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Efficacy of Complementary Food Supplementation with Lipid-Based Nutrient Supplements on Growth of Malawian Children

ACADEMIC DISSERTATION
To be presented, with the permission of the Faculty of Medicine of the University of Tampere, for public discussion in the Jarmo Visakorpi Auditorium, of the Arvo Building, Lääkärinkatu 1, Tampere, on September 25th, 2009, at 12 o’clock.

UNIVERSITY OF TAMPERE
SUMMARY

In low income countries, 170 million under-five year-old children are stunted. Prevalence of underweight and wasting are also high. In the past few years, several analyses have documented that at least 35% of all childhood deaths are attributable to underweight; that most of these deaths occur in moderate to severe underweight; that undernutrition (wasting, stunting, underweight) are common between 6 and 24 months of age; that stunting is associated with poor cognition and development, and lower physical capacity and income in adulthood; and that proper nutrient supplementation can mitigate the effects of undernutrition.

Efforts to promote normal infant growth have so far produced modest growth outcomes and infants in low income countries continue to experience growth faltering during this critical period. The relatively new supplementary strategy food products commonly known as the lipid-based nutrient supplements (LNS) have been successful in treating severe forms of acute malnutrition in the community and are well accepted, suggesting that similar products could be useful for promotion of growth and prevention of undernutrition in infancy. Therefore, the present study tested the effects of LNS as a complementary food on promotion of growth and development of infants and young children, and its effect as a supplementary food on secondary prevention of childhood undernutrition. Firstly, to test the effect of LNS as a supplementary food on secondary prevention of severe acute undernutrition we conducted a supplementary
feeding trial in which the infants received either daily doses of 50 g LNS called fortified spread (FS50) or 71 g iso-energetic corn-soy blend (CSB) called Likuni Phala (LP). Secondly, to test the effect of provision of LNS as a complementary food supplement for primary prevention of undernutrition and promotion of growth and development we conducted a randomised controlled complementary feeding trial in which the infants in 2 trial groups received LNS daily doses of either 50 g (FS50) or 25 g (FS25) and those in the control group received 71 g (LP) for 12 months.

In the supplementary feeding trial, mean weight-for-age increased by 0.22 (95% CI 0.07 to 0.37) and 0.28 (0.18 to 0.40) Z-score units in the LP and FS groups, respectively. Comparable increase for mean weight-for-height was 0.39 (0.20 to 0.57) and 0.52 (0.38 to 0.65) units. Recovery from underweight and wasting was 20% and 93% in the LP-group and 16% and 75% in the FS-group. Few individuals recovered from stunting and mean length-for-age was not markedly changed.

In the complementary trial the mean weight and length gains in LP, FS50 and FS25 groups were 2.37, 2.47, and 2.37 kg (P = 0.66) and 12.7, 13.5, and 13.2 cm (P = 0.23), respectively. In the same groups, cumulative 12-month incidence of severe stunting was 14.0%, 0.0% and 4.0% (P = 0.01), severe underweight 15.0%, 22.5% and 16.9% (P = 0.71), and severe wasting 1.8%, 1.9% and 1.8% (P > 0.99). There was a significant interaction between baseline length and intervention (P = 0.04); among children with below-median length at enrolment, those given FS50 gained on average 1.9 cm (0.3 to 3.5; P = 0.02) more than individuals receiving LP.
In the supplementary feeding trial, the mean gain in haemoglobin concentration after 12 weeks supplementation was 3.8 g/L in the FS50-group compared to 1.5 g/L in the LP-group. Compared to enrolment measurements in all the 3 trial groups, the mean haemoglobin concentration declined marginally at the end of the intervention. The smallest decline occurred in the LNS groups; <1 g/L, 4 g/L and 7 g/L in the FS50, FS25 and LP groups, respectively.

At 18-months of age, the mean ± SD mental ages in the LP, FS50 and FS25 groups were 17.9 ± 1.3, 17.9 ± 1.3 and 17.9 ± 1.2 months ($P > 0.99$), respectively. Length-for-age $Z$-score (LAZ) gain during the intervention period and maternal education were associated with developmental outcome at age of 18-months ($P = 0.03$ and $P = 0.04$; respectively).

