CHARLES BERNARD MANGANI

Promotion of Healthy Growth with Lipid-Based Nutrient Supplements Among Rural Malawian Children

ACADEMIC DISSERTATION

To be presented, with the permission of the Board of the School of Medicine of the University of Tampere, for public discussion in the Small Auditorium of Building B, School of Medicine of the University of Tampere, Medisiinarinkatu 3, Tampere, on May 8th, 2015, at 12 o’clock.

UNIVERSITY OF TAMPERE
CHARLES BERNARD MANGANI

Promotion of Healthy Growth with Lipid-Based Nutrient Supplements Among Rural Malawian Children

Acta Universitatis Tamperensis 2038
Tampere University Press
Tampere 2015
Supervised by
Professor Per Ashorn
University of Tampere
Finland
Professor Yin Bun Cheung
Duke-NUS Graduate Medical School
Singapore

Reviewed by
Docent Kaija-Leena Kolho
University of Helsinki
Finland
Professor Olli Vainio
University of Oulu
Finland

The originality of this thesis has been checked using the Turnitin OriginalityCheck service in accordance with the quality management system of the University of Tampere.

Copyright ©2015 Tampere University Press and the author

Cover design by
Mikko Reinikka

Distributor:
kirjamynti@juvenes.fi
http://granum.uta.fi

Acta Universitatis Tamperensis 2038  Acta Electronica Universitatis Tamperensis 1527
ISSN-L 1455-1616  ISSN 1456-954X
ISSN 1455-1616  http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2015
In memory of my beloved parents, Giles and Ivy Mangani
Abstract

Childhood stunting (length-for-age z-score of < -3.00) remains a global health priority affecting millions of children. About a third of all children younger than 5 years in low-income and middle-income countries (LMICs) are estimated to be stunted. Impaired development is common too, with an estimated 200 million of these children failing to attain their developmental potential. Stunting commonly occurs between the ages of 6 to 24 months, contributes to increased morbidity and mortality and is a risk factor for developmental delay or deficits. It also affects health and productivity in adulthood.

Stunting is associated with poor complementary feeding in children living in LMICs. Interventions addressing this have so far had modest success in promotion of linear growth and its prevention. Recently, lipid-based nutrient supplements (LNS) have emerged as a potential low-cost strategy for enrichment of local complementary foods. They have been used successfully in treatment of children with severe acute malnutrition. Evidence from subsequent studies seems to indicate that some versions of LNS products might promote healthy growth and also prevent the development of undernutrition. Nevertheless, evidence of this is modest and still developing. Furthermore, in most of these studies, the focus has been on outcomes related to anthropometric measurements (such as height, weight) or micronutrient status (mainly iron, hemoglobin concentration). Only a few of the studies have also evaluated the effect of supplementation of complementary diet with LNS on child development, or on morbidity. In addition, the safety of universal use of LNS products in malaria-endemic areas (in view of their iron-fortification) has scarcely been assessed. Excess iron availability in the body has been suggested to facilitate infection from malaria parasites, and growth of pathogenic intestinal bacteria, leading to increased morbidity from malaria and other infections.
The present study was therefore conducted first, to evaluate the effects of supplementation with LNS on the prevention of stunting, and promotion of growth among infants and young children. The second aim was to assess the effect of the supplementation on the achievement of developmental milestones. The third aim was to assess the safety of LNS products by evaluating if supplementation leads to more frequent morbidity compared to no supplementation, and in the absence of excess morbidity, to evaluate effect of LNS on morbidity reduction. To evaluate these aims, a randomized community-based clinical trial was conducted in rural Malawi.

A total of 840 6-month-old infants were enrolled and allocated to receive supplementation with either Milk-LNS, Soy-LNS, corn-soy blend (CSB) or no nutritional supplementation (control) for 12 months. Children were followed every 2-weeks at home on morbidity, and developmental outcomes. At every 12-weeks interval from enrolment, outcomes on growth, haemoglobin concentration, malaria parasitemia and development were assessed at trial office.

At the end of 12-month follow-up period, final growth measurements were obtained from 747 of the participants (88.9%). There was no significant differences between intervention groups on their success to follow-up ($P = 0.852$). The incidence of severe stunting was 11.8%, 8.2%, 9.1%, and 15.5% ($p=0.098$) and that of very severe stunting 7.4%, 2.9%, 8.0%, and 6.4% ($p=0.138$) in control, Milk-LNS, Soy-LNS, or CSB respectively during the follow-up period. In the same groups, the proportion of children who were severely stunted at the end of the intervention period was 14.1%, 11.0%, 16.0%, and 16.1% ($p=0.454$). Mean length and weight gains were 13.0 cm, 13.2 cm, 13.0 cm and 12.9 cm ($p=0.43$) and 2.42 kg, 2.53 kg, 2.46 kg and 2.32 kg ($p=0.12$) in control, Milk-LNS, Soy-LNS, or CSB respectively. Between 9 and 12 months of age, the mean change in length-for-age was -0.15 in control, -0.02 in milk-LNS, -0.12 in soy-LNS and -0.18 ($P = 0.045$) in CSB group. There were however no
significant differences between the groups in linear growth during the other age-intervals.

Related to the achievement of developmental milestones, the mean age at achievement of walking with assistance was 42.5, 42.3, 42.7, and 43.2 weeks in control, milk-LNS, soy-LNS and CSB, respectively (p=0.75). Similarly, in the same groups, no significant differences were observed in either the mean age at standing alone (45.0, 44.9, 45.1, and 46.3 weeks), walking alone (54.6, 55.1, 55.3, 56.5 weeks), or running (64.6, 63.7, 64.8, 65.9 weeks).

Regarding morbidity, the proportion of days with febrile illness between 6 and 18 months was 4.9% and there were no differences between the groups: 4.9% (95% CI 4.3, 5.5%), 4.5% (3.9, 5.1%), 4.7% (4.1, 5.3%), 5.5% (4.7-6.3%) in milk-LNS, soy-LNS, CSB and control, respectively. The proportion of days with respiratory problems and diarrhea between 6 and 18 months of age did not also differ between groups. The risk of clinical malaria was similar between the intervention groups and the control group over the 12 months follow-up period with 95% confidence intervals confirming non-inferiority (incident rate ratio [95%CI] for milk-LNS=0.80 (0.59, 1.09); 0.77 (0.56, 1.06) for soy-LNS; and 0.79 (0.58, 1.08) for CSB). Incidence of febrile episodes, respiratory problems or admission to hospital, prevalence of malaria parasitemia throughout the follow-up were also similar between the groups. There was non-significant increase in diarrhea incidence in the two LNS groups compared to the control group.

In conclusion, a year-long supplementation with LNS did not generally prevent incidence of stunting or result in growth promotion compared to CSB or no supplementation. However, the study findings suggest that supplementation with milk-LNS may slow down the process of infant growth faltering at the ages of 9-to-12 months. This period corresponds with the time when the normal growth of a child, as described by the infancy-childhood-puberty model, transition from the infancy phase into the childhood phase of growth. Secondly, provision of LNS or CSB did not have
an impact on the achievement of the selected developmental milestones with similar effect between the supplements and no supplementation on child development. Lastly, supplementation with LNS or CSB did not result in increases in malaria or respiratory morbidity. However, LNS supplementation was associated with modest increase in diarrhea incidence though the findings could not conclude non-inferiority of the LNS products in comparison to no supplementation.

The findings from the present study obtained from southern Malawi could be used for designing of programmes using LNS products that aim at prevention of undernutrition, and promotion of normal linear growth and early child development in similar settings. Such programmes however need to evaluate the benefits of incorporating other intervention strategies such as those that aim to improve water quality, general sanitation and hygiene, or prevent and control infections including subclinical conditions.
# Table of Contents

LIST OF ORIGINAL PUBLICATIONS..................................................................................viii
LIST OF FIGURES AND TABLES..................................................................................ix
ABBREVIATIONS............................................................................................................xi

1 INTRODUCTION .............................................................................................................1

2 REVIEW OF THE LITERATURE ...................................................................................3

  2.1 Approach to the literature review...........................................................................3

  2.2 Definition and measures of healthy growth ..........................................................3

      2.2.1 Assessment of child growth and nutritional status ......................................4

      2.2.2 Anthropometric classification of nutritional status .......................................5

      2.2.3 Assessment of early child development .........................................................6

  2.3 Epidemiology of stunted growth and impaired development ...............................7

      2.3.1 Timing of growth faltering: the first 1000 days critical window .....................7

      2.3.2 Prevalence and trends in undernutrition and impaired development ............8

  2.4 Determinants of healthy growth ............................................................................9

      2.4.1 The control of growth and development .......................................................10

      2.4.2 Environmental factors influencing growth and development ......................10

  2.5 Consequences of stunted growth and impaired development .............................14

      2.5.1 Short-term consequences ............................................................................14

      2.5.2 Long-term consequences ............................................................................15

  2.6 Strategies to improve healthy growth ....................................................................16

      2.6.1 Nutrition-specific interventions ....................................................................16

      2.6.2 Nutrition-sensitive interventions ..................................................................20

      2.6.3 Early child development programmes ..........................................................21

  2.7 Lipid-based nutrient supplements and promotion of healthy growth ....................22

      2.7.1 LNS products: overview and role in complementary feeding .......................22

      2.7.2 Safety of LNS products ................................................................................24

  2.8 Justification for the present study ........................................................................25

3 AIMS ..........................................................................................................................26
ACKNOWLEDGEMENTS ................................................................. 65
REFERENCES .............................................................................. 68
APPENDICES .............................................................................. 95
ORIGINAL PUBLICATIONS .......................................................... 96
LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the original articles as below which are referred to in the thesis by the roman numerals:


LIST OF FIGURES AND TABLES

FIGURES

**Figure 1.** Height gain of Malawi children (both sexes) compared to the median of the WHO Child Growth Standard (for girls). ................................................................. 8

**Figure 2.** WHO Conceptual framework on Childhood Stunting: Context, Causes and Consequences ................................................................................................. 12

**Figure 3.** Strategies to improve healthy growth ........................................................................................................... 18

**Figure 4.** Overall design of the thesis ....................................................................................................................... 28

**Figure 5.** Participant follow-up and data collection during the intervention .......... 34

**Figure 6.** Flow of participants in the study ............................................................................................................... 41

**Figure 7.** Cumulative incidence of very severe stunting (A), moderate to severe stunting (B) moderate to severe wasting (C) and moderate to severe underweight (D) in children in the control, milk-LNS, soy-LNS and CSB groups ........................................... 45

**Figure 8.** Prevalence of various forms of undernutrition at 18 months of age ...... 46

**Figure 9.** Geometric mean age at achievement of motor milestones for control, milk-LNS, soy-LNS and CSB groups ............................................................................. 47

**Figure 10.** Geometric mean age at achievement of social and language milestones for control, milk-LNS, soy-LNS and CSB groups ............................................................................. 47

**Figure 11.** Proportion of children with a delay in milestone achievement in control, milk-LNS, soy-LNS and CSB groups ............................................................................. 47

**Figure 12.** Overall geometric mean ratio of febrile illness alone, cough alone, acute respiratory infection, and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months ............................................................................................................. 50

**Figure 13.** Incidence of guardian-reported fever, cough, acute respiratory infection, and diarrhea for control, milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months ............................................................................................................. 51
Figure 14. Incidence rate ratios of febrile illness alone, cough alone, acute respiratory infection and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months.......................................................... 52

Figure 15. Incidence of malaria, acute respiratory problems and diarrhea diagnoses at health facilities for control, milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months.................................................................................. 53

Figure 16. Incidence rate ratios of malaria, acute respiratory problems and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months.............. 54

TABLES

Table 1. Classification of stunting, underweight and wasting based on Z-scores ............................................................................. 6
Table 2. Basic demographic, socio-economic and health indicators for Malawi .................................................................................. 30
Table 3. Nutrient composition of the 3 supplements used in the study......... 32
Table 4. Baseline characteristics of participants at enrollment.................. 43
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ARI</td>
<td>Acute Respiratory Infections</td>
</tr>
<tr>
<td>CSB</td>
<td>Corn-soy blend</td>
</tr>
<tr>
<td>HAZ</td>
<td>Height-for-age z score</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immune-deficiency virus</td>
</tr>
<tr>
<td>ICH-GCP</td>
<td>International Conference of Harmonization-Good Clinical Practice</td>
</tr>
<tr>
<td>LAZ</td>
<td>Length-for-age z score</td>
</tr>
<tr>
<td>LMICs</td>
<td>Low-and-middle-income countries</td>
</tr>
<tr>
<td>LNS</td>
<td>Lipid-based nutrient supplement</td>
</tr>
<tr>
<td>MDHS</td>
<td>Malawi Demographic and Health Survey</td>
</tr>
<tr>
<td>MGRSG</td>
<td>Multicenter Growth Reference Study Group</td>
</tr>
<tr>
<td>MNP</td>
<td>Micronutrient Powder</td>
</tr>
<tr>
<td>MMN</td>
<td>Multiple micronutrients</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid-Upper-Arm-Circumference</td>
</tr>
<tr>
<td>PAHO</td>
<td>Pan American Health Organization</td>
</tr>
<tr>
<td>RNI</td>
<td>Recommended Nutrient Intake</td>
</tr>
<tr>
<td>IRR</td>
<td>Incident Rate Ratio</td>
</tr>
<tr>
<td>RUSF</td>
<td>Ready-to-use supplementary food</td>
</tr>
<tr>
<td>RUTF</td>
<td>Ready-to-use therapeutic Food</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations International Children’s Emergency Fund</td>
</tr>
<tr>
<td>USD</td>
<td>United States Dollar</td>
</tr>
<tr>
<td>WAZ</td>
<td>Weight-for-age z score</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHZ</td>
<td>Weight-for-height z score</td>
</tr>
<tr>
<td>WLZ</td>
<td>Weight-for-length z-score</td>
</tr>
</tbody>
</table>
1 Introduction

Healthy growth, especially in the first 2 years of life is important for adequate organ and immune system development and function, physical wellbeing, and development (Piwoz et al. 2012). Attained height at a given age provides a useful summary measure on a child’s nutritional wellbeing and general health. In addition to linear growth, children’s healthy growth is also described by the timing of acquisition of cognitive, motor and socio-emotional skills. Healthy growth is a complex dynamic and malleable process under the influence of biological and environmental factors. Suboptimal environments, especially in the first 1000 days of the life cycle lead to unhealthy growth. On the other hand, it can also be enhanced by interventions affecting the child, the environment or both (Shrimpton et al. 2001, Thompson and Nelson 2001, Victora et al. 2010).

Almost one third of children below the age of five years in low-income and middle-income countries (LMICs) and about 35 % of those living in Africa were estimated to be stunted in 2011 (Black et al. 2013, de Onis et al. 2013). And in 2007, an estimated 200 million children under 5 years of age also failed to attain their developmental potential (Grantham-McGregor et al. 2007). Globally, there has been steady decline in stunting prevalence over the past two decades. Nevertheless, it still remains a major global health priority because of the large number of children affected by it and the associated severe short and long term health consequences (de Onis et al. 2013). In addition to reduced final adult height, stunting in childhood is also associated with increased morbidity, mortality, delay or deficits in development including lower cognitive function and poor school performance (Pelletier et al. 1995, Grantham-McGregor et al. 2007). Additionally, stunting has been associated with adult health and disease (Piwoz et al. 2012). Impaired early childhood development in turn has been
associated with final academic achievement, adult productivity, and earning potential (Victora et al. 2008)

On a population level, few nutrition-specific interventions have been successful with most having failed to markedly improve linear growth or prevent stunting (Dewey and Adu-Afarwuah 2008). Lipid-based nutrient supplements (LNS) have been used successfully to manage children with severe acute malnutrition (Diop et al. 2003). Additional subsequent studies seem to indicate that some versions of LNS products might be used to promote linear growth, prevent the development of undernutrition and improve development (Adu-Afarwuah et al. 2007, Phuka et al. 2008). Therefore, the present study was done to evaluate the effect of supplementation of complementary food with lipid-based nutrient supplements on healthy growth.
2 Review of the Literature

2.1 Approach to the literature review
The purpose of the literature review is to give the background for the present research. The review therefore defines the concept of healthy growth, methods of assessing healthy growth and nutritional status; describes the global status and trends of undernutrition and impaired development, factors that influence healthy growth, and the consequences of undernutrition and impaired development. Finally, the main strategies to promote healthy growth are reviewed and described including the role of lipid-based nutrient supplements, and the justification of conducting the current study given.

A search of electronic publications using relevant key words was performed to identify relevant literature for the review. The search was mainly done in electronic journals (e.g. the Lancet) and online databases (mainly PubMed). References from retrieved publications were also further reviewed for other relevant literature. Additional searches were also conducted for data and reports from international organizations (e.g. WHO, the World Bank, UNICEF), government and non-governmental organizations.

2.2 Definition and measures of healthy growth
The concept of healthy growth describes the achievement of a child’s full potential for growth and development (Stewart et al. 2013). Healthy growth has been defined as normal linear growth relative to the World Health Organization Child Growth Standards (Piwoz et al. 2012) including normal child development. Healthy linear
growth is considered as a marker of healthy growth because of its association with short and long term health and developmental consequences and future economic productivity (Piwoz et al. 2012, Victora et al. 2008). Child development consists of linked domains of sensori-motor, cognitive-language, and social-emotional function (Grantham-McGregor et al. 2007). Thus, stunted linear growth and impairments in acquisition of cognitive, motor and social-emotional skills in childhood describes a failure to achieve healthy growth, or a state of “unhealthy growth”.

Several methods are used to measure healthy growth and are described below with a focus on anthropometric assessment of somatic growth, classification of nutritional status based on the anthropometric assessment and finally methods in assessment of child development.

2.2.1 Assessment of child growth and nutritional status
Traditionally, assessment of a child’s growth status has been based on auxological methods: measures of weight, length/height, mid-upper arm circumference (MUAC) and head circumference (Ulijaszek and Kerr 1999). These anthropometric measures are simple, feasible in many settings, cheap, non-invasive, and easy to relate to by both parents and the assessors. Additionally, growth and nutrition status have also been measured by assessing body composition, evaluating clinical signs of deficiency, measurement of biochemical compounds and evaluation of diet (Cameron 2006).

Anthropometric measures in combination with age and sex are used to construct anthropometric indices. These indices allow for interpretation of body measurements. For example, height alone has no meaning unless it is related to an individual’s age (WHO 1995). Anthropometric indices are used to describe the nutritional status of an individual or population. The three most commonly used anthropometric indices in nutritional assessment of children are weight-for-height, height-for-age, and weight-for-age. These indices are expressed either as z-scores, percentiles, or percentage of
median, which enable comparison of a child or a group of children with a reference population (de Onis et al. 2003). Currently, the WHO growth standard is the recommended and commonly used growth standard (WHO 2006a). First, the standard was developed by assessing growth and development in children likely to achieve their full genetic growth; healthy breastfed children from non-smoking mothers growing normally and living in optimal conditions. In addition, the children were selected from a diverse set of countries, which allowed for genetic, ethnic and cultural variability strengthening their universal applicability (WHO 2006a).

2.2.2 Anthropometric classification of nutritional status

Deficits in one or more of the anthropometric indices indicate some level of undernutrition. The magnitude of the deficit is reflective of the degree of undernutrition. The WHO Global Database on Child Growth and Malnutrition uses a z-score cut-off point of < -2 SD to classify low height-for-age, low weight-for-age and low weight-for-height as stunting, underweight and wasting respectively, and z-score cut-off points of < -3 SD to define severe forms of the same undernutrition categories (Table 1; WHO 1995,WHO 1997). Stunting reflects the cumulative adverse effects of poor environmental factors and long-term restriction of a child's growth potential and wasting usually acute poor environmental factors. Underweight is a composite indicator indicating that a child may have acute weight loss, stunting, or both (WHO 1995, WHO 1997).
Table 1. Classification of stunting, underweight and wasting based on Z-scores

<table>
<thead>
<tr>
<th>Classification</th>
<th>Z-score values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate</td>
<td>-1 &lt; Z-score &lt;+2</td>
</tr>
<tr>
<td>Mild</td>
<td>-2 &lt; Z-score &lt;= -1</td>
</tr>
<tr>
<td>Moderate</td>
<td>-3 &lt; Z-score &lt;= -2</td>
</tr>
<tr>
<td>Severe</td>
<td>Z-score &lt;= -3</td>
</tr>
</tbody>
</table>

2.2.3 Assessment of early child development

Child development is often assessed by an inventory of milestones through testing and evaluation of a child’s ability in performing a series of tasks. A large number of standardised assessment tools are available to measure achievement of psychometric (psychological characteristics or domains such as language, cognitive, etc.) and motor developmental skills (Fernald et al. 2009). These can be used to evaluate children on a single developmental domain or across multiple domains.

Information on developmental status of a child can be obtained either through direct testing of the child, reports of the child’s behaviours or skills by key informants, e.g., parents, and observation of the child in daily or structured activities (Fernald et al. 2009). Direct tests assess infants either by presenting stimuli, e.g., an object, and note responses, or by asking children to complete tasks or activities e.g., stacking blocks. Key informants reports contain responses on specific questions about the child’s abilities and behaviour based on what parents or guardians know of the child, with no direct assessment of the child by an independent observer. Lastly, observational measures rely upon a trained observer to document the behaviour of a child. Most standardised tests combine two or more modes of assessment (Fernald et al. 2009).

Assessments of child development are done either as screening tests or tests to evaluate a child’s abilities (Johnson and Marlow 2006). Screening tests are usually brief assessments done to identify children who are at risk of impaired development in one or more domains. The most commonly used screening tests include the Denver
Developmental Screening Test (DDST) (Frankenburg et al. 1992), and the Ages and Stages Questionnaires (Bricker 1999). On the other hand, ability tests are designed to assess the maximum skills level for a child at any given age (including age at achievement of a skill or milestone). Examples are the Bayley Scales of Infant Development (Bayley 1969), the Griffiths Mental Developmental Scales-revised (Griffiths scales) among others (Johnson and Marlow 2006). Aggregation of individual assessment in a group or sample creates population-based measures. These can be used to compare a group of children (such as in a study sample, or within a country) to other groups of children or to a standard such as the World Health Organization Child Growth Standards (WHO 2006a).

2.3 Epidemiology of stunted growth and impaired development

2.3.1 Timing of growth faltering: the first 1000 days critical window
In low-income and middle-income countries, growth faltering often begins in utero, and generally continues until 40 months of age before reaching an apparent plateau at the age of 5 years (Shrimpton et al. 2001, Maleta et al. 2003a, Dewey and Huffman 2009, Victora et al. 2010). For example, it has been shown that average newborn’s length-for-age is approximately $-0.5$ SD of the WHO growth standard and declines further to almost $-2$ SD by the end of the second year. Most of the linear growth faltering in children under the age of five years occurs between 6 and 24 months (Figure 1) (Maleta et al. 2003a, Dewey and Huffman 2009, Victora et al. 2010). The first 1000 days, the period from conception to the child’s second birthday, is a critical window for healthy growth. During this period, there is rapid development of the brain and body which are also at their greatest vulnerability to malnutrition and infection. Therefore, impairments in their development have potentially irreversible long-term physical and mental damage. This period also represents the optimal “window of opportunity” with greatest potential for benefit from growth promoting interventions (Piwoz et al. 2012).
2.3.2 Prevalence and trends in undernutrition and impaired development

Child undernutrition still remains a major public health problem in LMICs. Globally, an estimated 165 million (26%) of children younger than 5 years were stunted, 100 million (16%) underweight and 52 million (8%) wasted in 2011. Approximately 90% of the stunted children live in Asia and Africa (de Onis et al. 2012). Since 1990, there has been a steady decline in stunting prevalence from an estimated 39.7% in 1990 to 26.7% in 2010. The decline has especially been notable in Asia (from 48% to 27%) compared to the modest decline in Africa (from 42% to 36%) (de Onis et al. 2013). There is a renewed focus to reduce stunting rates with a global target to reduce by 40% the number of stunted children under 5 years of age by 2025 (de Onis et al. 2013, WHO 2012). Based on current rate of progress, substantial reduction in prevalence is forecasted for Asia (from 28% in 2010 to 17% in 2025). On the contrary, only small reductions in prevalence are anticipated in Africa (from 36% in 2010 to 32% in 2025) with the total number of overall number of stunted children increasing because of
population growth (de Onis et al. 2013) highlighting the need for both innovative effective intervention strategies and their scaling up.

