Month 4	Month 6 (end)
Eggs	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Red meat (beef, pork, mutton) / stew / sausage / mince meat	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Chicken / poultry	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Liver (e.g. chicken liver, beef liver, sheep liver etc)	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Fish (fresh or canned)	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Sweets / Chocolates	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Chips / Cheese curls / Niknaks	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Fizzy cold drink e.g. Coke	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Juice concentrate, mix with water e.g. Oros	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Rooibos	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never
Tea, normal	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never	1__ Every day 2__ Most days 3__ Once a week 4__ Never

Baby's code:

--	--	--	--

Food item	Frequency of intake during the last week			
	Baseline	Month 2	Month 4	Month 6 (end)
Sugar (any type), eaten as such, in drinks (e.g. tea) or added to food	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
Salt (added to food)	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
How often did you use oil when preparing the <u>baby's food</u> ?	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
How often did you use margarine when preparing the <u>baby's food</u> ? [any type of margarine]	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never
How often did you use peanut butter when preparing the <u>baby's food</u> ?	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never	1__Every day 2__Most days 3__Once a week 4__Never

APPENDIX L: Morbidity Questionnaire



MORBIDITY

Baby's name & surname: Baby's code:

--	--	--	--

Date of the interview (dd/mm/yyyy):

		/			/	2	0	1	
--	--	---	--	--	---	---	---	---	--

Week number:

--	--

Fieldworker's code:

--	--

M1. During the past week (7 days), did your baby have diarrhoea (at least three watery stools per day)	1	Yes *	
	2	no (go to question M2)	
	3	don't know (go to question M2)	

M1a. For how many days did he/she have diarrhoea?	
---	--

When did your baby's diarrhoea start?	Date: ____/____/ 20__
When did your baby's diarrhoea stop?	Date: ____/____/ 20__
Or is it carrying on? _____	

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M2. During the past week (7 days), did your baby vomit?	1	Yes *	
	2	no (go to question M3)	
	3	don't know (go to question M3)	

M2a. For how many days did he/she vomit?	
--	--

When did your baby's vomiting start?	Date: ____/____/ 20__
When did your baby's vomiting stop?	Date: ____/____/ 20__
Or is it carrying on? _____	

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M3. During the past week (7 days), did your baby have a continuous cough (at least for one whole day)?	1	Yes *	
	2	no (go to question M4)	
	3	don't know (go to question M4)	

Baby's code:

--	--	--	--	--

M3a. For how many days did he/she cough continuously?	
---	--

M3b. Was the cough wet or dry?	1	Wet	
	2	Dry	

When did your baby's coughing start?	Date: ____/____/20____
When did your baby's coughing stop?	Date: ____/____/20____
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M4. During the past week (7 days), did your baby have wheezing or difficult breathing?	1	Yes *	
	2	no (go to question M5)	
	3	don't know (go to question M5)	

M4a. For how many days did he/she have wheezing or difficult breath?	
--	--

When did your baby's wheezing / difficulty in breathing start?	Date: ____/____/20____
When did your baby's wheezing / difficulty in breathing stop?	Date: ____/____/20____
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M5. During the past week (7 days), did your baby have a hot body?	1	Yes *	
	2	no (go to question M6)	
	3	don't know (go to question M6)	

M5a. For how many days did he/she have a hot body?	
--	--

When did you notice that your baby first have a hot body?	Date: ____/____/20____
When did you notice that your baby's body is no longer hot?	Date: ____/____/20____
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M6. During the past week (7 days), did your baby have any sores on the skin or rash?	1	Yes *	
	2	no (go to question M7)	
	3	don't know (go to question M7)	

M6a. For how many days did he/she have the sores or rash?	
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Baby's code:

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When did your baby first have a sore or rash?	Date: ____ / ____ / 20____
When did your baby's sore or rash stop?	Date: ____ / ____ / 20____ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M7. During the past week (7 days), did your baby have a continuous runny nose/ blocked nose?	1	Yes *	
	2	no (go to question M8)	
	3	don't know (go to question M8)	

M7a. For how many days did he/she have a runny nose/blocked nose?	
---	--

When did your baby's runny nose / blocked nose start?	Date: ____ / ____ / 20____
When did your baby's runny nose / blocked nose stop?	Date: ____ / ____ / 20____ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M8. During the past week (7 days), did your baby have any other illness?	1	Yes *	
	2	no (go to question M9)	
	3	don't know (go to question M9)	

M8a. Which illness did your baby have?	