The cumulative 36-month incidence of severe stunting was 19.6% in LP, 3.6% in FS50 and 10.3% in FS25 groups ($P = 0.03$). Mean weight for age $Z$-score (WAZ) change was -1.09, -0.76 and -1.22 ($P = 0.04$), mean LAZ change -0.47, -0.37, and -0.71 ($P = 0.10$), and mean weight for height $Z$-score WHZ change -1.52, -1.18, and -1.48 ($P = 0.27$). All differences were more marked among individuals with baseline LAZ below median. Differences in length developed during the intervention, at ages 10-18 months, whereas weight differences continued to increase after the intervention.
In conclusion, short-term supplementation with lipid-based nutrient supplements (LNS) or corn-soy blends (CSB) improve weight gain similarly but neither of them has short term effect on length. Long-term provision of LNS results in higher length gain, weight gain and prevents incidence of stunting than provision of iso-energetic CSB. Similarly, LNS may promote increased haemoglobin concentration more than CSB. Provision of the multiple micronutrients through fortified LNS and CSB supplements have similar effect on child development.
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LIST OF ORIGINAL ARTICLES

This thesis is based on the following original articles, referred to in the text of the article by their roman numerals.


IV. Phuka JC, Thakwalakwa C, Maleta K, Cheung YB, Briend A, Manary M, Ashorn P. Childhood development of 18 months old Malawians after a year of complementary feeding with lipid-based nutrient supplements or corn-soy blend. (Submitted)
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<td>ALA</td>
<td>Alpha-linolenic acid</td>
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<td>ARI</td>
<td>Acute respiratory infection</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>BDI-II</td>
<td>Battelle Developmental inventory II</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BSID-II</td>
<td>Bayley Scales if Infant Development II</td>
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<tr>
<td>CDC</td>
<td>United States Centers for Disease Control</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CRF</td>
<td>Case reporting form</td>
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<tr>
<td>CSB</td>
<td>Corn-soy blend</td>
</tr>
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<td>CSM</td>
<td>Corn-soy milk</td>
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<tr>
<td>DALY</td>
<td>Disability-adjusted life year</td>
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<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>DSMB</td>
<td>Data safety management board</td>
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<tr>
<td>DPT</td>
<td>Vaccine against Diphtheria, Pertussis and Tetanus</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded programme on immunization</td>
</tr>
<tr>
<td>FS</td>
<td>Fortified spread</td>
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<tr>
<td>GDP</td>
<td>Gross domestic product</td>
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<tr>
<td>GNI</td>
<td>Gross national income</td>
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<tr>
<td>HAZ</td>
<td>Height-for-age Z-score</td>
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<td>HIV</td>
<td>Human immunodeficiency syndrome</td>
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<tr>
<td>ICP</td>
<td>The infancy, childhood and puberty growth model</td>
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<tr>
<td>ITN</td>
<td>Insecticide-treated bed net</td>
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<tr>
<td>LA</td>
<td>Linoleic acid</td>
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<tr>
<td>LAZ</td>
<td>Length-for-age Z-score</td>
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<tr>
<td>LCPUFA</td>
<td>Longchain polyunsaturated fatty acid</td>
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<tr>
<td>LMS</td>
<td>Parameters need to generate percentile and Z-scores: median (M), the generalized coefficient of variation (S), and the power in the Box-Cox transformation (L)</td>
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<tr>
<td>LNS</td>
<td>Lipid-based nutrient supplement</td>
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<td>LP</td>
<td>Likuni Phala</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>MUAC</td>
<td>Mid-upper-arm circumference</td>
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<td>MGRS</td>
<td>The World Health Organisation (WHO) Multicentre Growth Reference Study</td>
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<td>MSEL</td>
<td>Mullen Scales of Early Learning</td>
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<tr>
<td>NCHS</td>
<td>The United States National Center for Health Statistics</td>
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<tr>
<td>NRU</td>
<td>Nutrition Rehabilitation Unit</td>
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<tr>
<td>NTD</td>
<td>Neural tube defect</td>
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<tr>
<td>ORS</td>
<td>Oral rehydration salts</td>
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<td>ODA</td>
<td>The net official development assistance</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PDCAAS</td>
<td>Protein digestibility-corrected amino acid score</td>
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<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>RUTF</td>
<td>Ready-to-use therapeutic food</td>
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<td>SAE</td>
<td>Serious adverse event</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SOP</td>
<td>Standard operating procedures</td>
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<td>TEM</td>
<td>Technical error of measurement</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>UNICEF</td>
<td>United Nations Children’s Fund</td>
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<tr>
<td>USAID</td>
<td>U.S. Agency for International Development</td>
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<tr>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
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<tr>
<td>WAZ</td>
<td>Weight-for-age <em>Z</em>-score</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>WHZ</td>
<td>Weight-for-height <em>Z</em>-score</td>
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<tr>
<td>WLZ</td>
<td>Weight-for-length <em>Z</em>-score</td>
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1 INTRODUCTION