Prevalence of underweight has also been decreasing steadily with a global reduction of 36% between 1990 and 2011. In 2011, the proportions of underweight children were highest in south-central Asia and western Africa with prevalences of 30% and 22% respectively (Black et al. 2013). Similar downward trend in the prevalence of wasting has also been noted with 11% decrease between 1990 and 2011 (Black et al. 2013).

In 2007, it was estimated that at least 200 million children aged less than 5 years fail to reach their potential in cognitive, socio-emotional and sensory-motor development. The risk of loss in developmental potential is highest among the disadvantaged children (stunted children plus non-stunted children living in poverty) (Grantham-McGregor et al. 2007). Prevalence of disadvantage children under 5 years was highest in Sub-Saharan Africa and south Asia; 61% and 52%, respectively (Grantham-McGregor et al. 2007), the same two regions also having the highest prevalence rates of stunting. Currently, there is paucity of regional data evaluating trends in prevalence rates of children with developmental delays or loss.

2.4 Determinants of healthy growth

Human growth and development is a process that begins at conception and continues into the late teens or early twenties. It involves a pattern of progressive increase in somatic size and functional maturation (Tanner 1963, Cameron 2006). In addition to somatic growth, the process of human growth and development also includes progressive advances in three interrelated domains of sensory-motor, cognitive-language and socio-emotional abilities (Grantham-McGregor et al. 2007).
2.4.1 The control of growth and development

The process of human growth and development is under the control of both genetic and environmental factors. The degree and timing of interaction between these two factors throughout the whole growth period influence the phenotypic variation observed among humans (Cameron 2006, Jelenkovic et al. 2011). Growth status in all anthropometrics has been shown to have significant heritability though the influence is greater in height compared to the other anthropometrics (Silventoinen et al. 2000, Silventoinen et al. 2003, Jelenkovic et al. 2011). However, genetic control in the phenotypic variation in traits such as stature is less significant in early childhood (Silventoinen et al. 2008) with children from diverse ethnic groups having similar growth pattern during the first 5 years of life in environments that support healthy growth (WHO 2006b).

2.4.2 Environmental factors influencing growth and development

Several environmental factors affect growth and development. The key factors as described in the WHO conceptual model (Figure 2) include the immediate household and family factors, nutrition especially breastfeeding and complementary feeding, morbidity, and the community and society contextual factors. In the following sections, the nutrition and health factors during the first 1000 days and the immediate home environment affecting healthy growth are described.

Maternal nutrition and health during pre-conception and pregnancy are important for intrauterine growth. Fetal growth restriction is an important contributor to later stunted growth and is estimated to contribute to about 20% of the childhood stunting (Black et al. 2013, Dewey and Huffman 2009). Intrauterine growth restriction has been associated with low maternal body-mass index and micronutrient deficiencies of iron, vitamin A and zinc (Black et al. 2008, Black et al. 2013). In addition to nutrition, maternal infections during pregnancy such as HIV and malaria are also associated with

In disadvantaged populations, suboptimal breastfeeding from either delayed initiation of breastfeeding, not breastfeeding, non-exclusive breastfeeding or early cessation of breastfeeding is associated with increased risk of morbidity resulting in increased likelihood of stunted growth (Onyango et al. 1999, Simondon et al. 2001, Black et al. 2008, Arpadi et al. 2009, Kramer and Kakuma 2012).

Most of the incident stunting occurs during the complementary feeding period between 6 and 24 months of age (Maleta et al. 2003a, Dewey and Huffman 2009, Victora et al. 2010). Inadequate complementary feeding occurs as a result of limitations in quality and quantity of diet (Onyango et al. 1998, Arimond and Ruel 2004, Krebs at al. 2007a, Krebs at al. 2007b), poor feeding practices (Umeta et al. 2003, Dewey and Adu-Afarwuah 2008, Islam et al. 2008) and food contamination including unhygienic food preparation and feeding practices (Figure 2).
Figure 2. WHO Conceptual framework on Childhood Stunting: Context, Causes and Consequences Source: Stewart et al. 2013
Children in LMICs suffer from a high burden of infections (Black et al. 2010). Infections impair growth first by decreasing dietary intake through reduced appetite, and impairing intestinal absorption leading to loss of nutrients (Scrimshaw et al. 1968, Welsh et al. 1998). Secondly, infections lead to increased metabolism as a result of the acute phase response (Cunningham-Rundles et al. 2005, Rodriguez et al. 2011). All common childhood infections contribute to growth faltering (Guerrant et al. 1992, Moore et al. 2001, Wamani et al. 2006). However, the association between diarrhea and stunted growth is more consistent and greater compared to the other illnesses (Black et al. 1984, Checkley et al. 2003, Assis et al. 2005, Checkley et al. 2008). Black et al. estimated that the odds of stunting at 24 months of age increased by 5% with each episode of diarrhea in the first 24 months (Black et al. 2008), while Checkley et al. estimated that 25% of the burden of stunting could be attributed to five or more episodes of diarrhea occurring prior to the age of 2 years (Checkley et al. 2008).

In the immediate home environment, several psychosocial and economic factors can affect healthy growth (Figure 1). Caregiver education, especially maternal has been associated with improved nutrition and growth (Fotso and Kuate-Defo 2005, Hatt and Waters 2006, Casanovas et al. 2013) possibly through improved dietary and feeding practices, health seeking and promotion behaviors and household incomes among others (Frost et al. 2005). Developmentally, mothers who showed more sensitivity and positive response to their children have been shown to have more secure attachment and increased cognitive ability among their infants (Walker et al. 2005, Walker et al. 2007). Significantly higher cognitive functions have also been observed in young children given cognitive stimulation (Walker et al. 2005, Walker et al. 2007). Finally, household poverty which leads to household food insecurity, limited access to nutrient rich diets, poor housing, poor hygiene and sanitation among others provide a home environment that contribute to unhealthy growth (Hong 2007, Iannotti at al. 2012, Casanovas et al. 2013).
2.5 Consequences of stunted growth and impaired development

Stunting is an important determinant of impaired development. However, stunting and impaired development often coexist, both in early childhood and in later life because of shared biological and psychosocial risk factors (Fernald et al. 2006, Walker et al. 2007, Abubakar et al. 2010). Linear growth faltering especially in early childhood is simultaneously associated with structural and functional damage to the brain resulting in both, developmental delay and permanent deficits in cognitive functions (Grantham-McGregor et al. 2007, Thompson and Nelson 2001). In terms of this thesis, short term consequences are those that occur in early childhood and long term consequences are those that occur in adolescence and adulthood.

2.5.1 Short-term consequences

Child undernutrition including stunted growth is associated with impairments in both innate and acquired immune function resulting in increased risk of infection (Rodriguez et al. 2011). For example, a study in Nigeria found that duration of diarrhea was affected by pre-existing malnutrition, the duration being 33% longer in underweight children, 37% longer in stunted children, and 79% longer in wasted children (Tomkins 1981). Furthermore, all anthropometric measures of undernutrition increase the risk of death with stunting and underweight each accounting for between 14-17% of child deaths (Black et al. 2013).

In young children, stunting is associated with delayed cognitive development including poorer cognitive ability (Mendez and Adair 1999, Berkman at al. 2002, Grantham-McGregor et al. 2007). It has also been associated with poorer motor development (Sigman et al. 1989, Grantham-McGregor and Fernald 1997, Kariger et al. 2005, Siegel et al. 2005) and altered behavior including attachment and levels of play (Graves 1978, Gardner at al. 1999). Finally, in later childhood, stunted children are less likely to be enrolled in school, more likely to enrol late, to repeat grades and to attain lower achievement levels or grades for their age (Grantham-McGregor et al. 2007).
2.5.2 Long-term consequences

Stunting in childhood increases the likelihood of short adult stature with worsening reductions in adult height the earlier in life stunting occurs. Pooled data from five birth cohorts showed that a 1 SD lower height-for-age at 2 years was associated with 3.2 cm lower adult height and a 1.9 cm lower adult height for a 1 SD lower height-for-age during mid-childhood (Adair et al. 2013).

Among women, maternal stunting (height <145 cm) increases the risk of adverse pregnancy outcomes mainly from cephalopelvic disproportion (Black et al. 2008, Lee et al., 2009) and also the risk of perinatal deaths (Lawn at al. 2006, Ozaltin at al. 2010). Furthermore, short mothers are more likely to have small babies who have increased risk of becoming stunted (Victora et al. 2008, Ozaltin et al. 2010).

Malnutrition in early childhood may be a risk factor for chronic metabolic diseases in adulthood (Gluckman at al. 2007, Uauy at al. 2008). Childhood undernutrition has been associated with impaired glucose metabolism, increased risk of cardiovascular disease and harmful lipid profiles in adulthood (Huxley et al. 2000, Victora et al. 2008, Whincup et al. 2008). Furthermore, stunted growth has also been associated with later mental health problems (Cheung and Ashorn 2009, Cheung et al. 2013).

Stunting and the associated impaired development in early childhood affect academic achievement, adult productivity and earning potential. Stunting in early childhood has been associated with reduced achieved learning (as measured by vocabulary or non-verbal tests) and years of schooling (Grantham-McGregor et al. 2007, Martorell et al. 2010, Adair et al. 2013). Short adult stature has also been linked to low skilled employment and lower wage earnings (Grantham-McGregor et al. 2007, Hoddinott et al. 2008, Hoddinott et al. 2013).
2.6 Strategies to improve healthy growth

Stunting and impaired development represents the outcome of a complex interaction of biological and psychosocial factors that require multiple interventions to manage. Several evidence-based interventions have been implemented to promote healthy growth at different levels of scale using a range of delivery platforms and strategies. The available interventions can simply be divided into either nutrition-specific or nutrition-sensitive interventions (Figure 3) (Black et al. 2013). In general nutrition-specific interventions directly affect nutrient intake and include among others prenatal nutrition, promotion and support for optimal breastfeeding, promotion of appropriate complementary feeding practices, provision of complementary foods, micronutrient supplementation or fortification of local diets and treatment of severe acute malnutrition (Piwoz et al. 2012, Black et al. 2013). Nutrition-sensitive interventions on the other hand are aimed at improving nutritional status and promoting healthy growth through non-dietary means by addressing underlying determinants across multiple sectors. These involve making improvements in the health and healthcare, political environment, or social and economic context in which a child is born and grows. These include agriculture and food systems; education, particularly of girls and women; water, sanitation, and hygiene, disease prevention and management and social protection programmes among others (Piwoz et al. 2012, Black et al. 2013, Casanovas et al. 2013). Some of the core interventions to promote healthy growth are reviewed below highlighting the evidence on their success and also any possible challenges.

2.6.1 Nutrition-specific interventions

Good prenatal nutrition provides a foundation for healthy growth in later life. Key prenatal strategies include iron or iron-folic acid supplementation, supplementation with multiple micronutrients (MMN), balanced protein-energy supplementation of pregnant women and nutrition education and counseling. A Cochrane review of daily iron supplementation to pregnant women reported a 19% reduction in the incidence of
low birth weight (Pena-Rosas et al. 2012). Evidence on effect of prenatal iron or iron-folic acid supplementation on childhood development is limited. In Nepal, iron and folic acid supplementation during pregnancy was associated with better cognitive and motor functioning at ages of 7–9 years compared to a non-supplemented group (Christian et al. 2010).

Prenatal MMN supplementations have also been shown to have an impact on fetal growth and could affect post-natal stunting. Findings from three meta-analyses reported reductions in incidence of low birth weight of between 11-17% (Fall et al. 2009, Ramakrishnan et al. 2012, Bhutta et al. 2013). Furthermore, they have also been shown to increase mean birth weight and mean birth length. Evidence on the effects of prenatal MMN supplementation on post-natal growth remains inconclusive; significant reduction in stunting in infancy was reported in Burkina Faso (Roberfroid et al. 2012) but not in Nepal (Stewart et al. 2009). A recent meta-analysis also reported no benefits on post-natal linear growth (Lu et al. 2014). Trials that have examined effect of MMN supplementation on development suggest potential benefits to motor development and mental development (McGrath et al. 2006, Tofail et al. 2008, Li et al. 2009).

Several studies have showed that balanced energy–protein supplementation benefit fetal growth, particularly in undernourished women. Significant gains have been reported in mean birth weight (Kramer and Kakuma 2003, Imdad and Bhutta 2012) and in reduction of incidence of low birth weight (Imdad and Bhutta 2012), and non-significant small gains in birth length (Kramer and Kakuma 2003). There is, however, limited information on associations between prenatal balanced energy–protein supplementation and early childhood development. In Bangladesh infants of undernourished mothers who had prenatal balanced energy–protein supplementation had improved problem-solving ability at 7 months of age compared to infants of mothers who were not supplemented (Tofail et al. 2008).
Figure 3. Strategies to improve healthy growth (Adapted from Bhutta et al. 2013)
Promotion of appropriate breastfeeding practices is another important strategy. Current recommendations on optimal breastfeeding include initiation of breastfeeding within one hour of birth, exclusive breastfeeding of infants till 6 months of age, and continued breastfeeding until 2 years of age or older (Dyson et al. 2005). Exclusive breastfeeding during the first 6 months have been shown to reduce morbidity and mortality among infants and young children in LMICs (Arifeen et al. 2001, Jones et al. 2003, Bahl et al. 2005). However, evidence on its benefits on post-natal growth is insufficient (Bhutta et al. 2008, Bhutta et al. 2013, Stewart et al. 2013). Breastfeeding has also been shown to benefit child development. A meta-analysis of 11 studies in developed countries reported cognitive benefits associated with breastfeeding (Anderson et al. 1999). Greater duration of breastfeeding was also associated with improvements in motor development (Daniels and Adair 2005, Dewey et al. 2001).

There are several intervention strategies to promote appropriate complementary feeding (Dewey and Adu-Afarwuah 2008). These include education about complementary feeding, increasing the energy density of complementary foods, and fortification of complementary foods (de Onis et al. 2013). Educational interventions promoting the use of complementary feeding have shown limited or no effect on linear growth (Dewey and Adu-Afarwuah 2008, Shi et al. 2010, Vazir et al. 2013). Similarly, limited or no effect on linear growth has been shown with interventions aimed at increasing energy density of complementary foods (Dewey and Adu-Afarwuah 2008). Micronutrient fortification of complementary foods has also generally failed to improve linear growth or prevent stunting (Mamiro et al. 2004, Dewey and Adu-Afarwuah 2008, Mazariegos et al. 2010, Ouedraogo et al. 2010, De-Regil et al. 2011). Studies on complementary food interventions have reported benefits to motor, mental development and cognitive performance (Waber et al. 1981, Grantham-McGregor et al. 1991, Husaini et al. 1991, Pollitt et al. 2000, Eilander et al. 2010).
2.6.2 Nutrition-sensitive interventions

An estimated seven million children die annually from infectious diseases and undernutrition (Black 2013 et al., UNICEF 2014). Most of the disease prevention and management interventions to promote healthy growth in LMICs have concentrated on efforts that aim at reducing the burden of disease from malaria, acute respiratory infections (ARI), diarrhea, helminthic infection, and HIV/AIDS (Lopez et al. 2006). Prenatal antimalarial interventions using intermittent preventive treatment for malaria and use of insecticide-treated bed nets (ITNs) during pregnancy have been associated with reductions in risk of low birth weight (Bhutta et al. 2008, Gamble et al. 2007). In children, these strategies have been associated with reduced morbidity from malaria episodes, anemia and hospital admissions (Meremikwu et al. 2008, Bhutta et al. 2013).

Deworming during pregnancy has been associated with prevention in the fall of hemoglobin (Bhutta et al. 2008). The benefits of this intervention on linear growth in children is, however, very small (Bhutta et al. 2008). Vaccination in children has been estimated to prevent approximately 6 million child deaths globally each year (Ehreth et al. 2003). Furthermore, vaccines may help prevent some of the chronic consequences of undernutrition (Anekwe and Kumar 2012).

Diarrhea is the most common illness among children in low-income and middle-income countries. Poor water supplies, sanitation and hygiene increase the frequency of diarrhea episodes (Checkley et al. 2004, Humphrey 2009). And as described above, frequent diarrhea illnesses are associated with unhealthy growth. Several studies report improvements in growth and reduction of stunting rates with improvements in hygiene and sanitation, and access to clean water (Casanovas et al. 2013).

Parental education, especially maternal have consistently been associated with improvements in child health and nutrition. Children of mothers with no schooling have greater risk of stunting independent of economic status or whether living in urban or rural areas (Pongou et al. 2006, Subramanyam et al. 2011).
The 2013 Maternal and Child Nutrition series reported that stunting prevalence among children younger than 5 years was 2.47 times higher in the poorest quintile of households than in the richest quintile (Black et al. 2013). Social protection programmes through household cash transfer is one strategy that have been used to address the effects of poverty. Evaluation of programmes implementing cash transfers have shown mixed results. However, in countries where the programmes have been successful, there have been reductions in the rates of stunting through positive benefits on household food consumption and dietary diversity, and increase in the utilization of health and nutrition services (Casanovas et al. 2013, Hoddinott et al. 2008, Ruel et al. 2013).

2.6.3 Early child development programmes

In addition to interventions in nutrition and health, early childhood development interventions are important in healthy growth by promoting cognitive and emotional development. Among the key intervention strategies are parenting and education support and center-based early learning programmes (Engle et al. 2011). Parenting interventions aim at promoting parent–child interactions leading to improved responsiveness and increased attachment by the child and also encourage learning among other outcomes (Cooper et al. 2009, Aboud and Akhter 2011). Evaluation of studies on parenting intervention has shown positive effects on children’s cognitive and psychosocial development (Engle et al. 2011). Centre-based early learning programmes include both formal preschools that have structured curricula and trained professional staff and the informal community based preschools. Assessment of these early training programmes has shown that they improve children’s cognitive functioning, readiness for school, and school performance (Engle et al. 2011). Larger positive effects from both parenting and center-based early learning interventions have been reported in more disadvantaged populations (Engle et al. 2011), making them one of priority intervention for promotion of healthy growth in LMICs.
2.7 Lipid-based nutrient supplements and promotion of healthy growth

2.7.1 LNS products: overview and role in complementary feeding

Lipid-based nutrient supplements (LNS) are a range of fortified, lipid-based products based on similar ingredients, but varying in energy dose and concentration of micronutrients (Briend 2001, Dewey and Arimond 2012, Arimond et al. 2013). LNS products have low water content hence limit bacterial growth, can mask the metallic taste of added micronutrients and can be stored in warm environment for a long time (Briend 2001). They also have the added advantage that they can be eaten without the need of cooking.

Three main LNS products are currently used in maternal and child nutrition; Ready-to-use therapeutic foods (RUTF), Ready-to-use supplementary foods (RUSF)/medium-quantity LNS and LNS for home fortification/small-quantity LNS (Arimond et al., 2013). RUTF are designed for treatment of severe acute malnutrition, provide almost all the energy needs from foods other than breast milk and are given in large daily doses of ~180–280 g (Diop et al. 2003, Giliberto et al. 2005). RUSF/medium-quantity LNS are designed for treatment of moderate acute malnutrition (Matilsky et al. 2009, LaGrone et al. 2010; LaGrone et al. 2012), prevention of seasonal wasting (Isanaka et al., 2010, Huybregts et al. 2012) or prevention of stunting and/or underweight or promotion of growth (Isanaka et al. 2010, Grellety et al. 2012, Huybregts et al. 2012). They provide 50–100% of energy needed from foods other than breast milk and are given in daily doses of ~45–90 g (Arimond et al. 2013). Finally, small-quantity LNS products are designed to prevent undernutrition and promote growth and development through home fortification of local diet, provide <50% of energy needed from foods other than breast milk and are given in daily doses of ~20 g (Adu-Afarwuah et al. 2007, Phuka et al. 2008).
Poor complementary feeding (during 6-24 months of age) is one of the key determinants of stunted growth and development. Vulnerable households in low-income and middle-income countries experience challenges to access sufficient quantities of nutrient-dense foods, especially animal-source foods (PAHO/WHO 2003). Children in the complementary feeding age range also present unique physiological challenges; they have high nutrient needs for growth and development, yet can consume small quantities of food. Therefore to meet their daily need, complementary food needs to have high nutrient density which is difficult to achieve in vulnerable populations without use of fortified products (Dewey and Brown 2003, Vitta and Dewey 2012).

Complementary food in most LMICs is cereal-based diets which are deficient in multiple micronutrients and have poor macro-nutrient quality. Furthermore, they also have low caloric density because they are often prepared as a thin porridge. A number of approaches have been used to try to increase the energy and nutrient density with limited success; increasing the thickness of porridge (better results obtained when amylase is added to reduce its consistency), addition of oil which is often too expensive for many households. Enrichment of the cereal based complementary foods through home fortification with fortified products such as small quantity lipid-based nutrient supplements or multiple micronutrient powders have been used increasingly to date (Dewey and Arimond 2012, Arimond et al. 2013). In addition to providing multiple micronutrients, LNS delivers fats (main energy source), essential fatty acids (alpha-linolenic acid and linoleic acid that may play a role in linear growth and development) and protein. Small and medium quantity LNS are designed to fill gaps between the home complementary diet and nutrient needs and not to meet all the daily energy needs of children (Arimond et al. 2013).

Evidence on effect of both medium-quantity and small-quantity LNS on healthy growth is still limited but growing. A study in Ghana reported positive benefits of LNS on growth and motor development (Adu-Afarwuah et al. 2007). Another study in
Malawi showed reduction in the odds of stunting with LNS supplementation compared to corn-soy blend with effects sustained even after a 2 year non-intervention period (Phuka et al. 2008, 2009). However, developmental scores on the Griffiths scale between the LNS and CSB groups were similar (Phuka et al. 2012). In Chad, providing LNS showed a small but significant increase in the height-for-age z score compared to the control group after a 4 month supplementation (Huybregts et al. 2012). More recently, a trial in conducted in Haiti after the present study reported positive benefits of LNS on linear growth, but not on development compared to a control group (Iannotti et al. 2014).