When did your baby's illness start?	Date: ____ / ____ / 20____
When did your baby's illness stop?	Date: ____ / ____ / 20____ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M9. Did your baby visit any health service during the last week (7 days) because he/she was ill?	1	Yes	
	2	no (end of questionnaire)	
	3	don't know (end of questionnaire)	

Baby's code:

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M10.	Which health services did your baby go to during the last week?		
	a. Clinic	yes = 1	no = 2
	b. Private physician / doctor	yes = 1	no = 2
	c. Hospital	yes = 1	no = 2
	d. Traditional healer	yes = 1	no = 2
	e. Other (specify):	yes = 1	no = 2

M11. Did your baby receive any medicine prescribed by a nurse or doctor for his/her illness during the last week (7 days)	1	yes	
	2	no (go to question M12)	

M11a.	What type of medicine?
.....	

M11b. For how many days did he/she receive medicine?	
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M12. Was your baby officially diagnosed with any illness during the last week (7 days)?	1	yes	
	2	no (go to question M14)	

M13.	With what illness was your baby diagnosed?
.....	

M14. Was your baby hospitalised during the last week (7 days)	1	Yes *	
	2	no (end of questionnaire)	

M15. How long was your baby in hospital? (days)	
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When did your baby's stay in hospital start?	Date: ____ / ____ / 20__
When did your baby's stay in hospital stop?	Date: ____ / ____ / 20__
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M16.	Why was your baby hospitalised?
.....	

APPENDIX M: Baseline Questionnaire



BASELINE QUESTIONNAIRE

Baby's code:

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Date of the interview (dd/mm/yyyy):

		/			/	2	0	1	
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Fieldworker's code:

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SOCIO-ECONOMIC INFORMATION

1. With whom is interview?	Mother of the baby (<i>go to question 3</i>)	1
	Caregiver of the baby	2

2. Caregiver's relationship to the child?	Father	1
	Aunt	2
	Uncle	3
	Grandmother	4
	Grandfather	5
	Not related caregiver	6

3.a How old are you? (in years)	1	2
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3.b What is your birth date?	1	9
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4. Are you married?	Yes, married	1
	Common-law husband/wife	2
	Living together	3
	No, unmarried	4
	Separated / divorced	5
	Widowed/widow	6

5 Did you ever attend school?	Yes	1
	No (<i>go to question 7</i>)	2

Baby's code

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6. What was the highest standard that you passed at school?	Sub A / Grade 1	01	
	Sub B / Grade 2	02	
	Standard 1 / Grade 3	03	
	Standard 2 / Grade 4	04	
	Standard 3 / Grade 5	05	
	Standard 4 / Grade 6	06	
	Standard 5 / Grade 7	07	
	Standard 6 / Grade 8 / Form I	08	
	Standard 7 / Grade 9 / Form II	09	
	Standard 8 / Grade 10 / Form III / NTC I	10	
	Standard 9 / Grade 11 / Form IV / NTC II	11	
	Standard 10 / Grade 12 / Form V / NTC III	12	
Higher qualifications	13		

7. How many people live in the house?	a. Total number of people		
	b. Babies and small children		
	c. Primary school children		
	d. High school children		
	e. Adults		
	f. Elderly people		

8.a How many people in the household are employed and earns a salary or wage (weekly or monthly income)		
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8.b How many children in the household gets a child grant		
---	--	--

8.c How many people in the household gets either an old age pension or disability grant		
---	--	--

9. Where does the household usually get its drinking water from? (Mark only one)	Own tap – inside the house	1	
	Own tap – outside the house	2	
	Neighbour's tap	3	
	Public tap	4	
	Borehole	5	
	Other, specify	6	