In absence of optimal environmental conditions, growth may either be down regulated by internal programming early in foetal growth, or may be impaired. The impaired growth may be due to inadequate substances for tissue accretion such as amino acids and micronutrients, due to inadequate energy to support the accretion process, or due to slower metabolism. The result of these adaptations or inadequacies may manifest as growth faltering or as inadequate neuro-motor development. After short exposure to undernutrition children commonly present with wasting. Subsequently after prolonged undernutrition, wasting may progress to underweight or stunting (a statural growth failure) and it is associated with poor child development.

Evidence from the WHO Global Database on Child Growth and Malnutrition based on a collection of surveys worldwide indicate that prevalence of undernutrition based on stunting, wasting and underweight is high in developing countries but on the decline in all other parts of the world except in Africa (de Onis and Blössner 2003, de Onis et al. 2004a, UNICEF 2007). Prevalence of stunting in developing countries in under-five years old children was estimated to decline from 30% in 2000 to 16% in 2020, and for the same period in Africa from 35% to 31% (de Onis and Blössner 2003). Similarly, worldwide forecast for underweight from 1990 to 2015 were 30% to 19 %, but that is rising in Africa from 24% to 27% (de Onis et al. 2004a). Stunting affects approximately 160 million children less than 5 years of age, with prevalence of
approximately 46% in southern Asia and 38% in sub-Saharan Africa (de Onis et al. 2004a, UNICEF 2007)

Maternal and childhood undernutrition remain a big cause of total global disease burden. In 2002, it was estimated that childhood and maternal underweight were together responsible for 9.5% of world disability-adjusted life years (DALY) (Ezzati et al. 2002). Recent estimates suggest no decline; in 2008, maternal and childhood undernutrition were estimated to be responsible for 11% of the total global disease burden and 35% of child deaths (Black et al. 2008).


Global strategies to promote child growth and development and to prevent undernutrition are at the top of global health priorities. Despite efforts dating to several decades to provide complementary food supplements, results on growth are modest and undernutrition remains a big cause of global disease burden (Marchione
2002, Black et al. 2008). Lipid-based nutrient supplements have been effective in treating severe undernutrition in the communities and they are generally well accepted by infants (Manary et al. 2004, Sandige et al. 2004, Ciliberto et al. 2005, Ndekha et al. 2005, Collins et al. 2006, Bhutta et al. 2008, Dewey and Adu-Afarwuah 2008). It has been suggested that this approach may also be useful in promotion of child growth and development and in prevention of undernutrition (Nestel 2003). Hence, the present population-based randomised controlled study was conducted to test the efficacy of the lipid-based nutrient supplements (LNS) as complementary food supplements in Malawi.
2 LITERATURE REVIEW