2.7.2 Safety of LNS products

All lipid-based nutrient supplements are fortified with iron. However, there is on-going concern regarding the use of iron-fortified supplements in malaria-endemic areas. A previous study in Tanzania reported excess morbidity and mortality following iron supplementation in children (Sazawal et al. 2006). Since then, evaluation of a number of intervention studies with iron-containing supplements have not shown adverse effects on morbidity (Dewey 2009, Dewey and Baldiviez 2012), with one trial reporting a reduction in proportion of children with diarrhea and incidence of fever (Sharieff et al. 2006). However, a recent large study in Pakistan reported excess diarrhea and respiratory illness (Soofi et al. 2013) among children receiving iron-containing multiple micronutrient powders for 12 months. Another recent trial in Ghana found no increased risk of malaria but showed significant increase in hospital admissions among children supplemented with iron containing micronutrient powder (Zlotkin et al. 2013). So far, four studies have examined the effect of preventive LNS supplementation on morbidity; three of them conducted before the current study and one afterwards. One study reported significant reductions in fever and diarrheal illness (Huybregts et al. 2012) while the other three showed no significant effect (Adu-Afarwuah et al. 2007, Iannotti et al. 2014, Isanaka et al. 2009).
2.8 Justification for the present study

Healthy growth is a complex process under the influence of both genetic and environmental factors. The first 1000 days of life are particularly important because of the adverse short and long term consequences of stunted growth and impaired development. Adequate complementary feeding between 6-24 months of age is recognised as one of the central pillars supporting healthy growth. However, many infants and young children in low-income and middle-income countries do not have access to nutrient rich complementary foods. Dietary complementation with LNS provides a potential low-cost strategy for enrichment of local diet. Few controlled trials have shown that addition of LNS to complementary food could prevent child stunting and support normal motor development. The present study was designed to contribute to this evidence base by confirming the potential of LNS for prevention of undernutrition and promotion of normal early child development. In addition, the study aimed at evaluating the safety of their use in malaria endemic areas, an area which has not been assessed before.
3 AIMS

The aim of the present study was to assess the effect of supplementation of local complementary diet with medium-quantity lipid-based nutrient supplements (LNS) or corn-soy blend (CSB) on infant and early childhood growth, prevention of undernutrition, promotion of development and morbidity. The specific objectives were:

1. To assess the effect of provision of either daily medium-quantity LNS or CSB on promotion of growth and reduction of incidence of undernutrition in rural Malawian infant and young children.

2. To assess the effect of provision of either daily medium-quantity LNS or CSB on timing and achievement of developmental milestones among rural Malawian young children.

3. To assess the safety of provision of either daily medium-quantity LNS or CSB, and in the absence of excess morbidity, to evaluate effect of LNS on morbidity reduction among rural Malawian young children.
4 METHODS

4.1 Approach to the study
The aim of the study was investigated through a community-based randomised nutritional intervention trial conducted among 6 to 18 months old infants. The overall design of the study is shown Figure 4.

4.2 Study settings and participants

4.2.1 Study area
The study was conducted in Lungwena and Malindi, two rural Malawian communities (appendix 1). Malawi is a low-income country in south-eastern sub-Saharan Africa with a per capita GDP of USD 320 (UNICEF 2014). The country has a population of approximately 16 million people and an annual population growth rate of 2.7% (NSO and OCR Macro 2010). About 85% of the population live in rural areas and is mainly involved in subsistence farming (NSO and OCR Macro 2010). Table 2 shows some key health, demographic and socio-economic indicators for Malawi.
Figure 4. Overall design of the thesis

**Aims**

To assess the effect of LNS on growth promotion and incidence of stunting

To assess the effect of LNS on timing and achievement of developmental milestones

To assess the effect of provision LNS products on morbidity and their safety

Children randomised to either milk-LNS, soy-LNS, CSB, control

**Study 1**
- Height and weight gain in anthropometric indices
- Prevalence and incidence of undernutrition

**Study 2**
Age at achievement of developmental milestones

**Study 3**
- Longitudinal prevalence of common reported illnesses
- Incidence and relative risk of reported morbidity in LNS groups compared to control group
- Risk of serious adverse outcomes in LNS group compared to control group
The study area had 91 villages with a total population of approximately 60,000 people. Malindi is about 17 km from Mangochi town and had on average better access to electricity, clean water, and sanitation and a more educated population than Lungwena, a more rural site about 32 km from the town. Both areas are along the shores of Lake Malawi. The main economic activity in the area was subsistence farming and fishing. Maize, the main staple food is normally grown and harvested between December and March. The area has a high prevalence of early childhood stunting, underweight and poor food security (Maleta et al. 2003b). Previous studies from the area have documented stunting rates of between 40-70% with up to 31% of the children severely stunted at 1 year of age (Espo et al. 2002, Maleta 2003b). Both sites have functional health facilities providing preventive and curative services in both maternal and child health.

4.2.2 Study participants

Children participating in the trial were identified through community census in the catchment areas of the 2 health facilities. Infants who fulfilled the enrolment criteria and attended the enrollment visit were invited to participate. The inclusion criteria included age 5.50–6.50 months, residence in the study area, and informed consent from at least 1 authorised guardian. The exclusion criteria were weight for length (WFL) < 80% of the World Health Organization (WHO) reference median, presence of oedema, severe illness warranting hospitalisation on the enrolment day, history of peanut allergy, concurrent participation in another clinical trial, and any symptoms of food intolerance within 30 min after ingesting a 5-g test dose of LNS (either milk- or soy-based) used in the trial. Age for all participants was verified before enrolment objectively by crosschecking documented birth dates in health passports and under-five cards.
<table>
<thead>
<tr>
<th>Indicator</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area (km$^2$)</td>
<td>118,484</td>
</tr>
<tr>
<td>Total population (2012 estimate)</td>
<td>15,906,000</td>
</tr>
<tr>
<td>Population density (per km$^2$)</td>
<td>128.8/km²</td>
</tr>
<tr>
<td>Population annual growth rate (%)</td>
<td>2.7</td>
</tr>
<tr>
<td>Urban population (%)</td>
<td>15</td>
</tr>
<tr>
<td>Average household size, persons</td>
<td>4.6</td>
</tr>
<tr>
<td>Total fertility rate (2010)</td>
<td>5.7</td>
</tr>
<tr>
<td>Per capita gross domestic product (USD)</td>
<td>320</td>
</tr>
<tr>
<td>Proportion below poverty line (&lt; USD 1.25, %)</td>
<td>62</td>
</tr>
<tr>
<td>Female literacy rate (%)</td>
<td>57</td>
</tr>
<tr>
<td>Male literacy rate (%)</td>
<td>74</td>
</tr>
<tr>
<td>HIV prevalence among adults (15-49 yrs of age, %)</td>
<td>11</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>55</td>
</tr>
<tr>
<td>Maternal mortality ratio (per 100,000 live births)</td>
<td>675</td>
</tr>
<tr>
<td>Infant mortality rate (per 1,000 live births)</td>
<td>66</td>
</tr>
<tr>
<td>Under-five mortality rate (per 1,000 live births)</td>
<td>112</td>
</tr>
<tr>
<td>Proportion fully immunised by 12 months of age (%)</td>
<td>72</td>
</tr>
<tr>
<td>Proportion children under age 5 stunted (%)</td>
<td>47</td>
</tr>
<tr>
<td>Proportion children under age 5 wasted (%)</td>
<td>5</td>
</tr>
<tr>
<td>Proportion children under age 5 underweight (%)</td>
<td>13</td>
</tr>
<tr>
<td>Proportion of infants under 6 mo exclusively breastfed (%)</td>
<td>71</td>
</tr>
</tbody>
</table>

Sources: UNICEF State of the world children report 2014, NSO and OCR Macro Malawi Demographic Health Survey 2010
4.3 Nutritional interventions

Participants were randomly assigned to 1 of 4 intervention groups for a 12-month period. Children allocated to a control group did not receive any supplementary food in the initial 12-month period but received supplementation from 18 to 36 months of age. Participants supplied with supplemental complementary food received either 54 g/day of Milk-LNS, or 54 g/day of Soy-LNS, or 71 g/day of CSB. LNS was made from peanut paste, milk powder or soy flour, vegetable oil, sugar, and multiple micronutrient mixture (Nutriset Inc, Malaunay, France) and produced at a Malawian non-governmental organization, Project Peanut Butter (Blantyre). The corn-soy flour was purchased from a local producer (Rab Processors, Blantyre, Malawi) and was made from a mixture of corn and soy flours and micronutrients. Milk-LNS and Soy-LNS contained an identical range and quantities of micronutrients; CSB contained fewer micronutrients and for most, at a lower daily dose than that for LNS. The daily CSB and LNS dose provided approximately 280 kcal (Table 3). CSB had to be prepared as porridge before serving while LNS could be mixed to porridge or other foods or eaten as is (plain).
Table 3. Nutrient composition of the 3 supplements used in the study

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Milk-LNS</th>
<th>Soy-LNS</th>
<th>CSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mass of food supplement (g)</td>
<td>54</td>
<td>54</td>
<td>71</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>285</td>
<td>276</td>
<td>284</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.2</td>
<td>7.5</td>
<td>10.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>17.9</td>
<td>18.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Retinol (µg RE)</td>
<td>400</td>
<td>400</td>
<td>139</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>160</td>
<td>160</td>
<td>43</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>6</td>
<td>6</td>
<td>3.5</td>
</tr>
<tr>
<td>Panthothenic acid (mg)</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>30</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>366</td>
<td>366</td>
<td>72</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>0.4</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Iodine (µg)</td>
<td>90</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>6</td>
<td>6</td>
<td>5.5</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>78.5</td>
<td>78.5</td>
<td>-</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>20</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>6.0</td>
<td>6.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>186</td>
<td>186</td>
<td>-</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>319</td>
<td>307</td>
<td>-</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>0.6</td>
<td>0.6</td>
<td>-</td>
</tr>
</tbody>
</table>
4.4 Data collection

4.4.1 Enrolment and follow-up

Trial participants were recruited from 28 January 2008 to 25 May 2009. Participants meeting the eligibility criteria were allocated to intervention groups using blocked randomization. Each block contained 16 allocations evenly distributed for the four groups. Randomisation codes containing an identification number and group allocation were placed into identical-appearing opaque envelopes. From a set of reshuffled envelopes, a guardian was requested to choose one. The randomization list and envelopes were made by an individual not involved in trial implementation. The code was not disclosed to the researchers until all data had been entered and verified in a database.

After enrolment, participants were visited every two weeks at their homes where food supplements were delivered, and data on supplement use, development of milestones, morbidity and possible adverse events was collected. At 12 weekly intervals after enrolment up to 18 months of age, participants visited trial offices at the two health facilities. In addition to data collected during every 2 weekly visits, anthropometrics, blood haemoglobin concentration, and peripheral blood malaria parasitemia were also assessed at these visits. The follow-up and data collection plan is schematically presented in Figure 5.
4.4.2 Anthropometric measurements

Anthropometric measurements collected were length/height, weight, head circumference and mid-upper arm circumference (MUAC). Unclothed infants were weighed using an electronic infant weighing scale (SECA 735; Chasmors Ltd, London, England), and weights were recorded to the nearest 10 g. Length was measured to the nearest 1 mm using a high-quality length board (Kiddimetre; Raven Equipment Ltd, Essex, England). All anthropometric measurements were done by three trained research assistants. Anthropometric indexes, length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) were calculated using WHO Child Growth Standards (2010 STATA igrowup package) (WHO 2010).

Figure 5. Participant follow-up and data collection during the intervention
4.4.3 Haematological measurements

Hemoglobin concentration was measured from a venous blood sample using cuvettes and a reader (HemoCue AB, Angelholm, Sweden). Malaria status including parasite specification and count was determined via microscopy. Thin films were fixed with methanol and both thick and thin films were stained with Giemsa. Each smear was read twice by independent microscopists and discordant results were re-read by a third microscopist.

4.4.4 Developmental assessment

Milestones were assessed every 2 weeks from the age of 6 months up to 18 months. A milestone was recorded achieved if either a guardian reported or a fieldworker observed the child successfully performing the activity of interest. The child’s performance was recorded as tried but failed to perform the milestone, able to perform the milestone or “not known” in cases where performance could not be assessed. All the milestones were assessed at each participant visit with no assumed progression or any hierarchy in their achievement. Each examination was carried out independently of all previous assessments.

4.4.5 Morbidity assessment

Morbidity was assessed using standardized data collection instruments and guidelines. Throughout the study, guardians were asked to record on a daily basis the presence or absence of illness symptoms in picture calendars that were provided every two weeks. The calendar had separate rows for different days up to two weeks and separate columns for the following symptoms: fever, cough, diarrhea, and other. The first three symptom columns had pictures describing them to assist the guardians in identifying the correct area to record the information.
Participants who visited the Lungwena and Malindi health facilities for medical consultation were assessed and treated according to the Integrated Management of Childhood Illness guidelines (WHO 2008). This was done by separate teams of clinicians and nurses unrelated to the study and unaware of which intervention was allocated to which participants. Diagnoses were coded and recorded into 6 major categories: clinical malaria, clinical pneumonia, diarrhea, trauma, other respiratory illness and other illnesses.

4.4.6 Training and quality control
Standard operating procedures (SOPS) were developed before commencement of the study and guided all data collection. Data was collected by research assistants, clinicians and nurses using standardised forms and instruments. All personnel involved in data collection were trained on the content and use of questionnaires, interviews and how to conduct the required procedures and physical examinations. The performance of the field data collectors was monitored by senior research team members that included the author. The author of the thesis was responsible for training of all data collectors and their field supervision. He was the trial field coordinator of the study for 3 years, supervising all data collection and entry.

4.5 Statistical approach

4.5.1 Sample size calculation
Sample size calculation was based on the primary outcome of the study: the prevalence of severe stunting. The sample size of 210 participants per group gave 85% power (5% two-sided type I error) to detect a difference in the incidence of severe stunting between 15% in the control group and 5% in the intervention groups. This sample size
also allowed for an attrition of 10% of the participants during the follow-up and exclusion of 5% who were severely stunted at baseline.

4.5.2 Data management and analysis

The collected data were recorded on paper forms, transcribed to paper case report forms, and double entered into a tailor made database (Microsoft Access 2003; Microsoft Corp, Redmond, Washington). The two entries were electronically compared, and extreme or otherwise suspicious values were confirmed or corrected.

Statistical analyses were performed using Stata 11.0 (Stata Corp, College Station, Texas) on an intention-to-treat basis. Analysis of variance (ANOVA) and student T-test was used to compare continuous outcomes between the four intervention groups, and the multi-group extension of Fisher exact test for categorical outcomes. Generalized estimating equation (GEE) approach with Huber-White robust standard error was used to assess for outcomes with correlated data (multiple visits per child).

Various forms of undernutrition were expressed as prevalence or incidence. For the calculation of incidence, participants with the particular form of malnutrition (e.g. very severe stunting, severe wasting) at enrolment were excluded from the specific analysis. Incidence was calculated as the first time a participant developed a given form of malnutrition (e.g. very severe stunting). Relapses after recovery from the malnutrition were not counted as “new” cases of malnutrition. Survival analysis was used to determine cumulative probability of very severe stunting or severe stunting, wasting and underweight among different groups and the difference were tested by the log-rank test.

Parametric time-to-event analysis for interval censored data was performed to examine the associations of intervention with age at attainment of developmental
milestone (Griffin et al. 2006). This was based on the generalized gamma distribution, which is very flexible in shape. A developmental milestone was defined as achieved when a participant was recorded to be able to perform the defined task on two consecutive visits. The measurement of age at attainment of each developmental milestone was censored in one of the following ways: (1) left censoring if the child had experienced the milestone by the first examination date; (2) interval censoring if the child had experienced the milestone between 2 examination dates and (3) right censoring if the child had not experienced the milestone by the end final visit during the intervention period.

Morbidity outcomes were expressed either as longitudinal prevalence or incidence of a specific symptom or condition. Longitudinal prevalence of an illness was defined as the percentage of all days of observation that the child suffered the illness (Morris et al. 1996). Geometric mean ratios (GMR) were calculated to compare the longitudinal prevalence of illness in the 3 intervention groups compared to the control group. Negative binomial regression modeling was used for analyses of disease incidence to obtain incidence rate ratios (IRR) (Glynn and Buring 1996).

Safety of either LNS or CSB compared to the control diet was assessed using non-inferiority analysis. The non-inferiority margin (Δ) was no more than 20% increase in geometric mean of longitudinal prevalence, or incidence rate ratios in guardian-reported or clinical morbidity, or proportion with malaria parasitemia in the intervention groups versus the control group. Non-inferiority was established if the 2-sided 95% CIs of the GMR or IRR for an intervention group compared to the control group fell entirely below 1.2.

4.6 Ethical Approval
The trial was performed according to International Conference on Harmonisation/Good Clinical Practice guidelines (ICH-GCP). The trial protocol was
reviewed and approved by the University of Malawi College of Medicine research and ethics committee and the ethical committee of Pirkanmaa Hospital District (Finland). Key details of the protocol were published at the clinical trial registry of the National Library of Medicine (National Library of Medicine NCT00524446).
5 RESULTS

5.1 Enrolment and follow-up

A total of 1385 infants were identified through community census. Seventy three (73) of the infants were too old (aged >6.50 months) and the remaining 1312 were invited to enrolment sessions. Four hundred and five (405) of the invited participants did not report at the enrolment session. Among the participants who came for enrolment, 15 were too young (aged<5.50 months), 16 too old (aged>6.50 months), 23 were severely stunted (LAZ < -3.00), 1 below the anthropometric cut-off for weight-for-length (WFL) < 80% of the WHO reference median and guardians of 12 infants refused to participate in the trial after receiving full information of the trial. The remaining 840 infants were randomized into four intervention groups (Figure 6).

During the 12 months follow up period, 25 children (3.0%) died and 68 (8.1%) dropped out. The proportion of participants who died and dropped out was similar in the different study groups (p=0.54, and p=0.99, respectively). Final anthropometric measurements were obtained from 747 of the enrolled 840 participants (88.9%) with no significant differences between intervention groups (p=0.85).
Figure 6. Flow of participants in the study

1385 infants screened
- 73 aged > 6.5 mo

1312 infants invited to enrolment session
- 405 did not show up for session
  - 12 parents refused to
- 23 severely stunted (LAZ< -3.00)
  - 16 aged > 6.5 mo
  - 15 aged < 5.50 mo
  - 1 WFH median reference < 80%

840 randomised
- 209 infants allocated to control
  - 8 deaths
  - 16 drop-outs
  - 185 finished follow-up
- 212 infants allocated to milk LNS
  - 4 deaths
  - 17 drop-outs
  - 191 finished follow-up
- 210 infants allocated to soy-LNS
  - 5 deaths
  - 17 drop-outs
  - 188 finished follow-up
- 209 infants allocated to CSB
  - 8 deaths
  - 18 drop-outs
  - 181 finished follow-up
5.2 Background information

Table 4 shows the summary of baseline characteristics of the participants by intervention group. The summary statistics were largely comparable in the four intervention groups.

5.3 Adherence to intervention

All guardians reported that the participants consumed the study supplements regularly and were well tolerated. Diversion of supplements to someone other than the intended beneficiary was reported at only 69 of 18,906 (0.36%) supplement delivery interviews: 29 in Milk-LNS, 19 in Soy-LNS, and 21 in CSB groups (p=0.38). Leftovers of trial products were found only at 1.3%, 1.3%, and 0.6% of the home visits in the Milk-LNS, Soy-LNS, and CSB groups, respectively (p<0.005).

5.4 Growth and incidence of undernutrition

Mean length gain at the end of the 12-month intervention period was 13.0 cm, 13.2 cm, 13.0 cm and 12.9 cm in control, milk-LNS, soy-LNS and CSB respectively. Similarly gains in weight in the 4 groups were 2.42 kg, 2.53 kg, 2.46 kg and 2.32 kg. Relative to the control group, the mean gains in length and weight were 0.2 cm (95% CI - 0.1 to 0.6) and 110 g (95% CI -0.044 to 264) in children who received Milk-LNS. Similarly, mean gains in LAZ and WAZ in milk-LNS compared to the control group were -0.08 (95% CI -0.22 to 0.07) and -0.09 (95% CI -0.25 to 0.06) respectively. Among the four intervention groups, children who received Milk-LNS had the most length gain in terms of cm and z-scores and CSB had the least. However, the differences between the intervention groups were not statistically significant.
Table 4. Baseline characteristics of participants at enrollment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Milk-LNS</th>
<th>Soy-LNS</th>
<th>CSB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($n=209$)</td>
<td>($n=212$)</td>
<td>($n=210$)</td>
<td>($n=209$)</td>
</tr>
<tr>
<td>Age in months, mean (SD)</td>
<td>6.02 (0.23)</td>
<td>6.02 (0.25)</td>
<td>6.04 (0.25)</td>
<td>6.03 (0.24)</td>
</tr>
<tr>
<td>Infant sex, male sex, %</td>
<td>53.1</td>
<td>50.5</td>
<td>49.1</td>
<td>46.9</td>
</tr>
<tr>
<td>Breastfed, %</td>
<td>100.0</td>
<td>99.5</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Anthropometric status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stunted, &lt;-2 LAZ WHO, %</td>
<td>34.0</td>
<td>34.0</td>
<td>39.1</td>
<td>39.7</td>
</tr>
<tr>
<td>Length-for-age z score, mean (SD)</td>
<td>-1.64 (0.97)</td>
<td>-1.59 (1.05)</td>
<td>-1.68 (1.11)</td>
<td>-1.72 (0.97)</td>
</tr>
<tr>
<td>Underweight, &lt;-2 WAZ WHO, %</td>
<td>13.4</td>
<td>10.4</td>
<td>14.3</td>
<td>18.2</td>
</tr>
<tr>
<td>Weight-for-age z score, mean (SD)</td>
<td>-0.80 (1.06)</td>
<td>-0.70 (1.10)</td>
<td>-0.80 (1.12)</td>
<td>-0.85 (1.21)</td>
</tr>
<tr>
<td>Wasted, &lt;-2 WHZ WHO,%</td>
<td>1.9</td>
<td>0.9</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Weight-for–length z score, mean (SD)</td>
<td>0.41 (1.05)</td>
<td>0.50(1.05)</td>
<td>0.46 (1.00)</td>
<td>0.42(1.11)</td>
</tr>
<tr>
<td>Mean hemoglobin (SD), g/dl</td>
<td>9.4 (1.7)</td>
<td>9.6 (1.7)</td>
<td>9.3 (1.7)</td>
<td>9.5 (1.2)</td>
</tr>
<tr>
<td>Proportion anemic, (Hb&lt;110g/dl),%</td>
<td>84.4</td>
<td>82.5</td>
<td>83.7</td>
<td>83.6</td>
</tr>
<tr>
<td>Proportion with malaria parasitemia,%</td>
<td>17.1</td>
<td>10.1</td>
<td>13.7</td>
<td>10.9</td>
</tr>
<tr>
<td>Maternal Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal BMI, kg/m², mean (SD)</td>
<td>20.6(2.4)</td>
<td>20.7(2.2)</td>
<td>21.0 (2.4)</td>
<td>20.7 (2.4)</td>
</tr>
<tr>
<td>Maternal education, years, mean (SD)</td>
<td>3.6 (3.4)</td>
<td>4.0 (3.7)</td>
<td>3.0 (3.1)</td>
<td>3.7 (3.1)</td>
</tr>
<tr>
<td>Household Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children &lt; 5 years of age, mean (SD)</td>
<td>1.6 (0.7)</td>
<td>1.6 (0.8)</td>
<td>1.6 (0.7)</td>
<td>1.7 (0.9)</td>
</tr>
<tr>
<td>Piped drinking water, %</td>
<td>4.0</td>
<td>5.4</td>
<td>2.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Availability of latrine,%</td>
<td>92.5</td>
<td>94.2</td>
<td>93.6</td>
<td>92.7</td>
</tr>
</tbody>
</table>
The cumulative change in LAZ in the study groups on consecutive measurements during the one year follow-up period was evaluated. The observed difference in the change in mean LAZ between the groups was most marked between 9 to 12 months (Figure 2, Publication Paper I). During this period, the mean change in LAZ in children who received Milk-LNS was very small (-0.02 Z-score units). On the other hand, the decrease in mean LAZ in children in the Control, Soy-LNS and CSB group were -0.15, -0.12 and -0.18 Z-score units, respectively (p=0.045). The differences in mean change in LAZ in this age interval were statistically significant in pairwise comparisons between Milk-LNS and control groups (p=0.029) and CSB group (p=0.014) but not with Soy-LNS group (p=0.079).