10. What type of toilet does the household have?	Flush toilet	1	
	Pit toilet	2	
	None	3	
	Other, specify	4	

11. Do you have electricity available inside your home?	Yes	1	
	No (go to question 13)	2	

12. During the last 4 weeks, were there times that you did not use electricity because you had not money to pay it?	Yes	1	
	No	2	

Baby's code

INFANT FEEDING

13. Are you currently breastfeeding your baby/ls the baby currently breastfed?	Yes (<i>go to question 15</i>)	1
	No	2
	Don't know (<i>go to question 15</i>)	3
14. How old was the baby when breastfeeding was stopped? (<i>in months</i>)		
15. What was the <u>first</u> drink other than breast milk that your baby was ever given to drink?		
16. How old was your baby when you gave this drink for the first time? (<i>in months</i>)		
17. At the moment, does your baby get any <u>milk feeds</u> other than breast milk?	Yes	1
	No (<i>go to question 20</i>)	2
	Don't know (<i>go to question 20</i>)	3
18. What type of milk, other than breast milk is your baby getting?	Cow's milk (full strength)	1
	Cow's milk (diluted)	2
	Klim / Nespray	3
	Infant formula	4
	Give name:	
	Other, specify:	5
19. How old was your baby when he/she was given this <u>milk feed</u> for the first time? (<i>in months</i>)		
20. At the moment, does your baby get any semi-solid or solid food (with a spoon)?	Yes	1
	No (<i>go to question 24</i>)	2
	Don't know (<i>go to question 24</i>)	3
21. What was the <u>first</u> semi-solid or solid food (with a spoon) that your baby ate?		
22. How old was your baby when he/she ate semi-solid or solid food (with a spoon) for the first time? (<i>in months</i>)		
23.a. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the day?		
23.b. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the night?		
24. Did your baby drink anything from a bottle with a teat yesterday during the day and/or the night?	Yes	1
	No	2
	Don't know	3
25. Does your baby get any dietary supplements (e.g. vitamin syrup, vitamin tablets)?	Yes, specify:	1
	No	2
	Don't know	3

Baby's code

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MORBIDITY

M1. During the past week (7 days), did your baby have diarrhoea (at least three watery stools per day)	1	Yes *	
	2	no (go to question M2)	
	3	don't know (go to question M2)	

M1a. For how many days did he/she have diarrhoea?	
---	--

When did your baby's diarrhoea start?	Date: ____ / ____ / 20__
When did your baby's diarrhoea stop?	Date: ____ / ____ / 20__
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M2. During the past week (7 days), did your baby vomit?	1	Yes *	
	2	no (go to question M3)	
	3	don't know (go to question M3)	

M2a. For how many days did he/she vomit?	
--	--

When did your baby's vomiting start?	Date: ____ / ____ / 20__
When did your baby's vomiting stop?	Date: ____ / ____ / 20__
	Or it is carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M3. During the past week (7 days), did your baby have a continuous cough (at least for one whole day)?	1	Yes *	
	2	no (go to question M4)	
	3	don't know (go to question M4)	

M3a. For how many days did he/she cough continuously?	
---	--

M3b. Was the cough wet or dry?	1	Wet	
	2	Dry	

When did your baby's coughing start?	Date: ____ / ____ / 20__
When did your baby's coughing stop?	Date: ____ / ____ / 20__
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

Baby's code

M4. During the past week (7 days), did your baby have wheezing or difficult breathing?	1	Yes *	
	2	no (go to question M5)	
	3	don't know (go to question M5)	

M4a. For how many days did he/she have wheezing or difficult breath?	
--	--

When did your baby's wheezing / difficulty in breathing start?	Date: ____ / ____ / 20__
When did your baby's wheezing / difficulty in breathing stop?	Date: ____ / ____ / 20__ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M5. During the past week (7 days), did your baby have a hot body?	1	Yes *	
	2	no (go to question M6)	
	3	don't know (go to question M6)	