2.1 Approach to the literature review

The purpose of this review is to give the background rationale for the current study. Thus, the review presents: the knowledge on fundamentals of human growth; the empirically guided methods of assessing human growth and nutritional status; the timing of growth faltering in developing countries; the consequences of growth faltering and its associated preceding factors; and the lessons learned from prior efforts to promote human growth and development. Subsequently, the justification of the current study, a feasibility assessment on nutrition strategies for promoting infant growth and development, is presented. To give adequate width and depth of background knowledge on promotion of growth and prevention of undernutrition, both systematic and random search procedures were used. Published electronic references were obtained by searching strategically relevant key words. The search was mostly conducted through but not restricted to the PubMed of U.S National Library of Medicine and the National Institutes of Health. Relevant references cited in retrieved literature were identified and reviewed further. The main focus was on primary results rather than secondary results, although findings from a few critical reviews or books were included. As efforts to promote growth through supplementation date back to at least 4 decades ago, accessible historical literature dating that far was reviewed but more emphasis was given to recent data.
2.2 Biological and environmental factors of growth

2.2.1 Pattern of human growth

Human growth, a stature increasing process, involves somatic accretion, differentiation and functional maturation of organs. Starting with conception, the growth process continues until cessation in late teens or early twenties (Tanner 1963, Karlberg 1989, Cameron 1992, Coley et al. 2006). Human postnatal growth is best represented by a trace on a height growth curve or distance growth chart from birth to adulthood (Tanner 1952). These charts are important tools for assessing the pattern of growth over time. Traditionally, the series of measurements for construction of the growth chart are conducted at intervals long enough to produce growth curves that look smooth and continuous curve. More detailed measurements that are carried out at shorter intervals, however, reveal that postnatal growth is not necessarily smooth, but it rather occurs by saltation spaced by stasis (Lampl et al. 1992). Further evaluation suggests that rather than one continuous curve starting from birth to adulthood, the growth curve is a composition of a series of different overlapping growth curves. A number of mathematical models demonstrating decomposed growth curves have been suggested and have been rigorously evaluated before (Preece and Baines 1978, Stützle et al. 1980, Karlberg 1989, van Dommelen et al. 2004).

The Infancy, Childhood, and Puberty Growth Model (ICP-Growth Model) constructed by Karlberg is the most conceptually convincing mathematical model of human
growth and it has been accepted by other authors (van Dommelen et al. 2004, Cameron 2007). This model superimposes curves from three independent growth phases which are summed up to produce one postnatal growth curve (Karlberg et al. 1989) (Figure 1). The three independent phases in this growth-model curve correlates better with regulatory growth mechanisms (Karlberg 1989) and it fits empirical data better when compared to other models (van Dommelen et al. 2004). Each phase is characterised by an initial rapid growth that later slows down before the next phase commences (Karlberg 1989, Karlberg et al. 1994). The fastest growth occurs in the first phase that is also characterised by rapid deceleration before it ceases at about 3-4 years of age (Karlberg et al. 1994) (Figure 2). The childhood growth phase starts at about one year of age and slowly decelerates until it is interrupted by the pubertal growth spurt at 13-15 years. However, rarely before the pubertal phases a small interruption may occur at 6-8 years and this is known as the juvenile growth spurt (Tanner and Cameron 1980).
Figure 1: The ICP growth model for boys. The mean functions are plotted for each of the three components as well as the combined growth $t_c$: Onset of Childhood component $t_E$: Age at end of growth. (Karlberg 1989)
Figure 2: Length and length velocity for a normal Pakistani
Monthly recorded values have been used in (a), every second month values in (b)
(Karlberg 1994)

2.2.2 Regulation of human growth

The phenotypic variation of height is a summation of the genetic variation and the random environmental effects (Silventoinen et al. 2008a). Intrinsic factors affecting growth from infancy to adulthood include at least genetics, morphogens and hormones (Owens 1991, Tabata and Takei 2004). Externally, the body obtains substrates for building or maintaining the body (Owen 1991). Additionally, the genetic control may interact with the environment independently; for example, the
genes may influence caloric intake, caloric expenditure and metabolism (Marti et al. 2004).