A total of 86 infants developed severe stunting during the intervention period and did not differ between the groups (16 in milk-LNS, 17 in Soy-LNS, 30 in CSB and 23 in Control group, p=0.098). Similarly, the proportion of children developing other forms of malnutrition did not differ between the groups though these events were less in children in the Milk-LNS group whereas those in the CSB group had more (except for very severe stunting) (Figures 7A-D). At the end of the 12-month intervention, the proportion of children who were severely stunted was 14.1%, 11.0%, 16.0%, and 16.1% (p=0.454) in control, Milk-LNS, Soy-LNS, or CSB respectively. Generally, the prevalence for all forms of undernutrition was slightly lower in the Milk-LNS group than the other groups (Figure 8). None of the differences however reached statistical significance (all p-values > 0.05).
Figure 7. Cumulative incidence of very severe stunting (A), moderate to severe stunting (B) moderate to severe wasting (C) and moderate to severe underweight (D) in children in the control, milk-LNS, soy-LNS and CSB groups.
5.5 Timing and achievement of developmental milestones

Figure 9 and Figure 10 show the distribution of the mean age of achievement of the motor milestones, and the social and language milestones, respectively. The mean age of achievement for the four motor, two social and two language milestones were not different across the intervention groups (all p values >0.05). No statistically significant differences were observed between the study groups in analyses that combined all the eight milestones (p= 0.36), or combined all milestones in the domains of motor (p=0.27), social (p=0.46) or language (p=0.28).
Figure 9. Geometric mean age at achievement of motor milestones for control, milk-LNS, soy-LNS and CSB groups.

Figure 10. Geometric mean age at achievement of social and language milestones for control, milk-LNS, soy-LNS and CSB groups.
In the study sample, the geometric mean (SD) age at achievement was 42.7 (9.5) weeks for walking with assistance, 45.4 (10.9) weeks for standing alone, and 55.4 (11.9) weeks for walking alone (Figure 7). Compared to the WHO multicentre study reference population, the median age at achievement of standing in the study sample was similar to that observed in the WHO reference population (difference -0.9 weeks) but slightly later for supported walking or walking alone (difference in medians 4.1 weeks for both milestones) (Figure 3, Publication Paper II). The proportion of children with delay in walking with assistance, standing alone and walking alone were 5.9%, 2.5% and, 6.6% respectively with no statistically significant differences between the study groups (Figure 11).

Figure 11. Proportion of children with a delay in milestone achievement in control, milk-LNS, soy-LNS and CSB groups (no statistically significant differences between the groups for all the milestones).
5.6 Haematological changes

The mean haemoglobin concentration on study entry was 9.5 g/dl and was similar between the study groups. At 18 months of age, the mean haemoglobin concentration was 10.2 g/dl in control, 10.3 g/dl in milk-LNS, 10.1 g/dl in soy-LNS and 10.3 g/dl in CSB; all below the cut-off point for anaemia of 11g/dl. There were modest increases in mean haemoglobin concentration between 6 and 18 months of age (0.8 g/dl in control, 0.6 g/dl in milk-LNS, and 0.8 g/dl in soy-LNS and 0.7 g/dl in CSB).

There was a significant reduction in proportion of children with anaemia (defined as haemoglobin <11 g/dl) between 6 months and 18 months in all four groups (all p-values<0.05) from 84.4 % to 65.1% in Control, 82.5 % to 63.4 % in Milk-LNS 83.7 % to 73.8 % in soy-LNS and 83.6 % to 63.5% in CSB.

5.7 Morbidity and safety of intervention

During the 12 months intervention period, guardians reported any illness on 14.5% of the days. The overall longitudinal prevalence of febrile illness, cough, acute respiratory infection and diarrhea in the study sample was 5.5 %, 6.1%, 1.6% and 4.5 % respectively. There were no statistically significant differences between the intervention groups in any of the reported illnesses (all p-values >0.05). Compared to the control group, the geometric mean ratio (95% CI) for guardian reported longitudinal prevalence of febrile illness alone was 0.91 (0.73-1.09), 0.90 (0.72, 1.08) and 0.91 (0.73, 1.09) in milk-LNS, Soy-LNS and CSB respectively. Similarly, there was also no evidence that either cough alone or ARI were more frequent in either of the LNS groups or CSB than in the control group with non-inferiority concluded. However, there was a slight increase in geometric mean ratio of longitudinal prevalence of diarrhea in the milk-LNS group compared to the control (GMR 1.06, 95% CI 0.87-1.25), and it could not be excluded that milk-LNS was associated with more frequent diarrhea compared to the control group (Figure 12).
Figure 12. Overall geometric mean ratio of febrile illness alone, cough alone, acute respiratory infection, and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months. The vertical dashed line represents the margin of non-inferiority, $\Delta$. The solid line represents the null effect. GMR and its corresponding 95% CI need to fit to the area left of the non-inferiority margin line to conclude that either LNSs or CSB is at least as safe as no supplement i.e. to conclude non-inferiority.

Between the ages of 6 and 18 months, guardian reported incidence of febrile illness, diarrhea, cough, ARI were 5.6, 4.6, 5.5 and 1.3 episodes per child-year respectively. The rates were similar between the intervention groups (Figure 13).
Figure 13. Incidence of guardian-reported fever, cough, acute respiratory infection, and diarrhea for control, milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months.

Compared to the control group, the incidence of guardian reported episodes of febrile illness alone, cough alone or ARI were not different in the either of the LNS groups or CSB. However, the incidence of diarrhea was slightly higher in milk-LNS compared to control (Figure 13). With the exception of diarrhea in milk-LNS, the non-inferiority at the pre-specified margin of 1.2 was concluded for guardian reported incidences of all morbidity (Figure 14).
Figure 14. Incidence rate ratios of febrile illness alone, cough alone, acute respiratory infection and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months. The vertical dashed line represents the margin of non-inferiority, Δ. The solid line represents the null effect. Incident rate ratio and its corresponding 95% CI need to fit to the area left of the non-inferiority margin line to conclude that either LNSs or CSB is at least as safe as no supplement i.e. to conclude non-inferiority.

A total of 2706 non-scheduled visits were made by study participants to health facilities in the study area for medical consultation and treatment. The overall incidences of clinical malaria, clinical pneumonia and diarrhea at these visits were 0.54, 0.92, 0.18 episodes per child-year respectively. The rates were similar between the groups except for diarrhea which were slightly higher in milk-LNS and soy-LNS (Figure 15). There was no evidence that clinical malaria or diarrhea were more frequent in either of the LNS groups or CSB than in the control group with non-inferiority concluded for the clinical malaria though was not concluded for diarrhea (Figure 16). The incident rate ratio (95% CI) of clinical pneumonia in CSB was 0.87 (0.68 to 1.11) and non-inferior to control and marginal in
Milk-LNS (IRR 0.95 and 95% CI 0.75 to 1.20; Figure 16). The incidence of admission to hospital was similar between the intervention groups.

**Figure 15.** Incidence of malaria, acute respiratory problems and diarrheal diagnoses at health facilities for control, milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months.
Figure 16. Incidence rate ratios of malaria, acute respiratory problems and diarrhea for milk-LNS, soy-LNS and CSB groups between the ages of 6-to-18 months. The vertical dashed line represents the margin of non-inferiority, Δ. The solid line represents the null effect. Incident rate ratio and its corresponding 95% CI need to fit to the area left of the non-inferiority margin line to conclude that either LNSs or CSB is at least as safe as no supplement, i.e., to conclude non-inferiority.

There were a total of 25 deaths (8 in control, 4 in Milk-LNS, 5 in Soy-LNS, 8 in CSB), 34 hospitalisations (12 in control, 7 in Milk-LNS, 6 in Soy-LNS, 9 in CSB) and 3 participants were recorded as having experienced another serious adverse event during the follow-up. Fifty-nine (95.2%) of the SAEs were considered “unrelated” and the rest “probably unrelated” to the trial interventions.
This study was carried out to evaluate the effect of complementary food supplementation with LNS on growth, development of undernutrition, achievement of developmental milestones and morbidity among rural Malawian children. It also evaluated the safety of the LNS products with regards to excess morbidity in comparison to no supplementation. In this chapter, the strengths and weaknesses of the studies are outlined. Secondly, the possible reasons for the observed findings are discussed and the findings compared with other studies. Lastly the general conclusions from the findings are presented.

6.1 Strengths and weaknesses of the study

The study had a number of strengths. First, allocation of participants to the study groups was randomized and the intervention groups were similar in terms of a wide range of participant characteristics at enrolment. Secondly, the trial had comprehensive follow-up with a low and balanced drop-out rate. Thirdly, reported adherence to the intervention was high with no apparent differences between the groups. Fourth, outcome assessors were blinded to the participant’s group’s allocation. Lastly, broad inclusion criteria for study participation make the findings generalizable to a wide target population.

Despite the strengths, the study had some limitations that should be considered when interpreting the present findings. First, there was no direct observation of supplement consumption. Therefore, there was a possibility of incomplete compliance bias to the intervention, especially since an earlier study from the same area suggested that supplementary LNS and CSB given to infants at a daily ration size similar to that used in this study are indeed shared with other household members and possibly other individuals
Secondly, although assessors for anthropometric and facility-based morbidity outcomes were completely blinded to the group allocation of participants, there was insufficient blinding of the fieldworkers who conducted assessment of developmental outcomes. This weakness was due to the logistical difficulties of arranging two separate teams of assistants; one to deliver the supplements and another to assess milestone achievement at the participants’ homes. However, the likelihood of observation bias was minimised by constant rotation of field workers and blinding them to all previous milestone assessment results. Lastly, the study participants were not masked with respect to the intervention assignment because of the type of supplement (paste, flour) provided to children, leading to possible differential reporting by guardians on some of the outcomes such as morbidity. However, objective measures such as malaria parasitemia in terms of morbidity and blinded assessor outcomes showed robustness in the finding when compared with similar outcomes from reported guardian data.

6.2 Growth and incidence of undernutrition

Children in the study grew more in length and weight after supplementation with medium-quantity milk-LNS compared to children with the other complementary diets, though the differences were small. They also had lower incidences of undernutrition; severe stunting, very severe stunting and severe underweight. Furthermore, compared to the other complementary diets, children who received milk-LNS did not experience linear growth faltering between the ages of 6 to 12 months, but had similar rate of faltering from 12 to 18 months of age. Lastly, growth outcomes were worse among children whose complementary diet was supplemented with fortified CSB than among the non-supplemented.

Several reasons could explain the findings. Both milk-LNS and soy-LNS had higher fat and protein content compared to CSB. Increase in essential fatty acid intake, especially α-linolenic acid has been associated with length gain (Udell et al. 2005). Crucially however,
milk-LNS also contained milk protein. Several studies have suggested that animal source foods, especially bovine milk promote length or height gain in human children (Hoppe et al. 2006, de Beer 2012). Furthermore, soy products contain several antinutrients that might negatively affect linear growth, such as enzyme inhibitors, phytate and lectins (Sarwar 1997, Hoppe et al. 2008).

In terms of the observed pattern of linear growth between 6 and 18 months of age, the infancy-childhood-puberty model describes the interval from 6 to 12 months of age as the period when an infant changes from the infancy phase of growth to the childhood phase (Karlberg, 1989, Liu et al. 1998a). A delay in this shift (called the infancy-childhood spurt) is associated with the linear growth faltering (Liu et al. 1998a, Liu et al. 1998b). Milk products contain insulin-like growth factor (IGF-1) and other biologically active substances that have been suggested influence the infancy-childhood growth spurt (Low et al. 2001, Wiley et al. 2005, 2012).

In the present study, linear growth faltering occurred throughout the first year of life despite the complementary food supplementation except for milk-LNS where it occurred more prominently after the age of one year. This highlights the importance of other environmental factors that affect healthy growth such as infections (Waterlow 1994, Prentice and Paul 2000, Goto et al. 2009, Humphrey 2009), even though no significant differences were observed between the intervention groups in this study. Apart from clinical infections, subclinical disease, especially those associated with persistent gut inflammation including environmental enteropathy have also been shown to limit growth (Piwoz et al. 2012, Campbell et al. 2003). Limited success with single-pronged interventions to promote healthy growth (Dewey and Adu-Afarvuah 2008) demonstrates the need for more integrated approaches/strategies implementing appropriate nutrition-sensitive programmes that will complement nutrition-specific interventions (Casanovas et al. 2013, Ruel et al. 2013).
The nature and use of the LNS products (they were either added to a small amount of prepared porridge or eaten as is) meant that they were unlikely to displace or replace other foods or breast milk. On the contrary, CSB is typically fed as a dilute porridge. Therefore, to meet energy needs, an infant has to consume a large volume, which may result in displacement of breast milk intake and also minimise dietary diversity. It is also most likely to be shared with other family members. This could partly explain the worse outcomes observed in children who were supplemented with CSB compared to the non-supplemented group.

6.3 Timing and achievement of developmental milestones

Children in Lungwena and Malindi showed no differences in mean age at achievement of developmental milestones when supplemented with either LNS or CSB or not supplemented. Relative to the WHO MGRS, the proportion of children with developmental delay in the selected motor milestones between infants who received LNS, CSB and those who did not were also similar.

The timing of the achievement of the different developmental milestones was similar to other previous observational studies (Bayley 1969, Neligan and Prudham 1969,WHO 2006b). Furthermore, the distribution of the selected motor milestones among the children in this study was generally similar to the distribution in the WHO MGRS despite a ~4 week delay in walking with assistance and walking alone, and about 1 week advancement in standing alone. Whether this is a spurious finding or is indicative of an underlying impairment in the developmental process is unknown. These questions could better be evaluated through trials with longer post-intervention follow-up where more sensitive tools could be used to assess various aspects of development and the children are also older.
Only two earlier studies conducted in Malawi and Ghana had assessed the impact of preventive LNS provision on infant development (Adu-Afarwuah et al. 2007, Phuka et al. 2012). In Ghanaian study, LNS supplementation for 6 months was associated with a higher proportion of children who could walk independently at 12 months of age (twice as high compared to non-supplemented control infants; Adu-Afarwuah et al. 2007). However, similar effects were not observed in the other motor milestones of walking or standing with assistance or standing independently. In the Malawian study, LNS had no positive benefit on developmental outcomes measured on the Griffiths scale at 18 months of age (Phuka et al. 2012). A recent study in Haiti conducted after the present study did not find any differences in proportion of children achieving a number of motor milestones at about 12-18 months of age between LNS and non-supplemented controls (Iannotti et al. 2014). Assessment of these motor milestones in both the Ghana and Haiti studies was cross-sectional which, theoretically, could fail to capture differences that would have been evident at an earlier age compared to longitudinal assessment. In addition, most tools used to assess early child development e.g. the Griffiths scale in the Malawi study, are unbiased to the child’s prevailing culture hence less sensitive to reliably evaluate development.

6.4 Haematological changes

Mean haemoglobin concentration and prevalence of anaemia at the end of the intervention were observed to be similar between the groups. Also increases in mean haemoglobin concentration between 6 and 18 months of age between the groups were similar and only modest. Although there was a significant reduction in proportion of children with anaemia during the intervention period, overall prevalence of anaemia remained high.

This could be explained by a number of reasons. First, a lower daily dose of iron was used in the LNS products in the present study. This may not have been enough to increase haemoglobin concentration and resolve anaemia due to iron deficiency. Contrary
to our findings, other supplementation studies of LNS products with higher iron content (about 1.3 to twice the daily dose in our products) reported significant effects of LNS interventions on both mean haemoglobin concentration and prevalence of anaemia compared to non-supplemented controls (Kuusipalo et al. 2006, Adu-Afarwuah et al. 2007, Huybregts et al. 2012), suggestive of a trend of improved haemoglobin status with LNS supplementation. Second, aetiology of anaemia in children in low-income and middle-income countries is multifactorial (WHO 2007) and may not be amenable to iron supplementation alone. In Malawi for example, the background rate of iron deficiency anaemia among children less than 5 years in 2009 was estimated at 30.6% (MoH 2009).

6.5 Morbidity and safety of intervention

The study findings did not show that LNS reduced morbidity. Poor nutrition and frequent infection feedback upon each other, leading to a ‘vicious cycle’ (Brown 2003, Scrimshaw et al., 1968, Solomons 2007). Studies on supplementation of complementary diet with LNS have reported no positive benefit of supplementation on morbidity (Adu-Afarwuah et al. 2007, Isanaka et al. 2009, Iannotti et al. 2014). In Chad however, significant reductions in diarrhea and fever (29.3 % and 22.5 %, respectively) were observed in children supplemented with medium-quantity LNS for 4 mo compared to a control group on a local diet (Huybregts et al. 2012). However, their participants had a mean weight-for-height z-score of -1.1 at baseline, whereas mean WLZ among children in the present was + 0.45 at baseline. Furthermore, the participants in the Chad study were on average 24 months old at enrolment, older than the present target population. Their finding may suggest that in more wasted children and/or in older children, LNS could reduce morbidity.

The present study has documented that children supplemented with the two tested formulations of LNS did not experience more frequent infections compared to the non-supplementation children. Previous studies conducted in a variety of settings and using different doses of LNS products to prevent undernutrition also reported no excess
morbidity (Adu-Afarwuah et al. 2007, Isanaka et al. 2009, Iannotti et al. 2014). Supplementation with LNS did not result in excess morbidity from malaria or respiratory illness. Excess circulating iron has been hypothesized to be harmful by increasing sequestration of red bloods infected by malaria parasites and facilitating their infection of liver cells. This in turn facilitates development of malaria morbidity, including its severe forms (Hurrell 2010, Dewey and Baldiviez 2012). A study in Niger evaluated the safety of LNS supplementation with regards to malaria morbidity. A 3-month preventive supplementation with 92 g/d dose of LNS among healthy 6-60 month-old children did not result in increased risk of malaria (Isanaka et al. 2009). A recent study in Ghana (Zlotkin et al. 2013) where they used an iron-containing MNP for home fortification of complementary diet reported increase in hospitalizations in supplemented children, but no associated increase in the risk of malaria. One possible explanation for the finding in our study is the lower daily dosage of iron used. In addition, the LNS intake was in smaller amount throughout the day, which may help to minimize the risk of adverse effect of iron (Dewey and Baldiviez 2012, WHO 2007). However, there was no conclusive evidence on whether LNS would or would not increase diarrhea morbidity. Iron supplementation has been shown to modify the intestinal microbial flora by stimulating growth and pathogenic potential of diarrhea causing pathogenic enteric bacteria (Zimmermann et al. 2010, Kortman et al. 2012)
7 SCIENTIFIC CONCLUSIONS

The current study was conducted to compare the effect complementary food supplementation with either LNS or iso-energetic multiple micronutrient fortified CSB or no supplementation on healthy growth. After a one-year long implementation the results show that;

1. Complementary food supplementation with milk-LNS may slow down the process of infant growth faltering around the time of the transition from the infant into the childhood phase of growth compared to soy-LNS or CSB or no supplementation. However, the supplement appeared insufficient to promote catch-up growth or even to maintain normal growth after the first year.

2. Complementary food supplementation with either milk and soy-LNS or CSB does not have an impact on the achievement of the selected developmental milestones among young children compared with no supplementation.

3. Complementary food supplementation with LNS products does not reduce morbidity in rural Malawian children.

4. Complementary food supplementation with milk or soy-LNS did not result in increases in malaria or respiratory morbidity in children in a malaria endemic setting compared to no supplementation. There was however a modest but non-significant increase in morbidity from diarrhea.
The present study have shown that complementary food supplementation with lipid-based nutrient supplements (LNS) did not promote growth or prevent development of stunting among 6-to-18 months old infants and young children compared to CSB or no supplementation. However, at age-interval 6 to 12 months, milk-LNS prevented growth faltering but these gains were not sustained beyond this age. The study therefore demonstrated that as a single-pronged nutrition intervention, LNS supplementation is insufficient to promote long term healthy linear growth. Nevertheless, it did show the potential of milk-LNS in promoting healthy growth. Therefore, LNS supplementation when combined with other interventions, especially those that address the problem of high infectious morbidity burden, and water, sanitation and hygiene could still be a possible solution to prevent development of stunting and promote healthy growth in low and middle income countries.

Developmentally, the study showed that the time at achievement of developmental milestones in infants and young children provided with LNS was similar to those provided with CSB or not supplemented. Additionally, all the children were not worse than the WHO reference population on achievement of selected motor milestones. This however may need further exploration by using more sensitive tools for developmental assessment during early childhood and also within the cultural context. In addition further follow-up assessments need to be conducted at older ages (after the intervention has ceased) using multiple methodologies to evaluate the long term effect of the intervention on the development of human capital at various ages.
The present study also demonstrated that while LNS provision did not reduce morbidity, it did not either result in the increased risk of malaria or respiratory morbidity. However, the findings did not show if LNS provision would or would not increase the risk of morbidity from diarrhea, or increase risk of hospital admissions. This therefore leaves an unresolved question which needs further evaluation. Nevertheless, the study potential show that universal supplementation with iron at the dosage used and also in the context of home fortification can be safely implemented in a malaria endemic setting.

From the findings of the current study, the following have been identified as areas that need further research to evaluate the mechanisms that impair healthy growth and interventions that may promote it in infants and young children.

- Studies looking at the independent and combined effects of complementary food supplementation or fortification with LNS, and water, sanitation and hygiene interventions on prevention of stunting and promotion of healthy linear growth evaluating and with demonstration of possible synergies.

- Studies aimed at evaluating the combined effect of nutritional intervention and reduction of gut inflammation from environmental enteropathy on prevention of stunting and promotion of healthy linear growth.

- To conduct trials with larger sample size to determine the effect of LNS supplementation on morbidity and their safety in terms of excess morbidity and incidence of serious adverse events in malaria-endemic settings.
9 ACKNOWLEDGEMENTS

This study is as a result of collaboration between the Department for International Health at the University Of Tampere School Of Medicine, the University Of Tampere School Of Public Health, and the College of Medicine at the University of Malawi. Most of my course work was done at the School of Public Health, through the International Postgraduate Programme in Epidemiology (IPPE) and Doctoral Programme in Public Health. The research was carried out in the Department of International Health at University of Tampere Medical School and the School of Public Health and Family Medicine of the College of Medicine Malawi. Several people supported me during my studies and it is impossible to individually acknowledge all of them. But I would like to express my heartfelt gratitude to them all. However, the following people deserve specially mention:

Professor Per Ashorn, for his overall supervision and guidance throughout my studies and being my mentor. Per introduced me to the conduct of community based research and clinical trials, scientific thinking and working and helped to developed my passion in research. His patience, encouragement, and practical help at many times were priceless in producing this work and bringing it to completion.

Professor Yin Bun Cheung, for co-supervision my studies. His guidance and encouragement were invaluable in completing this work. His expertise and guidance in statistical analysis and interpretation of the research data were eye opening and I benefited from his vast knowledge and experience.

Professor Kenneth Maleta, College of Medicine Malawi for introducing and bringing me into the research team in Lungwena and providing encouragement, support and advice during my studies.
Docent Kaija-Leena Kolho and Professor Olli Vainio the official reviewers of this thesis, for their constructive criticism and expert advice on the final manuscript.

Faculty and staff from the Department of International Health, Tampere University. Special thanks to Ulla Ashorn; her friendship and support was always greatly appreciated. My colleagues Anna Pulakka, Ulla Harjunmaa, Kirsi-Maarit Lehto and Hanna Peevo for their friendship, multifaceted support and many interesting discussions.

To Professor Kathryn Dewey, staff and students in the Program in International and Community Nutrition at University of California Davis and Professor Steve Vosti; I learnt a lot in international nutrition and its economics during my short stay in UC Davis.

I also extend my gratitude to the IPPE and Doctoral Programme in Public Health staff in introducing me to the field of epidemiology and biostatistics. Special thanks to Dr. Patrik Finne and Ms. Catarina Stahle-Niemin for support offered during the course work and to my fellow PhD students in the program for a wonderful and friendly learning environment.