M5a. For how many days did he/she have a hot body?	
--	--

When did you notice that your baby first have a hot body?	Date: ____ / ____ / 20__
When did you notice that your baby's body is no longer hot?	Date: ____ / ____ / 20__ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M6. During the past week (7 days), did your baby have any sores on the skin or rash?	1	Yes *	
	2	no (go to question M7)	
	3	don't know (go to question M7)	

M6a. For how many days did he/she have the sores or rash?	
---	--

When did your baby first have a sore or rash?	Date: ____ / ____ / 20__
When did your baby's sore or rash stop?	Date: ____ / ____ / 20__ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

Baby's code

M7. During the past week (7 days), did your baby have a continuous runny nose/ blocked nose?	1	Yes *	
	2	no (go to question M8)	
	3	don't know (go to question M8)	

M7a. For how many days did he/she have a runny nose/blocked nose?	
---	--

When did your baby's runny nose / blocked nose start?	Date: ____ / ____ / 20__
When did your baby's runny nose / blocked nose stop?	Date: ____ / ____ / 20__ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M8. During the past week (7 days), did your baby have any other illness?	1	Yes *	
	2	no (go to question M9)	
	3	don't know (go to question M9)	

M8a. Which illness did your baby have?
.....
.....

When did your baby's illness start?	Date: ____ / ____ / 20__
When did your baby's illness stop?	Date: ____ / ____ / 20__ Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M9. Did your baby visit any health service during the last week (7 days) because he/she was ill?	1	Yes	
	2	no (end of questionnaire)	
	3	don't know (end of questionnaire)	

M10. Which health services did your baby go to during the last week?	a. Clinic		yes = 1	no = 2	
	b. Private physician / doctor		yes = 1	no = 2	
	c. Hospital		yes = 1	no = 2	
	d. Traditional healer		yes = 1	no = 2	
	e. Other (specify):		yes = 1	no = 2	

Baby's code

M11. Did your baby receive any medicine prescribed by a nurse or doctor for his/her illness during the last week (7 days)	1	yes	
	2	no (go to question M12)	

IM11a. What type of medicine?
.....
.....

M11b. For how many days did he/she receive medicine?	
--	--

M12. Was your baby officially diagnosed with any illness during the last week (7 days)?	1	yes	
	2	no (go to question M14)	

M13. With what illness was your baby diagnosed?
.....
.....

M14. Was your baby hospitalised during the last week (7 days)	1	Yes *	
	2	no (end of questionnaire)	

M15. How long was your baby in hospital? (days)	
---	--

When did your baby's stay in hospital start?	Date: ____ / ____ / 20__
When did your baby's stay in hospital stop?	Date: ____ / ____ / 20__
	Or is it carrying on? _____

* If marked "yes": Fieldworkers please collate information for study nurse to complete AE form

M16. Why was your baby hospitalised?
.....
.....



Symposium: Lipid nutrition – new insights

Lipid-based nutrient supplements and linear growth in children under 2 years: a review

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The prevalence of stunting remains high in low- and middle-income countries despite adoption of comprehensive nutrition interventions, particularly in low-income countries. In the present paper, we review current evidence on the acceptability and efficacy of small-quantity lipid-based nutrient supplements (SQ-LNS) on preventing stunting in children under 2 years, discuss the factors that affect their efficacy, highlight the implications of the current findings at pragmatic level and identify research priorities. Although the present paper is not a generic systematic review, we used a systematic approach to select relevant literature. The review showed that there is growing interest in the potential benefits of using SQ-LNS to prevent growth faltering. Acceptability studies showed that SQ-LNS are generally well accepted. However, results on the efficacy of SQ-LNS on improving linear growth or preventing growth faltering in infants and young children are still inconclusive. Factors that may affect efficacy include the duration of the trial, composition and dosage of SQ-LNS given, and baseline demographics and nutritional status of research participants. Future research should focus on controlled and long-term follow-up trials to obtain more conclusive results. In the long term, there will be need for studies to investigate how provision of SQ-LNS can be integrated with existing strategies to prevent stunting in low- and middle-income settings.