Regulation of heritability of human growth is to a large extent determined by genetics. Evidence of the role of heritability on growth comes from strong height correlation between siblings or between parents and their children. In adulthood, height heritability ranges from 50% in full sibling to above 90% in monozygous twins (Silventoinen et al. 2003a, 2008a, Czerwinski et al. 2007). Additional evidence of strong genetic influence on growth traits such as height is a phenomenon known as canalisation; this phenomenon refers to the tendency of growth traits to follow a certain tendency or trajectory over time (Tanner 1963, Cameron 1992, Cameron 2007). Follow-up studies have shown that children failing to grow because of medical conditions have attained expected heights after a correction of their medical condition (Tanner 1963, Cameron 1992). The genetic control of stature has been shown to increase with age (Dubois et al. 2007, Silventoinen et al. 2008a), suggesting that environmental effect on growth is more important in early childhood. Apart from height, other characteristics of growth showing strong heritability include muscle strength, body weight, body mass index (BMI) and body composition (Schousboe et al. 2004, van Dommelen et al. 2004, Dubois et al. 2007, Silventoinen et al. 2007, 2008b).

Several hormones participate in intrinsic regulation of human growth. Some hormones like growth hormone are produced specifically to promote growth while for others
like insulin this is a secondary effect. Correlation of some hormones with different phases of growth helps in understanding the timing of their hormonal influence on growth. Each phase of the ICP growth model, for example, is regulated by at least one hormone. However, the effects of some hormones are not limited to a single phase (Karlberg 1989). Hormones regulating prenatal life are thought to continue to infancy growth phase up to the end of the first postnatal year (Karlberg 1989). Animal studies suggest that the prenatal growth is regulated by at least morphogens and hormones (Fowden 1995). Morphogens are thought to be responsible for organ differentiation and hormones are considered to be responsible for the general growth process. Insulin like-growth factors 1 and 2, insulin and glucocorticoids are regarded as the most important hormones regulating growth during this phase (Fowden 1995, 2003, Kaku et al. 2007). Although growth hormone is present in foetal life, its effect has at least not been shown to include regulation of prenatal growth (Owens 1991, Lee at al. 2003). After the first year of life, growth hormone in the presence of thyroid hormone regulates growth until puberty starts at about 11-15 years (Karlberg 1989), when additional growth effect comes from sexual hormones (Silventoinen 2003b).

2.2.3 Environmental factors influencing human growth

External effects from the environment compliment the intrinsic growth factors. The environmental effects on growth include at least nutrition, geographical location, physical environment, social economic status, morbidity, and education (Martorell 1995a, Silventoinen 2003b, Black et al. 2008). These environmental factors influence both the intrauterine and the postnatal growth phases. In foetal life, uterine conditions
account for approximately half of the variation in birth weight, an indication of a strong environmental influence on intrauterine growth (Adair and Pollitt 1985, Dubois et al. 2007). Postnatally, the environment influence on growth is well known and its largest impact is thought to be in the first few years (Martorell 1995a, Silventoinen 2003b, 2008a, Black et al. 2008). During the first 2 years of life, environmental factors account to about half or more of the growth variation; thereafter, the environmental influence on growth gradually decline with age to an extent that they accounts for less than 10% growth variation towards adulthood (Demerath et al. 2007, Dubois et al. 2007, Silventoinen et al. 2008a). Growth status is, thus, a good indicator of a child’s general health, nutrition and socioeconomic status (Silventoinen 2003b).

Inadequate nutrition or undernutrition, a major risk factor of growth failure, may occur due to disturbance at any point or level along the nutrient’s pathway from the environment into the body (Figure 3). Along this pathway, three possible levels are notable and will be described further below: the population, the household and the individual levels (UNICEF 1998). Firstly, at the population level, basic causes operate by reducing the quantity, the quality and the utilisation of potential resources; and these basic causes include at least political, cultural, religious, economic and social systems, including women’s status. Likewise, at the population level, inadequate or inappropriate knowledge and discriminatory attitudes limit household access to the actual resources. Secondly, at the household level, underlying causes operate through insufficient access to food, inadequate maternal and child care practices, poor water and sanitation, and poor health services. Thirdly, at the individual level, the immediate causes of undernutrition operate through inadequate food intake and
morbidity. Thus, in low income settings, all these environmental factors act through diseases or inadequate food intake or interaction of the diseases and inadequate intake (Black et al. 2008, UNICEF 1998) to cause undernutrition and the subsequent growth failure.