I thank all the staff in the Tampere University Mother and Child Health Project (TUMCHP) research team in Lungwena, Malindi and Mangochi for their work in the data collection and entry. Special thanks to all the previous researchers who did earlier work in Lungwena and laid the foundation for my study.

All the children and their families who participated in the clinical trial. Without them this study would not have been possible.

All the co-authors who collaborated in the original manuscripts that contributed to the thesis. Your advice, encouragement and timely comments were always greatly appreciated.
Thanks to my Malawian colleagues; John Phuka, Chrissie Twakwalakwa, Chiza Kumwenda, Jaden Bendabenda, Austrida Gondwe and Minyanga Kachale, for your friendship, support and sharing the ups and downs of the PhD process!

My siblings (Patricia, George, Martha, Sarah, Baldwin, Dennis and Rachel), cousins (Chikondi and Chawezi) and their families for their love and encouragement. My Aunt Mrs Lostina Chapola for her love and support and care for my son without which, this work would have been very hard to complete. My beloved son Benjamin, the light of my life, for enduring frequent and long periods of absence during the study. Lastly, but the greatest of all, I thank God the Father and Christ the Lord for the abundance of His never-ending grace. For in Him indeed I live, move and have my being!!

Tampere, January 2015

Charles Bernard Mangani
10 REFERENCES


malnutrition and morbidity: modification of the household-level effects by the 
community SES. Health Place 11:205-225.

major revision and restandardization of the Denver Developmental Screening Test. 


prevention of malaria in pregnancy: a systematic review of randomised controlled 

development of stunted and nonstunted Jamaican children. J Child Psychol 
Psychiatry 4: 819-827.

Gluckman PD, Hanson MA, Beedle AS (2007): Early life events and their consequences 
for later disease: a life history and evolutionary perspective. Am J Hum Biol 19:1- 
19.

Glynn RJ and Buring JE (1996): Ways of measuring rates of recurrent events. BMJ 312: 
364-367.

Goto R, Mascie-Taylor CG, Lunn PG (2009): Impact of intestinal permeability, 
inflammation status and parasitic infections on infant growth faltering in rural 


Martorell R, Horta BL, Adair LS, Stein AD, Richter L, Fall CH, Bhargava SK, Biswas SK, Perez L, Barros FC, Victora CG; Consortium on Health Orientated Research in Transitional Societies Group (2010): Weight gain in the first two years of life is an
important predictor of schooling outcomes in pooled analyses from five birth cohorts from low- and middle-income countries. J Nutr 140: 348-354.


National Statistical Office (Malawi) and OCR Macro. Malawi Demographic and Health Survey 2010. NSO and ICF Macro, Calverton (MD), Maryland, USA; 2011.

Neligan G and Prudham D (1969): Norms for four standard developmental milestones by sex, social class and place in family. Dev Med Child Neurol 11: 413-422.


Waber DP, Vuori-Christiansen L, Ortiz N, Clement JR, Christiansen NE, Mora JO, Reed RB, Herrera MG (1981): Nutritional supplementation, maternal education, and


Appendix I Map of research area
ORIGINAL PUBLICATIONS
Effect of complementary feeding with lipid-based nutrient supplements and corn–soy blend on the incidence of stunting and linear growth among 6- to 18-month-old infants and children in rural Malawi

Charles Mangani*,†, Kenneth Maleta*, John Phuka®, Yin Bun Cheung†‡, Chrissie Thakwalakwa*, Kathyrn Dewey§, Mark Manary¶, Taneli Puumalainen** and Per Ashorn†††

*College of Medicine, University of Malawi, Blantyre, Malawi, †University of Tampere, School of Medicine, Tampere, Finland, ‡Duke-NUS Graduate Medical School, National University of Singapore, Singapore, Singapore, §University of California, Davis, California, USA, ¶Washington University School of Medicine, St. Louis, Missouri, USA, **Ministry for Social Affairs and Health, Tampere, Finland, and †††Department of Paediatrics, Tampere University Hospital, Tampere, Finland

Abstract

Low nutritional value of complementary foods is associated with high incidence of childhood growth stunting in low-income countries. This study was done to test a hypothesis that dietary complementation with lipid-based nutrient supplements (LNS) promotes linear growth and reduces the incidence of severe stunting among at-risk infants. A total of 840 6-month-old healthy infants in rural Malawi were enrolled to a randomised assessor-blinded trial. The participants received 12-month supplementation with nothing, milk–LNS, soy–LNS, or corn–soy blend (CSB). Supplements provided micronutrients and approximately 280 kcal energy per day. Outcomes were incidence of severe and very severe stunting [length-for-age z-score, (LAZ)< -3.00 and < -3.50, respectively], and change in LAZ. The incidence of severe stunting was 11.8%, 8.2%, 9.1% and 15.5% (P = 0.098) and that of very severe stunting 7.4%, 2.9%, 8.0% and 6.4% (P = 0.138) in control, milk–LNS, soy–LNS, or corn–soy blend (CSB) groups, respectively. Between 9 and 12 months of age, the mean change in LAZ was -0.15, -0.02, -0.12 and -0.18 (P = 0.045) for control, milk–LNS, soy–LNS and CSB groups, respectively. There was no significant between-group difference in linear growth during other age-intervals. Although participants who received milk–LNS had the lowest incidence of severe and very severe stunting, the differences between the groups were smaller than expected. Thus, the results do not provide conclusive evidence on a causal association between the LNS supplementation and the lower incidence of stunting. Exploratory analyses suggest that provision of milk–LNS, but not soy–LNS promotes linear growth among at-risk infants mainly between 9 and 12 months of age.

Keywords: complementary feeding, lipid-based nutrient supplements, infants, children, linear growth, stunting.

Introduction

Almost one-third of all under 5-year-old children in low- or middle-income countries and over 40% of those living in Africa are estimated to be stunted, i.e. they have suffered from linear growth failure that has made them shorter than expected for their age (Black et al. 2008). Stunting is not only associated with reduced final height, but also with increased morbidity, mortality, developmental delay or deficits, poor school performance and lower cognitive function in childhood, and less income as an adult (Pelletier et al. 1995; Grantham-McGregor et al. 2007; Victora et al. 2008). Given these adverse outcomes and the frequency of the condition, it is not surprising that prevention of childhood stunting has been identified as a major global health priority (UNICEF 2009).
On a population level, stunting is associated with a number of harmful exposures, including inadequate diet and frequent infections (Martorell et al. 1994). However, most interventions addressing these risk factors have so far failed to markedly improve linear growth or prevent stunting among infants and young children (Dewey & Adu-Afarwuah 2008). Recently results from studies in Ghana and Malawi suggested that consumption of lipid-based nutrient supplements (LNS), a novel class of micronutrient–fortified, ready-to-use products, might boost length gain and reduce growth failure and the incidence of severe stunting among 6–18-month-old infants (Adu-Afarwuah et al. 2007; Phuka et al. 2008; Phuka et al. 2009). While these findings were encouraging, they are also limited; the Malawi study did not include a parallel no-intervention control group and the non-intervention group in Ghana study had no weekly follow-up visits compared with the intervention groups.

The main aim of the present study was to test a hypothesis that provision of LNS would reduce the incidence of severe stunting or other forms of linear growth faltering among 6–18-month-old infants in Malawi, southern Africa. Two separate LNS products were tested, one that contained dried skim milk and thus resembled products used in earlier prevention trials with LNS (Adu-Afarwuah et al. 2007; Phuka et al. 2008), and another one in which the milk powder was substituted with soy flour. The latter choice was motivated by the use of soy in nationally recommended complementary foods (Malawi MoHP 2010), its cheaper price and also its acceptability to vegetarians.

To improve on the earlier trials, we also designed the study with two control groups: one of these included children who received no intervention during the period of interest while participants in the other control group were given rations of micronutrient-fortified corn–soy blend (CSB), a nationally recommended complementary food for infants and young children (Malawi MoHP 2010). Because of our earlier finding that LNS provision might have the strongest impact on the most disadvantaged infants (Phuka et al. 2009), we selected the incidence of severe stunting as the primary outcome measure.

Methods

Study area

The study was conducted in Lungwena and Malindi, two rural Malawian communities with a total population of approximately 60,000 people. Infant undernutrition in the area is common with a high prevalence of early childhood stunting and underweight. Almost all children are breastfed until two years, but exclusive breastfeeding period is normally very short and almost all children are introduced to complementary foods by 4 months of age. The principal complementary food is maize, usually first served as a thin porridge and later in infancy substituted by a thicker porridge and complemented with soups from vegetables and fish (Vaahtera et al. 2001). The main staple, maize, is normally grown and harvested between December and March and dietary inadequacies in food consumption and nutrient intakes are common especially in the months preceding the only annual harvest (Hotz & Gibson 2001).

Key messages

- Dietary complementation with lipid-based nutrient supplements (LNS) has been suggested to improve linear growth and reduce the incidence of stunting.

- In the current sample, statistically significant between-group differences were observed in the mean length-for-age change between 9 and 12 months of age. There were parallel differences in the mean length gain, incidence of severe or very severe stunting or several other growth outcomes over the entire 12-month supplementation period, but none of these results reached statistical significance.

- The study findings suggest that provision of milk-containing LNS may slow down the process of infant growth faltering around the time of the transition into the childhood phase of growth.
Eligibility criteria, enrolment, and randomisation

The study was designed as a community-based randomised trial comparing the efficacy of four intervention schemes involving three intervention groups and one delayed-intervention group. Trial participants were recruited from 28 January 2008 to 25 May 2009. The inclusion criteria included age 5.50–6.50 months, residence in the study area, and informed consent from at least 1 authorised guardian. The exclusion criteria were weight for length (WFL) < 80% of the World Health Organization (WHO) reference median or presence of oedema, severe illness warranting hospitalisation on the enrolment day, history of peanut allergy, concurrent participation in another clinical trial, and any symptoms of food intolerance within 30 min after ingesting a 5-g test dose of LNS (either milk- or soy-based) used in the trial.

Initially the exclusion criteria also included severe stunting (length-for-age z-score; LAZ < -3.00), but this criterion was dropped at 6 weeks into the trial (17 March 2008, when 97 of the 840 participants had been enrolled). This change in eligibility criteria was prompted by our observation that approximately 23% of the infants who underwent the eligibility assessment met this criterion. The proportion was much higher than we had anticipated, mostly due to the fact that in this study we were using the 2006 WHO Child Growth Standards (World Health Organisation (WHO) Multicentre Growth Reference Study Group 2006), whereas our earlier studies and estimates had used the Centers for Disease Control and Prevention (CDC) 2000 references (Kuczmarski et al. 2002). Our earlier study (Phuka et al. 2009) suggested that the more severely stunted infants grew more when receiving the LNS intervention and, hence we wanted to include such infants who might benefit most from it.

Potentially eligible participants were identified through community census in the study area with a preliminary screening done to assess for eligibility. During recruitment, trained data collectors contacted all the families of children of eligible age whose parents showed a preliminary interest in the trial. Infants were invited to an enrolment session, where they were screened for eligibility, and guardians were given detailed information on the trial contents. Before enrollment, a guardian signed a written consent form for trial participation.

Blocked randomisation, with each block containing 16 allocations evenly distributed for the four groups, was used to assign participants to intervention groups. A set of identical-appearing opaque envelopes from one randomisation block was shuffled and a guardian was requested to choose one envelope. The envelope contained an identification number and the allocation to one of the four interventions. The randomisation list and envelopes were made by an individual not involved in trial implementation, and the code was not disclosed to the researchers or to those assessing the outcomes until all data had been entered and verified in a database.

Interventions and follow-up

Eligible infants were randomly assigned to 1 of 4 intervention schemes for a 12-month period. Infants in the control group were not provided with any supplemental complementary food during the primary follow-up, but received a delayed supplementation with 71 g per day-fortified corn–soy flour between 18 and 30 months of age. Participants in the other three groups received either 71 g day⁻¹ of micronutrient-fortified CSB, 54 g day⁻¹ of micronutrient-fortified LNS with milk protein base (milk–LNS) or 54 g day⁻¹ of micronutrient-fortified LNS with soy protein base (soy–LNS) between 6 and 18 months of age.

The supplements were home delivered at 2-week intervals (at each supplement delivery, either two 500-g bags of CSB, or five 150-g jars LNS were given). The corn–soy flour was purchased from a local producer (Rab Processors, Blantyre, Malawi). The investigational LNS were produced at a Malawian non-governmental organisation, Project Peanut Butter (Blantyre, Malawi), from peanut paste, milk powder (which made up 25% of the milk–LNS product weight), or soy flour (which made up 20% of the soy–LNS product weight), vegetable oil, sugar and multiple micronutrient mixture (Nutriset Inc, Malau- nay, France). The products were made mostly from the same ingredients as commercially available
Plumpy Nut™ or PumpyDoz™, but their nutrient composition was closer to the ‘preventive’ supplement named Nutributter™.

Table 1 shows the micronutrients and their quantities in the three supplements. The daily CSB and LNS ration provided approximately 280 kcal of energy and a sufficient dose of selected micronutrients to meet their recommended daily allowances when added to intakes from breast milk and complementary foods. Milk–LNS and soy–LNS contained an identical range and quantities of micronutrients. CSB contained fewer micronutrients and for most nutrients, the daily supplement ration contained a lower dose than that for LNS. Phytate content of the supplements was not assessed.

Guardians for infants in the intervention groups were provided with spoons and advised to feed their babies with normal healthy diets and additionally offer them daily either 10 spoonfuls of CSB, cooked into a complementary porridge, or eight spoonfuls of milk–LNS or soy–LNS, divided into two to four daily servings. All mothers were encouraged to continue breastfeeding on demand and to feed their infants only as much of the food supplement as the infants wanted to consume at a time.

Participants were visited every 2 weeks at their homes to collect information on supplement use and possible adverse events. Empty food containers were collected at these visits. At 12-week intervals after enrolment up to week 52, participants had a visit at the trial office where they underwent an anthropometric assessment. Unclothed infants were weighed using an electronic infant weighing scale (SECA 735; Chasmors Ltd, London, England), and weights were recorded to the nearest 10 g. Length was measured to the nearest 1 mm using a high-quality length board (Kiddimetre; Raven Equipment Ltd, Essex, England). All anthropometric measurements were done by three trained research assistants. The technical error of each research assistant’s measurements and the coefficient of reliability were assessed during weekly standardisation measurements from at least three participants. Anthropometric indexes LAZ, weight-for-age (WAZ) and weight-for-length (WLZ) were calculated using WHO Child Growth Standards (2010 STATA igrowup package) (WHO 2010).

At enrolment, the participants’ blood haemoglobin concentration was measured from a venous sample using cuvettes and a reader (HemoCue AB, Angelholm, Sweden). Malaria was diagnosed microscopically from Giemsa stained thick and thin blood films. Malaria treatment was provided according to the national guidelines to all participants with clinical malaria. All participants found to have a blood haemoglobin concentration below 80 g l\(^{-1}\) were treated with iron supplementation in accordance with the national treatment guidelines (1–6 mg per kilogram body weight per day for one month). Participants developing moderate or severe wasting (WFL < 80% of the WHO reference median) during the intervention were temporarily suspended from the study and referred for appropriate management but continued follow-up and resumed trial supplementation after nutrition treatment.
Outcome measures

The primary outcome was the incidence of severe stunting (LAZ <-3.00) during the 12-month follow-up. To allow direct comparison with the results from our earlier trials in which severe stunting was defined using an older CDC growth reference (13), we used the incidence of very severe stunting (LAZ <-3.5) as our first secondary outcome. In the studied age group, a length that gives a z-score of -3.0 when using the older reference corresponds to a z-score of -3.5 if calculated with the WHO growth reference (12). Other secondary outcomes included the incidence of moderate-to-severe stunting (LAZ <-2), mean change in LAZ, and the prevalence of various forms of stunting at the end of the intervention. Hence, the focus of the study was on stunting and linear growth, but for completeness, we also described changes in weight and the incidence of underweight and wasting.

For the calculation of incidence, participants with the particular form of malnutrition (e.g. very severe stunting, severe wasting) at enrolment were excluded from the specific analysis. Incidence was calculated as the first time a participant developed a given form of malnutrition (e.g. very severe stunting). Relapses after recovery from the malnutrition were not counted as ‘new’ cases of malnutrition.

Data management and statistical methods

The sample size of 210 participants per group gave 85% power (5% two-sided type I error) to detect a difference in the incidence of severe stunting between 15% in the control group and 5% in the intervention groups. This allowed for an attrition of 10% during the follow-up and exclusion of 5% who were severely stunted at baseline, based on the initial enrollment criterion.

The collected data were recorded on paper forms, transcribed to paper case report forms, and double entered into a tailor made Microsoft Access 2003 database (Microsoft Corp., Redmond, WA, USA). The two entries were electronically compared, and extreme or otherwise suspicious values were confirmed or corrected.

Statistical analyses were performed using Stata 11.0 (Stata Corp, College Station, TX, USA) on an intention-to-treat basis. Analysis of variance and Student’s t-test was used to compare continuous outcomes between the four intervention groups, and the multi-group extension of Fisher’s exact test for categorical outcomes. To take into account multiple comparisons between the different groups, we used a gatekeeping procedure of first testing the global null hypothesis of all four groups being equal. Pairwise comparisons were considered confirmatory only if the global null hypothesis was rejected.

Participant’s compliance to the trial was assessed in terms of compliance to scheduled health facility visits, food sharing and food leftover at each 2-week visit. Compliance in terms of observed leftovers and mothers’ report of usage was analysed using the generalised estimating equation approach with Huber–White robust standard error to allow for correlated data (multiple visits per child).

Ethics, study registration, and participant safety

The trial was performed according to International Conference on Harmonisation/Good Clinical Practice guidelines, and regulatory guidelines in Malawi. The trial protocol was reviewed and approved by the University of Malawi College of Medicine research and ethics committee and the ethical committee of Pirkanmaa Hospital District (Finland). Key details of the protocol were published at the clinical trial registry of the National Library of Medicine.

A data safety and monitoring board continuously monitored the incidence of suspected serious adverse events (SAEs), defined as any untoward medical occurrence that either resulted in death or was life threatening or required inpatient hospitalisation or prolongation of existing hospitalisation or resulted in persistent or significant disability or incapacity or other serious medical conditions.

Results

Of the 1385 infants who were identified through community census, 490 were either ineligible or not brought to an enrolment session. A further 55 were found ineligible in a more detailed assessment. The remaining 840 infants were randomised into four
intervention groups as shown in Fig. 1. None of the infants was allergic to a 5-g test dose of milk–LNS.

Table 2 shows the baseline demographics, anthropometrics and parental characteristics of the participants by intervention group. The summary statistics were largely comparable in the four groups although the CSB group had slightly fewer boys and lower mean initial weight and LAZ. At enrolment, the prevalence of severe and very severe stunting in the combined sample was approximately 8.5% and 3.5%, respectively. All except one child were breastfeeding at enrolment, but none of them exclusively. The proportion of children receiving breast milk remained very high throughout the study (99.7% at 12 months and 96.8% at 18 months of age).

During the 12 month follow up, 25 children (3.0%) died and 68 (8.1%) dropped out (Fig. 1). Hence, final measurements were obtained from 747/840 (88.9%) participants. The success of follow-up was not significantly different between intervention groups ($P = 0.852$). A total of 45 participants had no anthropometric data at all after enrolment (13 deaths, 32 drop-outs) and there was no difference in this proportion between the intervention groups ($P = 0.847$).
Table 2. Baseline characteristics of participants at enrolment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 209)</th>
<th>Milk–LNS (n = 212)</th>
<th>Soy–LNS (n = 210)</th>
<th>CSB (n = 209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex, male [n (%)]</td>
<td>111 (53.1)</td>
<td>107 (50.5)</td>
<td>103 (49.1)</td>
<td>98 (46.9)</td>
</tr>
<tr>
<td>Age, months (mean ± SD)</td>
<td>6.02 ± 0.23</td>
<td>6.02 ± 0.25</td>
<td>6.04 ± 0.25</td>
<td>6.03 ± 0.24</td>
</tr>
<tr>
<td>Weight, kg (mean ± SD)</td>
<td>7.02 ± 0.89</td>
<td>7.09 ± 0.97</td>
<td>7.00 ± 0.88</td>
<td>6.95 ± 1.01</td>
</tr>
<tr>
<td>Length, cm (mean ± SD)</td>
<td>63.2 ± 2.2</td>
<td>63.2 ± 2.4</td>
<td>63.0 ± 2.4</td>
<td>62.9 ± 2.3</td>
</tr>
<tr>
<td>Weight-for-age z score (mean ± SD)</td>
<td>-0.80 ± 0.16</td>
<td>-0.70 ± 1.10</td>
<td>-0.80 ± 1.12</td>
<td>-0.85 ± 1.21</td>
</tr>
<tr>
<td>Length-for-age z score (mean ± SD)</td>
<td>-1.64 ± 0.97</td>
<td>-1.59 ± 1.05</td>
<td>-1.68 ± 1.11</td>
<td>-1.72 ± 0.97</td>
</tr>
<tr>
<td>Weight-for-length z score (mean ± SD)</td>
<td>0.41 ± 1.05</td>
<td>0.50 ± 1.05</td>
<td>0.46 ± 1.00</td>
<td>0.42 ± 1.11</td>
</tr>
<tr>
<td>Very severely stunted [n (%)]</td>
<td>7/209 (3.4)</td>
<td>6/212 (2.8)</td>
<td>10/210 (4.8)</td>
<td>6/209 (2.9)</td>
</tr>
<tr>
<td>Severely stunted [n (%)]</td>
<td>14 (6.7)</td>
<td>17 (8.0)</td>
<td>24 (11.4)</td>
<td>16 (7.7)</td>
</tr>
<tr>
<td>Blood hemoglobin concentration, g/dl (mean ± SD)</td>
<td>9.4 ± 1.7</td>
<td>9.6 ± 1.7</td>
<td>9.3 ± 1.7</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>Maternal BMI, kg m⁻² (mean ± SD)</td>
<td>20.6 ± 2.4</td>
<td>20.7 ± 2.2</td>
<td>21.0 ± 2.4</td>
<td>20.7 ± 2.4</td>
</tr>
<tr>
<td>Maternal education, years (mean ± SD)</td>
<td>3.6 ± 3.4</td>
<td>4.0 ± 3.7</td>
<td>3.0 ± 3.1</td>
<td>3.7 ± 3.1</td>
</tr>
</tbody>
</table>

CSB, corn–soy blend; milk–LNS, milk powder containing lipid-based nutrient supplement; soy–LNS, soy-flour containing lipid-based nutrient supplement; SD, standard deviation.

Table 3. Change in weight and length among participants during the 12-month intervention, by study group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Milk–LNS</td>
</tr>
<tr>
<td>Change in length, cm (mean ± SD)</td>
<td>13.0 ± 2.0</td>
<td>13.2 ± 1.7</td>
</tr>
<tr>
<td>Change in length-for-age z-score (mean ± SD)</td>
<td>-0.31 ± 0.72</td>
<td>-0.23 ± 0.68</td>
</tr>
<tr>
<td>Change in weight, kg (mean ± SD)</td>
<td>2.42 ± 0.77</td>
<td>2.53 ± 0.78</td>
</tr>
<tr>
<td>Change in weight-for-age z-score (mean ± SD)</td>
<td>-0.30 ± 0.72</td>
<td>-0.21 ± 0.77</td>
</tr>
<tr>
<td>Change in weight-for-length z-score (mean ± SD)</td>
<td>-0.66 ± 0.93</td>
<td>-0.57 ± 1.02</td>
</tr>
</tbody>
</table>

CSB, corn–soy blend; milk–LNS, milk powder containing lipid-based nutrient supplement; soy–LNS, soy-flour containing lipid-based nutrient supplement; SD, standard deviation. P-value obtained by analysis of variance.