Lipid nutrient supplements: Stunting: Linear growth faltering: Fortification

Global context and consequences of stunting

In 2015, stunting affected approximately 159 million children under the age of 5 years worldwide and an important proportion of these children were in sub-Saharan Africa and South-central Asia⁽¹⁾. It is projected that about 127 million children under 5 years will be stunted in 2025 if no meaningful preventive actions are taken⁽²⁾. In low- and middle-income countries, stunting is a huge public health burden that has consequences on long-term health⁽³⁾. In addition, linear growth faltering has multiple causal factors⁽²⁾, and is associated with poverty and hence a critical development indicator⁽⁴⁾.

In vulnerable populations, intra-uterine growth restriction is often associated with maternal undernutrition⁽⁵⁾

and this may result in a vicious cycle of cross-generational stunting⁽⁶⁾. The incidence of stunting usually peaks around age 6–23 months as result of the transition from exclusive breastfeeding to introduction of complementary foods, which may be of poor nutritional quality^(7,8). In addition, infections can aggravate children's nutritional status and can contribute to stunting indirectly via the environmental enteric dysfunction mechanism⁽⁹⁾.

Growth retardation, reduced work capacity and poor mental and social development can occur as a result of poor dietary intake during early childhood⁽¹⁰⁾. In addition, growth faltering is also affected by several non-dietary factors that are closely linked and multifaceted⁽¹¹⁾. Nevertheless, the consequences of stunting may include

Abbreviations: LAZ, length for age z-score; LNS, lipid-based nutrient supplements; SQ-LNS, small-quantity lipid-based nutrient supplements.

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APPENDIX O: DOCTORAL CITATION FOR TONDERAYI MATHEW MATSUNGO

Luke 23:46

“Then Jesus called out in a loud voice, “Father, into Your hands I commit My Spirit.” And when He had said this, He breathed His last.”

Efficacy of lipid nutrient supplements on growth and micronutrient status in infants

Tonderayi “Tonde” Matsungu is a Nutrition Scientist, Academic and Consultant from Rusape, Zimbabwe. He was born on the 12th of May 1981 to Monica (*nee* Bukuta) and the late Clever Murombo Chatseya “CMC” Matsungu. Tonde is married to Forget Matsungu (*nee* Choto) and they are blessed with two children, Delbert (son) and Gabriella (daughter). He holds an M.Phil. in Human Physiology (2010) and a BSc in Nutrition (2004) all from the University of Zimbabwe.

He is currently a Senior Lecturer (Nutrition) with the Institute of Food, Nutrition and Family Sciences (IFNFS) at the University of Zimbabwe.



Tonde was the co-project manager (2013 to 2016) for the Tswaka study a *randomised controlled trial in South Africa comparing the efficacy of complementary food products on child growth* in 6-12 months old infants. His research focuses on stunting prevention strategies in low income settings. He is also passionate about capacity and leadership development for nutrition practitioners in Africa and globally. Tonde is the current Secretary General of the Zimbabwe Nutrition Association (ZimNA) and a committee member for the Education Committee for Nutritionists and Dieticians for the Allied Health Practitioners Council of Zimbabwe (AHP CZ). He also sits in the Executive committee for the African Nutrition Society (ANS).

His thesis, *Efficacy of lipid nutrient supplements on growth and micronutrient status in infants*, investigated the factors associated with stunting at age 6 months and evaluated the potential of two novel lipid-based nutrient supplements on promoting linear growth and improving iron status among 6-month old infants followed for 6 months. The cross-sectional findings showed that stunting (28.5%) was associated with lower birth weight, shorter maternal height and male sex. The efficacy trial showed that one of the lipid-based nutrient supplements did show an intervention effect on growth at 8 months and 10 months of age, but this was not sustained at 12 months of age. However, both lipid-based nutrient supplements significantly decreased the risk of infants for anaemia, iron deficiency and iron deficiency anaemia. Tonde can be reached via email at: tmatsungo@gmail.com

Isaiah 60:1

“Arise, shine; for thy light is come, and the glory of the LORD is risen upon thee”.



The End