All mothers reported that their infants readily ate the provided supplement and diversion of any portion to someone other than the intended beneficiary was reported at only 69 of 18906 (0.36%) supplement delivery interviews; 29 in milk–LNS, 19 in soy–LNS, and 21 in CSB groups (P = 0.383). From the 2-weekly home visits during which leftover trial products were checked, the percentage of visits with leftovers found were 1.3%, 1.3%, and 0.6% in the milk–LNS, soy–LNS, and CSB groups respectively (P < 0.001).

During the one year intervention period, children who received milk–LNS had the most length gain both in terms of cm and z-scores whereas children who received CSB had the least. None of the differences between the intervention groups and the control children was, however, statistically significant (Table 3). Looking into the consecutive measurements within the one year period, the biggest difference was seen in the age period between 9 to 12 months (Fig. 2). During this time children who received milk–LNS had a negligible change in their mean LAZ (−0.02 z-score units) whereas children in the Control, soy–LNS and CSB group dropped their mean LAZ (−0.12 and −0.18 z-score units, respectively (P = 0.045). (Sample size varied from 520 to 747 across age intervals of measurements.) Pairwise comparisons showed statistically significant differences in change in LAZ between milk–LNS and control groups (P = 0.029) and CSB group (P = 0.014), but not soy–LNS group (P = 0.079) in this age interval.

A total of 86 infants developed severe stunting during the intervention. The number of those who developed very severe stunting, moderate-to-severe
stunting, severe underweight, and severe wasting were 50, 177, 36, and 11, respectively. Children in the milk–LNS group had less of these events whereas those in the CSB group had more (except very severe stunting), but none of the differences between the groups was statistically significant (Table 4). At the end of the 12-month intervention, the prevalence of severe stunting and other forms of undernutrition was slightly lower in the milk–LNS group than the other groups but again the differences were not statistically significant (Table 5).

All interventions were well tolerated by the participants. Besides the 25 deaths (8 in control, 4 in milk–LNS, 5 in soy–LNS, 8 in CSB; Fig. 1), 34 other participants were hospitalised (12 in control, 7 in milk–LNS, 6 in soy–LNS, 9 in CSB) and 3 were recorded as having experienced another SAE during the follow-up. Fifty-nine (95.2%) of the SAEs were

---

**Table 4.** Incidence of severe undernutrition during the 12-month intervention, by study group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Control</th>
<th>Milk–LNS</th>
<th>Soy–LNS</th>
<th>CSB</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever developed severe stunting (LAZ &lt; -3.00)</td>
<td>23/195 (11.8%)</td>
<td>16/195 (8.2%)</td>
<td>17/186 (9.1%)</td>
<td>30/193 (15.5%)</td>
<td>0.098</td>
</tr>
<tr>
<td>Ever developed very severe stunting (LAZ &lt; -3.50)</td>
<td>15/202 (7.4%)</td>
<td>6/206 (2.9%)</td>
<td>16/200 (8.0%)</td>
<td>13/203 (6.4%)</td>
<td>0.138</td>
</tr>
<tr>
<td>Ever developed moderate-to-severe stunting (LAZ &lt; -2.0)</td>
<td>41/138 (29.7%)</td>
<td>40/140 (28.6%)</td>
<td>46/128 (35.9%)</td>
<td>50/126 (39.7%)</td>
<td>0.177</td>
</tr>
<tr>
<td>Ever developed severe underweight (WAZ &lt; -3.00)</td>
<td>11/205 (5.4%)</td>
<td>3/208 (1.4%)</td>
<td>10/206 (4.9%)</td>
<td>12/200 (6.0%)</td>
<td>0.107</td>
</tr>
<tr>
<td>Ever developed severe wasting (WLZ &lt; -3.00)</td>
<td>2/209 (1.0%)</td>
<td>2/212 (0.9%)</td>
<td>4/210 (1.9%)</td>
<td>3/209 (1.4%)</td>
<td>0.797</td>
</tr>
</tbody>
</table>

CSB, corn–soy blend; LAZ, length-for-age z-score; milk–LNS, milk-containing lipid-based nutrient supplements; soy–LNS, soy-containing lipid-based nutrient supplements; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score. P-value obtained by Fisher’s exact test.
considered unrelated and the rest probably unrelated to the trial interventions.

**Discussion**

This trial was carried out to test a hypothesis that 12-month-long dietary supplementation with LNS would promote linear growth and reduce the incidence of severe growth faltering among rural Malawian infants and young children. Although statistically not significant, the sample findings were consistent with the hypothesis, as participants receiving dietary complementation with milk-containing LNS gained on average approximately 0.2 cm more in length and 110 g more in weight and had a lower incidence of severe stunting during the follow-up than control children. The internal validity of the trial was high because of broad inclusion criteria, random group allocation, similarity of the intervention groups at enrolment, comprehensive follow-up, and blinding of the outcome assessors. The between-group differences were, however, smaller than anticipated and in many analyses the probability of the observed differences being due to random variation was above the traditionally accepted 5% threshold. Because of this, the primary null-hypothesis of no difference between the groups could not be conclusively rejected. Nevertheless, the inherent consistency of the findings on various aspects of growth, as well as their biological plausibility and coherence with earlier studies (Adu-Afarwuah *et al*. 2007; Phuka *et al*. 2008; Phuka *et al*. 2009) suggest a causal relationship between the milk–LNS intervention and moderately improved growth outcomes.

Age–interval-specific analyses from our sample indicated that the mean length-for-age remained constant from 6 to 12 months among participants who received milk–LNS (i.e. no growth faltering occurred), whereas it decreased by 0.1–0.2 z-score units between 9 and 12 months of age among other infants. From the age of 12–18 months, the mean length-for-age fell at approximately equal rate in all groups. One possible explanation for these findings is a specific effect of milk powder on linear growth in early childhood. According to the Infancy–Childhood–Puberty model of growth, the interval from 6 to 12 months of age is the period when an infant changes from the infancy phase of growth to the childhood phase (Karlberg 1989; Liu *et al*. 1998a, 2000). A delay in this shift [called the infancy-childhood (IC) spurt] has been associated with the high incidence of growth retardation in some low-income settings (Liu *et al*. 1998a,b). Factors leading to the IC spurt remain unclear, but insulin-like growth factor and numerous other biologically active substances that are present in cow’s milk – and hence also in milk–LNS – have been suggested to play a role (Low *et al*. 2001; Wiley 2005; Wiley 2012). This hypothesis is supported by several studies suggesting that animal source foods, especially bovine milk promote length or height gain in human children (Hoppe *et al*. 2006; de Beer 2012).

The apparent difference in linear growth and the incidence of severe stunting between participants who received milk-containing as opposed to soy-containing LNS could be a spurious finding or related to the lack of milk or other differences in the composition of the soy–LNS. Depending on the type

---

**Table 5.** Prevalence of severe undernutrition at the end of 12-month intervention, by study group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of participants with the indicated outcome / total number of participants (%)</th>
<th>Control</th>
<th>Milk–LNS</th>
<th>Soy–LNS</th>
<th>CSB</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severely stunted (LAZ &lt; -3.00)</td>
<td>26/185 (14.1%) 21/191 (11.0%) 30/188 (16.0%) 29/180 (16.1%)</td>
<td>0.454</td>
<td>0.290</td>
<td>0.066</td>
<td>0.370</td>
<td></td>
</tr>
<tr>
<td>Very severely stunted (LAZ &lt; -3.50)</td>
<td>12/185 (6.5%) 8/191 (4.2%) 17/188 (9.0%) 11/180 (6.1%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately-to-severely stunted (LAZ &lt; -2.00)</td>
<td>81/185 (43.8%) 79/191 (41.4%) 89/188 (47.3%) 98/180 (54.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severely wasted (WLZ &lt; -3.00)</td>
<td>2/184 (1.1%) 0/191 (0%) 3/188 (1.6%) 3/180 (1.7%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSB, corn–soy blend; milk–LNS, milk-containing lipid-based nutrient supplements; LAZ, length-for-age z-score; soy–LNS, soy-containing lipid-based nutrient supplements; WLZ, weight-for-length z-score. P-value obtained by Fisher exact test.
and extent of processing, soy products contain antinutrients that might negatively affect linear growth, such as enzyme inhibitors, phytate and lectins (Sarwar 1997; Hoppe et al. 2008). Although soy is cheaper than milk, has a favorable protein profile, and is increasingly used in therapeutic LNS preparations (de Pee & Bloem 2009), our results do not support its use in the formulation for preventive LNS. Similarly, the observation that the growth outcomes were worse among participants who received fortified CSB than among non-supplemented controls (e.g. incidence of moderate-to-severe stunting was 39.7% vs. 29.7%, respectively) does not lend support to the Malawian national recommendation of using CSB as a complementary food (Malawi MoHP 2010). Unlike the energy- and nutrient-dense LNS, the intake of which does not appear to alter breast milk intakes (Galpin et al. 2007), CSB is typically fed as a high-volume dilute porridge, which may more easily result in displacement of breast milk or other nutritious foods in the child’s diet. The relatively poor growth outcomes in the CSB group could also explain why the effects observed in the current trial (in which the LNS groups were compared with a no-supplement control group) were smaller than those observed in a previous trial conducted in the same area in which the LNS groups were compared with a group given CSB (Phuka et al. 2009). However, these potential explanations are speculative and require confirmation. The current study was designed to compare each intervention group to the control group, but not to make direct comparisons between the intervention groups.

As a whole, the results from the current trial and those of the two earlier ones (Adu-Afarwuah et al. 2007; Phuka et al. 2008; Phuka et al. 2009) are consistent with a hypothesis that provision of milk-containing LNS may slow down the process of infant growth faltering around the time of the transition into the childhood phase of growth in environmental conditions similar to those in rural Malawi. Unfortunately the intervention appears insufficient to reverse linear growth faltering is not surprising, because stunting typically involves multiple aetiologies (Waterlow 1994) and very few single-pronged interventions have ever achieved catch-up growth in length, either in a trial or programmatic setting (Dewey & Adu-Afarwuah 2008). Long-term reduction in stunting most likely requires a health and nutrition package that comprehensively covers the critical ‘1000 days’, i.e. the period from conception to approximately the point when the child turns 2 years old (Piwoz et al. 2012).

Acknowledgements

We are grateful to the participants and people of Lungwena and Malindi, the staff at the Lungwena Training Health Centre and St. Martin’s Hospital, our research assistants for their positive attitude, support, and help in all stages of the study.

Source of funding

This study was supported by Academy of Finland (grants 200720, 108873, 111685 and 109796), Foundation for Pediatric Research in Finland, Medical Research Fund of Tampere University Hospital, and the American people through the support of the Office of Health, Infectious Disease, and Nutrition, Bureau for Global Health, United States Agency for International Development (USAID), under terms of Cooperative Agreement No. GHN-A-00-08-00001-00, through the FANTA-2 Project, and operated by the Academy for Educational Development (AED). The contents are the responsibility of the authors and do not necessarily reflect the views of the funders, USAID or the United States Government. The micronutrient mixture used in the production of LNS was provided free of charge by Nutriset Inc. (Malanay, France). John Phuka and Chrissie Thakwalakwa received personal stipends from Nestle Foundation. Yin Bun Cheung was supported by the Singapore Ministry of Health’s National Medical Research Council under its Clinician-Scientist Award.

© 2013 John Wiley & Sons Ltd Maternal and Child Nutrition (2013), 10, pp. **--**
Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

All authors participated in the design of the trial. PA, KM coordinated and supervised the research team. CM, CT coordinated the research team at local centres and were responsible for data collection. YBC, CM designed the details of statistical analysis and analysed the data. CM, KM, PA wrote the first draft of the manuscript. All authors commented on the analysis, reviewed and approved the final manuscript.

References


with fortified spread and the incidence of severe stunting among 6-to-18 month-old rural Malawians. *Archives of Pediatrics and Adolescent Medicine* 162, 619–626.


Providing lipid-based nutrient supplements does not affect developmental milestones among Malawian children

Charles Mangani (cmangani@medcol.mw)\textsuperscript{1,2}, Yin Bun Cheung\textsuperscript{3}, Kenneth Maleta\textsuperscript{1}, John Phuka\textsuperscript{1}, Chrissie Thakwalakwa\textsuperscript{1,2}, Kathryn Dewey\textsuperscript{4}, Mark Manary\textsuperscript{5}, Taneli Puumalainen\textsuperscript{6}, Per Ashorn\textsuperscript{2,7}

\textsuperscript{1}.College of Medicine, University of Malawi, Blantyre, Malawi
\textsuperscript{2}.School of Medicine, University of Tampere, Tampere, Finland
\textsuperscript{3}.Duke-NUS Graduate Medical School, National University of Singapore, Singapore City, Singapore
\textsuperscript{4}.University of California, Davis, CA, USA
\textsuperscript{5}.Washington University School of Medicine, St. Louis, MI, USA
\textsuperscript{6}.Ministry for Social Affairs and Health, Tampere, Finland
\textsuperscript{7}.Department of Paediatrics, Tampere University Hospital, Tampere, Finland

Keywords
Child development, Complementary feeding, Developmental milestones, Infants and young children, Lipid-based nutrient supplements

Correspondence
Charles Mangani, College of Medicine, University of Malawi, Mahatma Gandhi Road, Private Bag 360, Blantyre 3, Malawi. Tel: +265 1 871 911 | Fax: +265 1 874 700 | Email: cmangani@medcol.mw

Received
13 June 2013; revised 8 September 2013; accepted 27 September 2013.

DOI:10.1111/apa.12443

ABSTRACT

Aim: To assess whether using lipid-based nutrient supplements (LNS) to complement the diets of infants and young children affected when they achieved selected developmental milestones.

Methods: In rural Malawi, 840 6-month-old healthy infants were enrolled to a randomised trial. Control participants received no supplements, others were provided with milk-containing LNS, soy-containing LNS or corn–soy blend (CSB) for 12 months. Outcomes were the age at which they achieved key milestone: motor (walking with assistance, standing and walking alone, running), social (drinking from a cup and eating by themselves) and language (saying single comprehensible words and waving goodbye).

Results: The mean age at which the subjects walked with assistance was 42.5, 42.3, 42.7 and 43.2 weeks in the control, milk-LNS, soy-LNS and CSB groups, respectively ($p = 0.748$). There were also no significant differences in the mean age at standing alone (45.0, 44.9, 45.1 and 46.3 weeks), walking alone (54.6, 55.1, 55.3, 56.5 weeks), running (64.6, 63.7, 64.8, 65.9 weeks) or any other social or language milestones (each $p > 0.10$).

Conclusion: The findings do not support a hypothesis that providing tested formulations and doses of micronutrient-fortified LNS or CSB would have an impact on when young children in rural Malawi achieved selected developmental milestones.

INTRODUCTION

Development of cognitive, motor and socio-emotional skills during infancy affects later childhood development, academic achievement, adult productivity and earning potential (1–3). An estimated 200 million children under 5 years of age in developing countries fail to attain their developmental potential (2). The period from conception to the age of 2 years has been considered critical time for child development (4). Unfortunately, undernutrition during pregnancy and early childhood are also common (5). Some intervention studies that provided food supplementation to improve children’s nutritional status and development have demonstrated benefits in motor development, mental development, cognitive ability and adult economic productivity (6,7).

Recently, there has been a growing interest in the use of lipid-based nutrient supplements (LNS) to combat child

Key notes
- Research has shown that cognitive, motor and socio-emotional development during infancy affects subsequent child development and is associated with later academic achievement, adult productivity and earning potential.
- Few nutritional intervention studies have assessed the potential of lipid-based supplements (LNS) in promoting early child development.
- This study showed that children receiving a LNS or corn–soy blend supplement for a year achieved developmental milestones at similar ages to those not receiving supplements.
undernutrition (8). This interest was originally derived from the successful use of LNS in the management of children with severe acute malnutrition (9,10). Subsequent studies have suggested that some versions of LNS might also be applied to promote healthy growth and to prevent the development of various forms of undernutrition in childhood, at least in sub-Saharan Africa (11,12). So far, however, most studies have looked at anthropometric or micronutrient outcomes, whereas data on the impact of LNS supplementation on child development are still scarce (11,13).

We recently completed a pragmatic trial in which we assessed the growth promoting effect of a 1-year-long provision of LNS and corn-soy blend at six to 18 months of ages in rural Malawi as compared to just the ordinary diet (14). The results from that trial suggested that providing milk powder containing LNS but not soy flour containing LNS promotes linear growth among at-risk infants. However, the growth benefit seemed mostly confined to the time when the infants were between nine and 12 months of age, during which period LNS provision seemed to almost completely halt the process of linear growth faltering. Thereafter, the LNS effect was much less, and therefore, the impact on mean length gain or the incidence of severe stunting over the entire intervention period was smaller than expected. In the present study, we report the impact of the LNS intervention on the participants’ age of achievement of selected motor and social milestones. As the impact on growth was most evident during the latter part of infancy, we focused our analysis especially on milestones that are usually achieved at or soon after this age range.

**METHODS**

**Study design and location**

We carried out a randomised, four-arm controlled trial in Lungwena and Malindi, two rural Malawian communities with a total population of approximately 60 000 people. In earlier studies, 40–60% of all 6–18 month children in the study area have been identified as stunted, whereas wasting has typically been quite infrequent (15). Few infants in the area are exclusively breastfed for more than 1–2 months. Thereafter, infants typically receive thin maize-based porridge and often also other complementary foods in addition to breast milk (16).

**Eligibility criteria, enrolment and randomization**

The study participants were healthy infants who met the following inclusion criteria: age 5.5–6.5 months, residence in the study area, informed consent from at least one authorised guardian. The exclusion criteria were weight-for-length (WFL) <80% of the World Health Organization (WHO) reference median, presence of oedema, severe illness warranting hospitalisation on the enrolment day, history of peanut allergy, concurrent participation in another clinical trial and any symptoms of food intolerance within 30 min of ingesting a 5-g test dose of LNS (either milk or soy-based) used in the trial.

To identify potentially eligible participants, we conducted community surveys in the study area. During recruitment, trained fieldworkers contacted all the families of children of the approximate right age whose parents showed a preliminary interest in the trial. Infants were invited to an enrolment session, where they were screened for eligibility, and guardians were given detailed information on the trial contents. Before enrolment, at least one guardian signed a written consent for trial participation.

We used blocked randomization, with each block containing 16 allocations for the four groups at 1:1:1:1 ratio. A set of identical-appearing opaque envelopes from one randomization block was shuffled, and the infant’s guardian was requested to choose one envelope. Each envelope contained the allocation information for one participant. The randomization list and envelopes were put together by an individual not involved in trial implementation. Randomisation of participants into the trial groups was carried out by a research assistant, and fieldworkers distributed the study food supplements. The development assessment was carried out by the same fieldworkers who distributed the study supplements, that is, they were not masked to the group allocation. The code was not disclosed to the researchers until all data had been entered and verified in a database.

The sample size estimation for the study was based on the prevalence of severe stunting, the primary outcome of the trial. It was based on the assumption of a prevalence of severe stunting of 15% in the control group and 5% in the intervention groups, a type I error of 5% and power of 85%. Allowing for an attrition rate of 10%, the targeted sample size per group was 210.

**Interventions and follow-up**

Two separate LNS products were tested in this trial, one that contained dried skimmed milk and thus resembled products used in earlier prevention trials with LNS (11,12) and another one in which the milk powder was substituted with soy flour. The latter choice was motivated by the use of soy in nationally recommended complementary foods (17), the fact that it is less expensive and its acceptability to vegetarians. We also designed the study with two control groups: one of these included children who received no intervention during the period of interest while participants in the other control group were given rations of micronutrient-fortified corn–soy blend (CSB), a nationally recommended complementary food for infants and young children (17). Thus, the trial participants were randomly assigned to receive for 12 months, from the age of 6 until 18 months, supplemental complementary food with either 71 g/day of micronutrient-fortified CSB, 54 g/day of micronutrient-fortified LNS with milk protein base (Milk-LNS), or 54 g/day of micronutrient-fortified LNS with soy protein base (Soy-LNS), or to a control group that did not receive any supplemental complementary food in the initial 12-month period but received a delayed supplementation from the age of 18–36 months. The daily CSB and LNS dose provided approximately 280 kcal.
The study examined the age of achievement of the following three gross motor developmental milestones, which corresponded to those assessed in the WHO Multicentre Growth Reference Study (MGRS) (18): walking with assistance, standing alone and walking alone. In addition, the study examined one extra gross motor milestone (running), two early social milestones (drinking from a cup and eating by themselves) and two early language milestones (saying single comprehensible words and waving goodbye) selected from the Denver Development Screening Test II (19). These milestones have previously been validated in rural Malawi among 6–18-month-old infants and showed variation in this age range (20).

Trained fieldworkers assessed milestone achievement at scheduled home and clinic visits every 2 weeks from 6 months up to the age of 18 months. A milestone was recorded achieved if either a guardian reported or a fieldworker observed the child successfully performing the activity of interest. The child's performance was recorded as tried, but failed to perform the milestone, able to perform the milestone or ‘not known’ in cases where performance could not be assessed, such as an uncooperative child. To minimise observational errors among fieldworkers, there were standardised criteria for assessing whether each developmental skill was demonstrated and the study team had monthly standardisation sessions between the senior research team (CM, CT) and the fieldworkers.

All the milestones were assessed at each participant visit with no assumed progression or any hierarchy in their achievement. Each examination was carried out independently of all previous assessments. To minimise the possibility of fieldworkers knowing the family and participant and hence being able to remember some or all of the previous results from past assessments, the allocation of fieldworkers to the participants’ home visits was rotated weekly.

**Measurement of covariates**

Weight was measured from unclothed infants using an electronic infant weighing scale (SECA 735; Chasmos Ltd, London, UK) and was recorded to the nearest 10 g. Length was measured to the nearest one mm using a high-quality length board (Kiddimetre; Raven Equipment Ltd, Essex, UK). Anthropometric indexes length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) were calculated using WHO Child Growth Standards (21).

**Data management and statistical analysis**

All collected data were recorded on paper forms, transcribed to paper case report forms and double entered into a tailor made database (Microsoft Access 2003; Microsoft Corp, Redmond, Washington, DC, USA). The two entries were electronically compared, and extreme or otherwise suspicious values were confirmed or corrected. All statistical analyses were performed using Stata 11.0 (Stata Corp, College Station, TX, USA). Parametric time-to-event
analysis (22) for interval-censored data was performed using a Stata program developed by Griffins et al. (23).

A developmental milestone was defined as achieved when a participant was recorded to be able to perform the defined task on two consecutive visits. The measurement of age at attainment of each developmental milestone was censored in one of the following ways: (i) left censoring if the child had experienced the milestone by the first examination date, (ii) interval censoring if the child had experienced the milestone between two examination dates and (iii) right censoring if the child had not experienced the milestone by the end final visit during the intervention period.

We used parametric interval-censored time-to-event analyses to examine the associations of intervention with age at attainment of developmental milestone (22,23). As parametric time-to-event analysis is based on time on the log-scale, the parameters estimated are geometric mean ages and differences in geometric mean age. Geometric mean has the advantage of being robust to outliers and positive skewness. We report the differences in geometric mean age (95% CI) in weeks in the three intervention groups compared with the control group. Medians were calculated by plugging the estimated parameters into the cumulative distribution function (22). For graphical plots, we used the estimated parameters to establish the percentiles in the cumulative distribution. Using likelihood ratio test, with data on each milestone as a stratum, we tested the global null hypothesis of all four groups being the same for all eight milestones, and also for the domains of motor, social and language. We also assessed the proportion of children with developmental delay on the three gross motor milestones corresponding to those assessed in the WHO MGRS (18). The WHO MGRS uses the values corresponding to the 1st and the 99th percentile as ‘normative’ for attainment of these developmental milestones. We considered a child to have a delay if the mean of the ages at consecutive visits before and after observing the milestones was later than the WHO MGRS 99th percentile. This approximate method was used because parametric models for interval-censored data are oriented towards testing difference in geometric means instead of difference in proportions.

We also assessed the validity of our assessment of age at achievement of each milestone using an interval-censored time-to-event regression model with LAZ at enrolment, which is known to be positively associated with developmental outcomes, as an explanatory variable. Fisher’s exact test (r-by-c extension) was used to compare categorical variables between the four intervention groups.

The participant’s adherence to the trial in terms of compliance to scheduled visits, food sharing and food leftovers at each 2-week visit was assessed using generalised estimating equation and has been described in detail elsewhere (14) including assessment of stunting, and comparison of continuous and categorical variables.

**RESULTS**

Between January 28, 2008 and May 25, 2009, a total of 1385 screened infants were identified for possible inclusion in the study and 840 of them were enrolled. The full trial profile, group allocation and reasons for exclusions are indicated in Figure 1. At baseline, the four study groups did not differ from each other in demographic, anthropometric or socio-economic characteristics (Table 2) except for a slightly lower proportion of boys and a lower mean initial weight in the CSB sample. At enrolment, the prevalence of stunting was 56.7%.

A total of 25 children (3.0%) died, 11 developed severe wasting and 68 (8.1%) dropped out during the study. The proportion of participants who died, developed severe wasting and dropped out was similar in the different study groups (Fig. 1, p = 0.536, p = 0.797 and p = 0.988, respectively). The completion rate for all the study visits was also balanced between the groups (p = 0.1940).

According to the guardians, the participants consumed the study supplements regularly and tolerated them well. Leftovers were found only at 1.3%, 1.3% and 0.6% of the home visits in the Milk-LNS, Soy-LNS and CSB groups, respectively (p < 0.001).

The distribution of the ages of achievement for the nine studied developmental milestones is illustrated in Figure 2. The geometric mean (SD) age at achievement was 42.7 (9.5) weeks for walking with assistance, 45.4 (10.9) weeks for standing alone and 55.4 (11.9) weeks for walking alone. The median value and the sample distribution for standing were similar to that observed in the WHO multicentre study (difference -0.9 weeks); for supported walking or walking alone, the study sample achieved the milestone slightly later than the reference population (difference in medians 4.1 weeks for both milestones, Fig. 3).

Table 3 shows the mean age of achievement for the four motor, two social and two language milestones by study group. As shown, there were practically no differences...
between the groups, for any of the individual milestones. There were also no statistically significant differences between the study groups in analyses that combined all the eight milestones \((p = 0.362)\) or combined all milestones in the domains of motor \((p = 0.272)\), social \((p = 0.462)\) or language \((p = 0.283)\).

The percentages of children with delay in walking with assistance, standing alone and walking alone were 5.9%, 2.5% and 6.6%, respectively. The proportion of individuals with a delay in milestone achievement was similar between the groups (Table 4).

As a validity check, we examined the association between the length-for-age z score at enrolment and the age at achievement of each milestone. For all but one milestone (saying single comprehensible words), the two variables were positively and statistically significantly associated with each other (Table 5).

**DISCUSSION**

We conducted this community-based trial to assess the effects of a 12-month-long dietary supplementation with

---

**Table 2** Baseline characteristics of participants at enrolment, by intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Milk-LNS</th>
<th>Soy-LNS</th>
<th>CSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex, No./total No. (%)</td>
<td>111/209 (53.1)</td>
<td>107/212 (50.5)</td>
<td>103/210 (49.1)</td>
<td>98/209 (46.9)</td>
</tr>
<tr>
<td>Mean (SD) age, months</td>
<td>6.02 (0.23)</td>
<td>6.02 (0.25)</td>
<td>6.04 (0.25)</td>
<td>6.03 (0.24)</td>
</tr>
<tr>
<td>Mean (SD) weight, kg</td>
<td>7.02 (0.89)</td>
<td>7.09 (0.97)</td>
<td>7.00 (0.88)</td>
<td>6.95 (1.01)</td>
</tr>
<tr>
<td>Mean (SD) length, cm</td>
<td>63.2 (2.2)</td>
<td>63.2 (2.4)</td>
<td>63.0 (2.4)</td>
<td>62.9 (2.3)</td>
</tr>
<tr>
<td>Mean (SD) weight-for-age z score</td>
<td>-0.80 (1.06)</td>
<td>-0.70 (1.10)</td>
<td>-0.80 (1.12)</td>
<td>-0.85 (1.21)</td>
</tr>
<tr>
<td>Mean (SD) length-for-age z score</td>
<td>-1.64 (0.97)</td>
<td>-1.59 (1.05)</td>
<td>-1.68 (1.11)</td>
<td>-1.72 (0.97)</td>
</tr>
<tr>
<td>Mean (SD) weight-for-length z score</td>
<td>0.41 (1.05)</td>
<td>0.50 (1.05)</td>
<td>0.46 (1.00)</td>
<td>0.42 (1.11)</td>
</tr>
<tr>
<td>Proportion stunted, No./total No. (%)</td>
<td>71/209 (34.0)</td>
<td>72/212 (34.0)</td>
<td>82/212 (39.1)</td>
<td>83/209 (39.7)</td>
</tr>
<tr>
<td>Mean (SD) children below 5 years of age in the household</td>
<td>1.6 (0.7)</td>
<td>1.6 (0.8)</td>
<td>1.6 (0.7)</td>
<td>1.7 (0.9)</td>
</tr>
<tr>
<td>Mean (SD) maternal education, years</td>
<td>3.6 (3.4)</td>
<td>4.0 (3.7)</td>
<td>3.0 (3.1)</td>
<td>3.7 (3.1)</td>
</tr>
<tr>
<td>Mean (SD) paternal education, years</td>
<td>3.7 (3.6)</td>
<td>4.3 (4.1)</td>
<td>3.7 (3.9)</td>
<td>4.7 (4.6)</td>
</tr>
<tr>
<td>Mean (SD) Socio-economic Index z score</td>
<td>-0.02 (1.00)</td>
<td>0.02 (1.03)</td>
<td>0.03 (1.08)</td>
<td>-0.04 (0.89)</td>
</tr>
</tbody>
</table>

CSB = Corn–soy blend; Milk-LNS = Milk lipid-based supplements; Soy-LNS = soy-containing lipid-based supplements.
LNS on the time to achievement of motor, language and social developmental milestones of rural Malawian infants and young children. Primary endpoints were three of the milestones in the WHO MGRS, and one extra gross motor milestone (running), two early social milestones, and two early language milestones from selected questions from the Denver Development Screening Test II. These milestones were selected because they are familiar to families everywhere, simple to test and evaluate, and have been previously validated in Malawi (20). For each of these milestones, we found practically no differences in the mean age of achievement or proportion with developmental delay.
### Table 3  Geometric mean of age (SD) of achievement of motor, social and language development milestones, by intervention group

<table>
<thead>
<tr>
<th>Developmental Milestone</th>
<th>All groups</th>
<th>Control</th>
<th>Milk-LNS</th>
<th>Soy-LNS</th>
<th>CSB</th>
<th>p-value*</th>
<th>Control vs. milk-LNS</th>
<th>Difference in geometric means (95% CI)</th>
<th>Control vs. soy-LNS</th>
<th>Difference in geometric means (95% CI)</th>
<th>Control vs. CSB</th>
<th>Difference in geometric means (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking with assistance</td>
<td>42.7 (9.5)</td>
<td>42.5 (9.4)</td>
<td>42.3 (9.4)</td>
<td>42.7 (9.5)</td>
<td>43.2 (9.6)</td>
<td>0.7480</td>
<td>0.2 (-1.5, 1.8)</td>
<td></td>
<td>-0.3 (-1.9, 1.4)</td>
<td>-0.7 (-2.3, 1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing alone</td>
<td>45.4 (10.9)</td>
<td>45.0 (10.9)</td>
<td>44.9 (10.8)</td>
<td>45.1 (10.9)</td>
<td>46.3 (11.2)</td>
<td>0.4089</td>
<td>0.3 (-1.8, 1.8)</td>
<td></td>
<td>-0.3 (-2.0, 1.7)</td>
<td>-1.4 (-3.2, 0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking alone</td>
<td>55.4 (11.9)</td>
<td>54.6 (11.8)</td>
<td>55.1 (11.9)</td>
<td>55.3 (12.0)</td>
<td>55.6 (12.2)</td>
<td>0.3041</td>
<td>-0.5 (-2.5, 1.5)</td>
<td></td>
<td>0.7 (-2.7, 1.3)</td>
<td>-1.9 (-3.9, 0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>64.6 (12.0)</td>
<td>64.6 (11.8)</td>
<td>63.7 (11.7)</td>
<td>64.8 (11.9)</td>
<td>65.9 (12.1)</td>
<td>0.2339</td>
<td>0.9 (-1.2, 2.9)</td>
<td></td>
<td></td>
<td>-0.2 (-2.3, 1.8)</td>
<td></td>
<td>-1.3 (-3.4, 0.5)</td>
</tr>
<tr>
<td>Social</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating by themselves</td>
<td>43.6 (8.3)</td>
<td>43.6 (8.3)</td>
<td>43.0 (8.2)</td>
<td>44.1 (8.4)</td>
<td>43.5 (8.3)</td>
<td>0.5493</td>
<td>0.7 (-0.8, 2.1)</td>
<td></td>
<td>-0.4 (-1.9, 1.1)</td>
<td>0.2 (-1.3, 1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking from a cup</td>
<td>50.6 (7.9)</td>
<td>50.9 (8.0)</td>
<td>50.0 (7.8)</td>
<td>51.0 (8.0)</td>
<td>50.5 (7.9)</td>
<td>0.5812</td>
<td>0.8 (-0.7, 2.4)</td>
<td></td>
<td>-0.1 (-1.7, 1.4)</td>
<td>0.4 (-1.2, 1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave goodbye</td>
<td>45.2 (9.9)</td>
<td>43.9 (9.6)</td>
<td>45.2 (9.9)</td>
<td>45.5 (9.9)</td>
<td>46.0 (10.0)</td>
<td>0.1082</td>
<td>-1.3 (-3.1, 0.4)</td>
<td></td>
<td>-1.6 (-3.4, 0.1)</td>
<td>-2.1 (-3.9, -0.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saying single words</td>
<td>53.8 (11.4)</td>
<td>53.4 (11.3)</td>
<td>53.5 (11.4)</td>
<td>54.3 (11.5)</td>
<td>53.8 (11.4)</td>
<td>0.8854</td>
<td>-0.1 (-2.3, 2.2)</td>
<td></td>
<td>-0.8 (-3.1, 1.5)</td>
<td>-0.4 (-2.7, 1.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSB = Corn-soy blend; Milk-LNS = Milk lipid-based nutrient supplements; soy-LNS = soy lipid-based nutrient supplements.

*Likelihood ratio test.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.

The main possible causes of bias in our study design were the insufficient blinding of the fieldworkers to the group allocation and the lack of information on the actual use of the supplements. These weaknesses were due to the logistic difficulties of assigning two separate teams of assistants to deliver the supplements and to assess milestone achievement at the participants' homes, the fact that the diet composition of LNS or CSB would influence the age of achievement of the tested milestones in rural Malawi.
the Ghanaian results suggest that in selected settings LNS provision may promote some aspects of motor development in infancy. However, caution is needed also when interpreting the Ghanaian results as there were no comparable differences in the proportions of infants who could walk or stand with assistance or stand independently (11). Theoretically, this could be explained by a failure of a cross-sectional assessment to capture differences that would have been evident at an earlier age. As assessment of development is notoriously difficult especially among infants and young children (26), it would be beneficial in future trials to combine many different approaches and methods to better clarify the impact of LNS supplementation on healthy growth and development and to conduct follow-up assessments at older ages (after the intervention has ceased) when it is possible to use more sensitive tools to assess various aspects of development.

One methodological issue warrants some further discussion in the present study. Using interval-censored time-to-event regression, we developed windows of milestone achievement that allowed descriptive comparison with the WHO MGRS for the three gross motor milestones of walking with assistance, standing alone and walking alone. To our knowledge, this is the first study that has reported this despite interval-censored regression having been used before in child development studies about associations. Displacement of our study population’s windows of achievement to the right relative to the WHO MGRS reflects developmental delay, its extent reflecting degree of retardation (18). It is unclear whether the ~4-week delay in walking with assistance and walking alone, and about 1-week advancement in standing alone, has any long-term clinical significance. The validity of our method for developmental assessment was supported both by the similarity of motor milestone achievement distribution between our sample and that in the WHO study.
and other previous observational studies (18,27,28) and by the observed associations between age of milestone achievement and participant length for age, a known predictor of development (2).

Interval censoring is a common problem in developmental assessment, but it is not often discussed. When the time interval is small, some random assignment of a value within the interval or mid-point assignment can give reasonable approximation to the true values. For example, the WHO MGRS (18) randomly assigned a value within a 1-month interval for plotting the windows of developmental milestone achievement. For studies with less frequent planned assessment or no-show in visits, the interval becomes larger, and the simple methods may not be justifiable. The robustness and informativeness of interval-censored time-to-event regression are well known among statisticians (29) and have been applied to studies of pubertal development (30). It deserves wider usage in studies of child development. Nevertheless, the regression method has its limitation, so does our study. The regression parameters concern mean log (time-to-event) and their differences between groups, which equivalently concerns the geometric mean of time-to-event and ratio of geometric means across groups. While these parameters are useful, they do not satisfy all the information needs of the users.

ACKNOWLEDGEMENT

We are grateful to the participants, parents and staff who participated in the study, to Laszlo Csonka for designing the collection tools and data entry programs and Tina Ying Xu (Center for Quantitative Medicine, Duke-NUS Graduate Medical School) for her input in part of data analysis. The research project was supported by Academy of Finland (Grants 200720,108873,111685 and 109796), Foundation for Pediatric Research in Finland, Medical Research Fund of Tampere University Hospital and the American people although the support of Office of Health, Infectious Disease and Nutrition, Bureau for Global Health, United States Agency for International Development (USAID), under terms of Cooperative Agreement No. GHN-A-00-08-00001-00, through the FANTA-2 Project and operated by the Academy for Educational Development (AED). John Phuka and Chrissie Thakwalakwa received personal stipends from Nestle Foundation. Yin Bun Cheung was supported by the Singapore Ministry of Health’s National Medical Research Council under its Clinician-Scientist Award.

References


Lipid-Based Nutrient Supplements Do Not Affect the Risk of Malaria or Respiratory Morbidity in 6- to 18-Month-Old Malawian Children in a Randomized Controlled Trial1–3

Charles Manganj,4,5* Per Ashorn,5,6 Kenneth Maleta,4 John Phuka,4 Chrissie Thakwalakwa,4,5 Kathryn Dewey,7 Mark Manary,6 Taneli Puurmalainen,9 and Yin Bun Cheung10

4College of Medicine, University of Malawi, Blantyre, Malawi; 5University of Tampere School of Medicine, Tampere, Finland; 6Department of Paediatrics, Tampere University Hospital, Tampere, Finland; 7University of California, Davis, Davis, CA; 8Washington University School of Medicine, St. Louis, MO; 9Ministry for Social Affairs and Health, Helsinki, Finland; and 10Duke–National University of Singapore Graduate Medical School, National University of Singapore, Singapore

Abstract

Background: There is evidence to support the use of lipid-based nutrient supplements (LNSs) to promote child growth and development in low-income countries, but there is also a concern regarding the safety of using iron-fortified products in malaria-endemic areas.

Objective: The objective of this study was to test the hypothesis that 6- to 18-mo-old rural Malawian children receiving iron-containing (6 mg/d) LNSs would not have excess morbidity compared with infants receiving no supplementation.

Methods: A randomized controlled trial allocated 840 children to receive daily supplementation with 54 g/d LNS with milk protein base (milk-LNS), 54 g/d LNS with soy protein base (soy-LNS), 71 g/d corn-soy blend (CSB), or no supplementation from 6 to 18 mo of age. Morbidity was compared using a non-inferiority margin set at 20% excess morbidity in supplemented groups compared with the nonsupplemented group.

Results: Baseline characteristics were similar across groups. The proportion of days with febrile illness between 6 and 18 mo was 4.9%, and there were no differences between the groups: 4.9% (95% CI: 4.3, 5.5%), 4.5% (95% CI: 3.9, 5.1%), 4.7% (95% CI: 4.1, 5.3%), and 5.5% (95% CI: 4.7–6.3%) in the milk-LNS, soy-LNS, CSB, and control groups, respectively.

Conclusions: Daily supplementation with 54 g of milk-based or soy protein–based LNS or 71 g of CSB did not result in increases in malaria or respiratory morbidity in children in a malaria-endemic setting. However, we could not conclude whether LNSs did or did not increase diarreal morbidity. This trial was registered at clinicaltrials.gov as NCT00524446. J. Nutr. 144: 1835–1842, 2014.
foods in the complementary diet, therefore providing the opportunity for dietary diversification (8).

There is ongoing concern regarding the use of iron-fortified supplements in malaria-endemic areas. Following the report by Sazawal et al. (14) on excess morbidity and mortality associated with iron supplementation in Tanzania, the risk of excess morbidity and mortality related to iron supplementation or iron-containing “home fortificants,” such as micronutrient powder (MNP), has been assessed in several studies (15). Previous studies on the use of home fortificants did not show adverse effects on morbidity (16), with 1 trial reporting a reduction in the proportion of children with diarrhea and incidence of fever (17). However, a recent large study in Pakistan reported excess diarrhea and respiratory illness (18) among children receiving iron-containing multiple MNPs for 12 mo. Another recent trial in Ghana found no increased risk of malaria, but it showed a significant increase in hospital admissions among children supplemented with iron-containing MNP (19). Four studies examined the effect of preventive LNS supplementation on morbidity; 1 found a reduction in fever and diarrheal illness (20), whereas the other 3 studies showed neither evidence of difference nor evidence of non-inferiority (21–23). However, the studies showed great variability especially with regard to the age of participants (from 6 to 60 mo), dose and duration of supplementation, and morbidity assessment (frequency of assessment, number of follow-up days, and morbidity assessed), making generalization of the findings difficult. Furthermore, only 1 of the studies assessed clinical malaria (22).

We aimed to determine the effect of provision of 54 g/d milk-based or soy protein–based LNSs, a quantity larger than used in several recent large-scale LNS trials (24), on the incidence of malaria and other common childhood illnesses among children residing in a high malaria-burden area. Malaria is endemic throughout Malawi, with an estimated 6 million cases occurring annually (25). Approximately 49% of children aged <5 y are estimated as residing in high-transmission intensity areas (26).

We reported previously that daily supplementation of a complementary local diet for 1 y with milk powder-containing LNSs could reduce linear growth faltering among at-risk children aged 6-18 mo compared with corn-soy blend (CSB) or the local complementary diet (27). In the current article, we report on the effect of daily consumption of LNSs on the secondary outcomes of morbidity in infants and young children using an non-inferiority analysis. In view of the recent accumulating evidence that raises concern about the safety of iron administration in highly malaria-endemic areas (18,19), the objective of the analysis was to evaluate the safety of providing an iron-containing LNS. We hypothesize that supplementation of the local complementary diet with fortified milk-based or soy protein–based LNSs would not result in excess morbidity.

Methods

Study setting. We performed the study in 2 rural health facility catchment areas, Lungwena and Malindi in Mangochi district, southern Malawi, from 28 January 2008 to 25 May 2009. Malindi is ~17 km from Mangochi town and had on average a more educated population and better access to electricity, clean water, and sanitation than Lungwena, a more rural site ~32 km from the town. Both sites have functional health facilities and are broadly representative of rural Malawi. All of Mangochi district had a high prevalence of infant stunting, underweight, and poor food security (28). Almost all children are breast-fed up to age 2 y; however, the duration of exclusive breastfeeding is generally no more than 1–2 mo. The principal complementary food is a thin maize-based porridge (29). The area has a holo-endemic malaria transmission pattern that peaks during the rainy season (November to March). An estimated 86% of the population in the district is under high-transmission intensity (26).

Study design and intervention. The study was designed as a community-based randomized trial comparing 3 nutritional supplementation groups and 1 control group. The protocol was described in detail previously (27). Briefly, 840 children aged 5.50–6.50 mo were randomly assigned into 4 treatment groups: 1) control; 2) micronutrient-fortified LNSs with milk protein base (milk-LNSs); 3) micronutrient-fortified LNSs with soy protein base (soy-LNSs); and 4) micronutrient-fortified CSB. Blocked randomization was used with each block containing 16 allocations evenly distributed for the 4 groups. The group allocation was masked to the investigators. However, fieldworkers and guardians knew whether their child was receiving supplementation with LNSs or CSB or was in the control group.

Children were supplemented with either 54 g/d milk-LNS, or 54 g/d soy-LNS or 71 g/d CSB, or were allocated to a control group that were not administered any supplemental complementary food in the initial 12-mo period but were given supplementation from ages 18–36 mo. Guardians were advised to offer the children daily either 10 spoonfuls of CSB, cooked into a complementary porridge, or 8 spoonfuls of milk-LNS or soy-LNS, divided into 2–4 daily servings mixed with a small amount of porridge. The daily LNS dose provided ~280 kcal and 6 mg of both iron and zinc (Supplemental Table 1). The daily CSB dose provided similar energy but lower iron (5.46 mg) and zinc (3.6 mg). Participants were followed every 2 wk between 6 and 18 mo and were provided with a 14-d supply of supplemental food. Child diet, feeding practices, and LNS use were also assessed at these visits.

The LNSs were produced locally by Project Peanut Butter from peanut paste, milk powder or soy flour, soybean oil (contributed ~57% of fat in the products), sugar, and a multiple micronutrient mixture (Nutriset).

Outcome measurements. Morbidity was assessed by trained fieldworkers every 2 wk using standardized data-collection instruments and guidelines. Throughout the study, the guardians were asked to record on a daily basis the presence or absence of illness symptoms in picture calendars that were provided every 2 weeks. The calendar had separate rows for different days up to 2 weeks and separate columns for the following symptoms: 1) fever; 2) cough; 3) diarrhea (≥3 stools/d); and 4) other. The first 3 symptom columns had pictures describing them to assist the guardians in identifying the correct area to record the information. The guardian reports on the calendars did not include the recording of temperature for fever or the number of stools for diarrhea. The recorded information was reviewed and cross-checked by fieldworkers at each 2 weekly food-delivery visit for completeness.

Participants who visited the Lungwena and Malindi health centers, the only formal health facilities in the study area, were assessed and treated according to the Integrated Management of Childhood Illness guidelines (30) by separate teams of clinicians and nurses unrelated to the study and unaware of which intervention was allocated to which participants. Data were retrieved from these health facilities. Diagnoses were coded and recorded into 6 major categories: 1) clinical malaria; 2) clinical pneumonia; 3) diarrhea; 4) trauma; 5) other respiratory illness; and 6) other illnesses.

Weight and length measurements were obtained at 12-wk intervals from enrollment up to 18 mo of age. The weight of unclothed infants was measured to the nearest 10 g using an electronic infant weighing scale (SECA 735; Chasmors). Length was measured to the nearest 1 mm using a high-quality length board (Kiddimetre; Raven Equipment). Anthropometric indexes, including length-for-age, weight-for-age, and weight-for-length, were calculated using WHO Child Growth Standards (STATA igrowup package) (31).

Diarrhea was defined as the passage of ≥3 loose or watery stools in a 24-h period. An acute respiratory illness (ARI) episode was defined as a minimum of 2 d with cough and fever. Febrile illness was defined as unusually high body temperature observed by the mother, in the absence of diarrhea or ARI. Recovery from an illness episode was considered if the child was free of symptoms for ≥3 consecutive days according to
definitions used in similar studies (32,33). Rapid breathing was defined as an elevated respiratory rate above the age-specific cutoff values of 50 breaths/min in infants and 40 breaths/min in older children. Clinical pneumonia was defined as a combination of cough with either rapid breathing or crepitations or bronchial breathing by auscultations or lower chest indrawing. Suspected severe pneumonia was defined as pneumonia associated with ≥1 of the following features: 1) convulsions; 2) extreme lethargy; 3) inability to drink or feed; 4) restlessness or irritability; or 5) abnormal sleeping as per the WHO Integrated Management of Childhood Illness guidelines (30). Clinical malaria was defined as a child with fever (axillary temperature > 37.5°C or reported a fever within the past 48 h) and a confirmed laboratory diagnosis of malaria parasitemia (at any parasite density).

Blood samples were obtained at baseline from all children and every 12 wk during the follow-up period. Malaria status, including parasite specification and count, was determined via microscopy. Thin films were fixed with methanol, and both thick and thin films were stained with Giemsa. Each smear was read twice by independent microscopists, and discordant results were re-read by a third microscopist. Blood hemoglobin concentration was measured from a venous sample using cuvettes and a reader (HemoCue). Malaria treatment was provided according to the national guidelines to all participants with clinical malaria. All participants found to have a blood hemoglobin concentration <80 g/dL were treated with iron supplementation in accordance with the national treatment guidelines (1–6 mg/kg body weight/d for 1 month).

Statistical analysis. Sample size calculation was based on the primary study outcome: the prevalence of severe stunting. Assuming a prevalence of severe stunting of 15% in the control group and 5% in the intervention groups and allowing for an attrition rate of 10%, the targeted sample size per group was estimated at 210 participants to achieve 85% power and control type I error rate at 5%. For this analysis, a non-inferiority margin (Δ) predefined in the statistical analysis plan of no more than 20% increase in geometric mean of longitudinal prevalence, incidence rates in guardian-reported or clinical morbidity, or proportion with malaria parasitemia in the intervention groups vs. the control group was used. We assumed that an increase in morbidity of ≥20% in the LNS groups relative to the control group would be clinically substantial with negative consequences to overall health and well-being of the children. There is no commonly agreed definition of non-inferiority in this context. Our definition is based on another non-inferiority safety trial that assessed the effects of supplementation of children aged 12–24 mo with an iron-containing MNP on infectious morbidities (34). Non-inferiority was established if the 2-sided 95% CIs of the geometric mean ratio (GMR), incidence rate ratio (IRR), or RR for an intervention group compared with the control group fell entirely below 1.2.

We analyzed data on an intention-to-treat basis, using Stata (version 11.2, StataCorp). Proportions, means, and SDs were presented for the baseline variables and compared between groups by using ANOVA for continuous variables and a χ² test for proportions. Longitudinal prevalence of an illness was defined as the percentage of all days of observation that the child suffered the illness (35). GMRs were calculated to compare the longitudinal prevalence of illness in the 3 intervention groups with the control group. For analyses of disease incidence, we used negative binomial regression modeling to obtain IRRs (36). The RR for prevalence of malaria parasitemia was estimated. Interactions between intervention and the child’s sex, study site, baseline length-for-age, and baseline hemoglobin were explored but not shown because of nonsignificant findings. The difference in morbidity between the 2 catchment areas was also explored through stratified analyses and calculation of rate ratios. No differences in morbidity were found between the sites (details not shown). Changes in anemia prevalence within intervention groups from 6 to 18 mo were analyzed by using McNemar’s test. We assessed the proportion of children having used a bed net in the previous 24 h and participant compliance to intervention (in terms of scheduled visit attendance, food sharing, and observed leftovers) at each 2-week visit. Bed net usage and compliance to intervention were analyzed using the generalized linear model with Huber-White robust SE to allow for correlated data (multiple visits per child) (37). Statistical tests were considered significant if P < 0.05.

All collected data were recorded on paper forms, transcribed to paper case report forms, and double entered into a custom-made database (Microsoft Access 2003; Microsoft). The 2 entries were electronically compared, and extreme or otherwise suspicious values were confirmed or corrected. The study was approved by the University of Malawi College of Medicine Research and Ethics Committee and the ethics committee of Pirkanmaa Hospital District (Finland). During the enrollment session, guardians were given detailed information on the trial contents and a consent form in the local language. A signed consent form was required for inclusion of a subject in the study. This trial was registered at clinicaltrials.gov as NCT00524446.

Results
A total of 1385 infants were screened, and 840 of them were enrolled. The recruitment, group allocation, reasons for exclusions, and dropouts are shown in Figure 1. The demographic, anthropometric, and environmental characteristics of the study population were similar between the groups at baseline (Table 1). Almost all the children were still breast-fed, 36.7% were stunted, 14.1% were underweight, and 1.7% were wasted. The prevalence of anemia and malaria parasitemia at baseline was similar between the groups. There was limited variability in the sample in drinking water source or toilet availability and type.

A total of 25 children (3.0%) died, and 68 (8.1%) dropped out during the study. The proportion of participants who died and dropped out was similar in the different study groups (P = 0.54 and P = 0.99, respectively) (Fig. 1). The completion rate for all the study visits was also balanced between the groups (P = 0.19).

Throughout the intervention period, reported adherence to the use of supplementary products was good in all the supplementation groups. During the 2 wk home visits, leftovers were found in only 1.3, 1.3, and 0.7% of the visits in the milk-LNS, soy-LNS, and CSB groups, respectively (P = 0.01). Diversion of any portion to someone other than the intended beneficiary was reported at only 69 of 18,906 (0.36%) supplement-delivery interviews: 29 in the milk-LNS, 19 in the soy-LNS, and 21 in the CSB groups (P = 0.52). The reported bed-net usage by participant in the previous night was 80.8, 78.5, 79.1, and 78.8% in the milk-LNS, soy-LNS, CSB, and control groups, respectively, across all the home visits (P = 0.79).

Longitudinal prevalence of febrile illness, cough, ARI, and diarrhea, or any reported morbidity was 5.5, 6.1, 1.6, 4.5, and 14.5%, respectively, in the control group and was similar to the 3 intervention groups (Table 2). Relative to the control group, the GMR (95% CI) for the longitudinal prevalence of febrile illness was 0.91 (0.73, 1.09) in the milk-LNS, 0.90 (0.72, 1.08) in the soy-LNS, and 0.91 (0.73, 1.09) in the CSB groups. The GMRs for longitudinal prevalence of cough, ARI, or any reported morbidity were also similar for all 3 intervention groups (Table 2); all 95% CIs confirmed non-inferiority. For diarrhea, the GMR (95% CI) was 1.06 (0.87, 1.25) in the milk-LNS, 0.99 (0.81, 1.17) in the soy-LNS, and 1.05 (0.86, 1.24) in the CSB groups. Because the 95% CIs for the milk-LNS and CSB groups crossed the non-inferiority margin of 1.2, non-inferiority could not be established for diarrhea.

Table 3 shows incidence and rate ratios for guardian-reported febrile illness, cough, ARI, and diarrheal episodes for the 3 treatment groups. During the 12-mo intervention period, overall incidence of reported febrile episodes was 5.6/child-year. Consistent with the findings on longitudinal prevalence, all except 1 of the 95% CIs of IRRs fell entirely below the non-inferiority margin. The exception was, again, for diarrhea in the
milk-LNS group, for which IRR was 1.12 (95% CI: 0.95, 1.32), crossing the non-inferiority margin of 1.2.

A total of 2706 nonscheduled visits were made by study participants to health facilities in the study area for medical consultation and treatment. Table 4 summarizes data for clinical malaria, respiratory problems, and diarrhea recorded at the clinic visits for the 4 groups. A total of 418 clinical malaria episodes were recorded during clinic visits, for an overall incidence of 0.54 episodes/child-year. All 3 intervention groups had lower incidence rate (IRR ranging from 0.77 to 0.80) than the control group; all 3 95% CIs fell entirely below the non-inferiority margin of <1.2. Similarly, non-inferiority of the 3 interventions in terms of rapid breathing was also established. The CSB group (IRR: 0.87; 95% CI: 0.68, 1.11) was non-inferior to the control group in clinical pneumonia incidence. Milk-LNS was marginal, with the IRR of clinical pneumonia at 0.95 (95% CI: 0.75, 1.20), just touching the non-inferiority margin. However, for chest indrawing, diarrhea, and hospital admission, neither inferiority nor non-inferiority could be concluded.

The overall proportion of children with malaria parasitemia during the intervention period was 12.4%. The proportion was slightly lower in the milk-LNS (11.0%) and soy-LNS (12.1%) groups than in the control (13.3%) and CSB (13.4%) groups. The 95% CIs of the RRs compared with the control group were as follows: 1) milk-LNS: 0.63, 1.06; 2) soy-LNS: 0.71, 1.17; and 3) CSB: 0.78, 1.28. Milk-LNS and soy-LNS were non-inferior; CSB was not.

At 18 mo, there were no significant differences between the intervention groups in mean hemoglobin concentration (10.2 g/dL in the control group, 10.3 g/dL in the milk-LNS group, 10.1 g/dL in the soy-LNS group, and 10.3 g/dL in the CSB group; P = 0.43). There were also no significant differences between the intervention groups in the mean gain in hemoglobin concentration between ages 6 and 18 mo (0.6 g/dL in the control group, 0.8 g/dL in the milk-LNS group, 0.8 g/dL in the soy-LNS group, and 0.7 g/dL in the CSB group; P = 0.45). There was a significant reduction in the proportion of children with anemia (defined as hemoglobin <11 g/dL) between 6 and 18 mo in all 4 groups (all P < 0.05) from 84.4 to 65.1% in the control group, 82.5 to 63.4% in the milk-LNS group, 83.7 to 73.8% in the soy-LNS group, and 83.6 to 63.5% in the CSB group.

**Discussion**

We reported the effect of supplementation with 54 g/d iron-containing milk-based or soy protein–based LNSs on the risk of
non-inferiority is concluded.

The number of days with illness.

The non-inferiority margin for the geometric mean ratio compared with control is 1.2.

lipid-based nutrient supplement with milk protein base; soy-LNS, lipid-based nutrient supplement with soy protein base.

Values are percentages (95% CIs) (ARI 1.6 (1.2, 1.9) (988) 1.3 (1.0, 1.6) (881) 1.2 (0.9, 1.5) (874) 0.9 (0.7, 1.2) (680) 0.82 (0.61, 1.03) 4 0.86 (0.63, 1.08)4 0.73 (0.54, 0.92)4

2 meals during the day is safe in malaria-endemic areas.

We did not find that LNSs reduced morbidity. These findings are consistent with most previous studies (21–23). In Chad at baseline. Furthermore, the participants in the Chad study were, on average, aged 24 mo at enrollment, older than the present target population. Their finding may suggest that, in more wasted children and/or in older children (even after the first 1000-d window), LNSs could reduce morbidity.

We assessed for the important morbidity outcomes of incidence of malaria among children who ever visited a health facility during the study period and prevalence of malaria parasitemia at 12-week intervals. In Niger, there was no increased risk of malaria associated with a 3-mo preventive supplementation with a 92-g/d dose of LNSs among healthy children aged 6–60 mo (22). Our findings are consistent with those results. A previous study reported a significant increase in malaria cases in groups supplemented with a tablet containing 12.5 mg/d iron once a day or half of a tablet if aged <1 y (14). The present study used a lower dosage of iron, and the LNS intake was in smaller amounts throughout the day, which may

common infections in young children in rural Malawi. The 2 tested formulations of LNSs were shown to be non-inferior to no supplementation with respect to several morbidities. Using different sources of information, the data consistently showed that LNSs with 6 mg/d iron would not result in excess morbidity from malaria or respiratory illness. However, there was no conclusive evidence on whether LNSs would or would not increase diarrhea morbidity.

The WHO expert panel in 2007 postulated that iron given with foods, either as home fortificants or centrally processed foods, would be a safe iron-delivery strategy in malaria-endemic areas (38). To our knowledge, this is the first study that assessed the non-inferiority of complementary diet supplementation with LNSs on excess morbidity. Our findings suggest that the use of LNS products that are fortified with iron at this amount and fed at ≈2 meals during the day is safe in malaria-endemic areas.

We did not find that LNSs reduced morbidity. These findings are consistent with most previous studies (21–23). In Chad however, Huybregts et al. (20) reported a significant 29.3 and 22.5% reduction in diarrhea and fever, respectively, in children supplemented with a 46-g/d dose of ready-to-use supplementary food, with 9 mg/d iron, for 4 mo compared with a control group on a local diet. However, their participants had a mean weight-for-height Z-score of −1.1 at baseline, whereas the mean weight-for-length among children in this study was +0.45 at baseline. Furthermore, the participants in the Chad study were, on average, aged 24 mo at enrollment, older than the present target population. Their finding may suggest that, in more wasted children and/or in older children (even after the first 1000-d window), LNSs could reduce morbidity.

We assessed for the important morbidity outcomes of incidence of malaria among children who ever visited a health facility during the study period and prevalence of malaria parasitemia at 12-week intervals. In Niger, there was no increased risk of malaria associated with a 3-mo preventive supplementation with a 92-g/d dose of LNSs among healthy children aged 6–60 mo (22). Our findings are consistent with those results. A previous study reported a significant increase in malaria cases in groups supplemented with a tablet containing 12.5 mg/d iron once a day or half of a tablet if aged <1 y (14). The present study used a lower dosage of iron, and the LNS intake was in smaller amounts throughout the day, which may

| TABLE 1 | Baseline characteristics of the participants1 |
| --- | --- | --- | --- | --- |
| Variable | Control (n = 209) | Milk-LNS (n = 212) | Soy-LNS (n = 210) | CSB (n = 209) |
| Age, mo | 6.02 ± 0.23 | 6.02 ± 0.25 | 6.04 ± 0.25 | 6.03 ± 0.24 |
| Male infants, % | 53.1 | 50.5 | 49.1 | 46.9 |
| Breast-fed, % | 100.0 | 99.5 | 100.0 | 100.0 |
| Anthropometric status | | | | |
| Stunted, < −2 LAZ WHO, % | 34.0 | 34.0 | 39.1 | 39.7 |
| LAZ | −1.64 ± 0.97 | −1.59 ± 1.05 | −1.68 ± 1.11 | −1.72 ± 0.97 |
| Underweight, < −2 WAZ WHO, % | 13.4 | 10.4 | 14.3 | 18.2 |
| WAZ | −0.80 ± 1.06 | −0.70 ± 1.10 | −0.80 ± 1.12 | −0.85 ± 1.21 |
| Wasted, < −2 WHZ WHO, % | 1.9 | 0.9 | 2.4 | 1.4 |
| Weight-for-length Z-score | 0.41 ± 1.05 | 0.50 ± 1.05 | 0.46 ± 1.00 | 0.42 ± 1.11 |
| Hemoglobin concentration, g/dL | 9.4 ± 1.7 | 9.6 ± 1.7 | 9.3 ± 1.7 | 9.5 ± 1.2 |
| Proportion anemic (Hb < 110 g/dL), % | 84.4 | 82.5 | 83.7 | 83.6 |
| Proportion with malaria parasitemia, % | 17.1 | 10.1 | 13.7 | 10.9 |
| Household characteristics | | | | |
| Children aged <5 y the household, n | 1.6 ± 0.7 | 1.6 ± 0.8 | 1.6 ± 0.7 | 1.7 ± 0.9 |
| Maternal years of education, n | 3.6 ± 3.4 | 4.0 ± 3.7 | 3.0 ± 3.1 | 3.7 ± 3.1 |
| Piped drinking water, % | 4.0 | 5.4 | 2.9 | 4.4 |
| Availability of latrine, % | 92.5 | 94.2 | 93.6 | 92.7 |

1 Values are means ± SDs or percentages. CSB, corn-soy blend; Hb, hemoglobin; LAZ, length-for-age Z-score; milk-LNS, lipid-based nutrient supplement with milk protein base; soy-LNS, lipid-based nutrient supplement with soy protein base; WAZ, weight-for-age Z-score; WLZ, weight-for-length Z-score.

<p>| TABLE 2 | Longitudinal prevalence of guardian-reported fever, respiratory illness, and diarrhea in Malawian children aged 6–18 mo given LNSs, CSB, or no supplement1 |</p>
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Milk-LNS</th>
<th>Soy-LNS</th>
<th>CSB</th>
<th>Milk-LNS vs. control</th>
<th>Soy-LNS vs. control</th>
<th>CSB vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibril infection</td>
<td>5.5 (6.7, 6.3) (3655) 2</td>
<td>4.9 (4.3, 5.5) (3523)</td>
<td>4.5 (4.0, 5.1) (3289)</td>
<td>4.7 (4.1, 5.3) (3287)</td>
<td>0.91 (0.73, 1.09) 1</td>
<td>0.90 (0.72, 1.08) 4</td>
<td>0.91 (0.73, 1.09) 1</td>
</tr>
<tr>
<td>Cough alone</td>
<td>6.1 (5.2, 7.0) (4116)</td>
<td>5.7 (4.9, 6.5) (4066)</td>
<td>5.3 (4.5, 6.0) (3762)</td>
<td>5.5 (4.5, 6.5) (3666)</td>
<td>0.88 (0.69, 1.07) 1</td>
<td>0.95 (0.67, 1.03) 4</td>
<td>0.86 (0.67, 1.04) 4</td>
</tr>
<tr>
<td>ARI</td>
<td>1.6 (1.2, 1.9) (988)</td>
<td>1.3 (1.0, 1.6) (881)</td>
<td>1.2 (0.9, 1.5) (874)</td>
<td>0.9 (0.7, 1.2) (680)</td>
<td>0.82 (0.61, 1.03) 4</td>
<td>0.98 (0.63, 1.08) 4</td>
<td>0.73 (0.54, 0.92) 4</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.5 (3.9, 5.1) (3155)</td>
<td>4.9 (4.3, 5.5) (3480)</td>
<td>4.5 (3.9, 5.0) (3165)</td>
<td>4.4 (3.8, 5.0) (3072)</td>
<td>1.06 (0.87, 1.25)</td>
<td>0.99 (0.81, 1.17) 4</td>
<td>1.05 (0.86, 1.24)</td>
</tr>
<tr>
<td>Any morbidity</td>
<td>14.5 (13.0, 16.0) (9969)</td>
<td>13.9 (12.5, 15.2) (9939)</td>
<td>13.0 (11.7, 14.3) (9289)</td>
<td>13.7 (12.0, 15.4) (9396)</td>
<td>0.97 (0.81, 1.14) 4</td>
<td>0.91 (0.75, 1.06) 4</td>
<td>0.90 (0.74, 1.05) 4</td>
</tr>
</tbody>
</table>

1 Values are percentages (95% CIs) (n) or geometric mean ratios (95% CIs). ARI, acute respiratory illness; CSB, corn-soy blend; LNS, lipid-based nutrient supplement; milk-LNS, lipid-based nutrient supplement with milk protein base; soy-LNS, lipid-based nutrient supplement with soy protein base.

2 The non-inferiority margin for the geometric mean ratio compared with control is 1.2.

3 The number of days with illness.

4 Non-inferiority is concluded.
help to minimize the adverse risk effect of iron (15). In the recent report from Ghana described previously (19), there was an increase in hospitalizations in children receiving iron-containing MNP but no associated increase in the risk of malaria.

We observed similar mean hemoglobin concentrations and prevalence of anemia at the end of the intervention between the groups. Previous studies in Chad (20), Ghana (21), and Malawi (39) showed substantial effects of LNS interventions on both mean hemoglobin concentration and prevalence of anemia compared with nonsupplemented controls. However, the iron content in our LNS daily dose was lower (approximately half of that in the previous Ghana and Malawi studies and 75% of that in the Chad study) and may not have been enough to increase hemoglobin concentration and resolve anemia due to iron deficiency. The proportion of anemia due to iron deficiency is unknown because hemoglobin was the only biomarker measured. The high prevalence of anemia at 18 mo of age (65% in the control group) suggests the coexistence of other etiologic causes of anemia not amenable to iron supplementation, given that the background rate of iron deficiency anemia among children aged <5 y in Malawi in 2009 was estimated at 30.6% (40).

This study has several limitations. First, we had no measures of iron status among the participants. Second, the sample size was calculated based on the primary outcome of the trial (linear growth) and not the morbidity outcomes. However, as Feinstein and Concato (41) maintained, after the completion of a trial, the intervention effect should be determined by CIs without and Concato (41) maintained, after the completion of a trial, the intervention effect should be determined by CIs without

### Table 3
Incidence of guardian-reported fever, respiratory illness, and diarrhea in Malawian children aged 6–18 mo given LNSs, CSB, or no supplement

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Incidence per child-year (n episodes)</th>
<th>Incidence rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (189.6 child-years)</td>
<td>Milk-LNS (198.0 child-years)</td>
</tr>
<tr>
<td>Febrile illness alone</td>
<td>5.88 (1116)</td>
<td>5.43 (1075)</td>
</tr>
<tr>
<td>Cough alone</td>
<td>5.82 (1104)</td>
<td>5.53 (1095)</td>
</tr>
<tr>
<td>ARI</td>
<td>1.49 (282)</td>
<td>1.32 (261)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.44 (843)</td>
<td>4.88 (966)</td>
</tr>
</tbody>
</table>

1 The non-inferiority margin for incident rate ratio compared with control is 1.2. Incidence per child-year was calculated as the number of episodes divided by child-years in a group.

### Table 4
Incidence of malaria, acute respiratory problems, and diarrhea in Malawian children aged 6–18 mo given LNSs, CSB, or no supplement

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Incidence per child-year (n episodes)</th>
<th>Incidence rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (189.6 child-years)</td>
<td>Milk-LNS (198.0 child-years)</td>
</tr>
<tr>
<td>Clinical malaria</td>
<td>0.64 (122)</td>
<td>0.51 (100)</td>
</tr>
<tr>
<td>Rapid breathing</td>
<td>1.18 (223)</td>
<td>1.06 (209)</td>
</tr>
<tr>
<td>Chest indrawing</td>
<td>0.10 (19)</td>
<td>0.08 (15)</td>
</tr>
<tr>
<td>Clinical pneumonia</td>
<td>0.96 (183)</td>
<td>0.91 (181)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0.15 (29)</td>
<td>0.23 (45)</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>12 (0.06)</td>
<td>7 (0.04)</td>
</tr>
</tbody>
</table>

1 The non-inferiority margin for the incident rate ratio compared with control is 1.2. Data are from clinician’s diagnosis at a health facility during unscheduled visits from any illness. Incidence per child-year was calculated as the number of episodes divided by child-years in a group. CSB, corn-soy blend; LNS, lipid-based nutrient supplement; milk-LNS, lipid-based nutrient supplement with milk protein base; soy-LNS, lipid-based nutrient supplement with soy protein base.

2 Non-inferiority is concluded.
Acknowledgments
The authors thank Laszlo Csonka for designing the data entry program. C.M., P.A., K.M., J.P., C.T., K.D., M.M., T.P., and Y.B.C. designed the trial; P.A. and K.M. coordinated and supervised the research team; C.M. and C.T. coordinated the research team at local centers and were responsible for the data collection; C.M., and Y.B.C. designed the details of statistical analysis and analyzed the data; C.M., Y.B.C., and P.A. wrote the first draft of the manuscript; Y.B.C. had primary responsibility for the final content. All authors read and approved the final manuscript.

References


24. iLiNS project. [cited 2014 March 5]; Available from: http://www.iilns.org/.