Figure 3: Causes and consequences of undernutrition.
Framework of the relations between poverty, food insecurity, and other underlying and immediate causes to maternal and child undernutrition and its short-term and long-term consequences (Black et al. 2008)

State of disease reduces in many occasions nutrient intake by reducing appetite, reducing absorption, and loss of nutrients (UNICEF 1998). Reduced food intake following loss of appetite is common in childhood illnesses including malaria, diarrhoea, respiratory tract infections and helminth infestations. Because of altered metabolism, nutrient demand also increases during childhood illnesses. The pathophysiology of infections such as malaria and acute respiratory tract infections (ARI) usually increases the energy demand of the body (Scrimshaw 1977).

Inadequate food intake negates nutrition; the process whereby living organisms take in and transform extraneous solid and liquid substances necessary for growth, maintenance of life, normal functioning of organs and production of energy. Short term undernutrition often causes preclinical micronutrient related medical conditions or wasting while prolonged exposure to undernutrition is often characterised by stunting. Both wasting and stunting may results in underweight. In the low income countries, micronutrient deficiencies commonly associated with undernutrition and the associated growth failure include at least zinc, iron, iodine, selenium, and essential fatty acids (Allen 1994).

Socio-economic status is another noticeable environmental factor that is associated with growth. Some of the elements contributing to socio-economic status include income, possessions, education, water, sanitation, family size and living environment. Poor economic status is probably the most important socio-economic factor as poverty is associated with several other factors: inadequate food, poor sanitation and hygiene,
infection, and maternal stress and depression (Fotso and Kaute-Defo 2005, Grantham-McGregor et al. 2007). Education, especially that of the mothers is another socio-economic factor strongly associated with growth and nutrition in childhood (Ivanans 1975, Liu et al. 1998, Pena et al. 2000, Griffiths et al. 2004, Larrea and Kawachi 2005, Walker et al. 2005, Hatt and Water 2006). Maternal education has been consistently associated with positive growth and other health outcomes despite being assessed differently in many studies (Fotso and Kaute-Defo 2005). Educated mothers are more likely to use health promotion facilities and programmes, and may as well earn more money enhancing their capacity to support children. Another factor influencing height is secular trends; over the years in most but not limited to high income countries, the mean population height has been increasing (Henneberg and Berg 1990, Silventoinen 2003b). Reduced stress from disease burden and improved nutritional environment are thought to contribute to secular trends (Silventoinen 2003b).

Taken together, human growth is genetically determined and biologically modified by intrinsic as well as environmentally determined growth factors. The average population growth potential of children is similar in both low and high income countries (Bhandari et al. 2002, WHO 2006). Therefore, childhood growth failure at population level in the low income countries is likely to be caused by environmental factors rather than the genetically determined intrinsic growth regulators. Thus, managing these environmental risk factors allow for an external opportunity for promoting growth, prevention of growth failure and stimulation of catch-up growth.
2.3 Assessment of growth and nutritional status

2.3.1 Methods of assessing human growth and nutritional status

Growth is often assessed using anthropometric measurements; of which common measurements include length/height, weight, head circumference and mid-upper arm circumference (MUAC). In auxology, anthropometry usually refers to measurements of external morphological (Ulijaszek and Deborah 1999) although anthropometry may more generally refer to all measurement of man which ideally includes psychological, physiological and anatomical traits. Collectively, these measurements are also important for nutritional assessment, screening, surveillance and monitoring.

Nutritional assessment methods, however, also take many other forms appropriate for characterising different stages of nutritional deficiency. These approaches include the clinical assessment, nutrient balance, functional indicators of nutritional sufficiency (physiological, biochemical and molecular techniques), anthropometric methods and measures of optimal nutrient intake (WHO/FAO 2004). In longitudinal studies and surveillance of undernutrition, anthropometric methods are widely used because they are technically easier to use than other methods. For surveillance, anthropometric data can easily be plotted on a distance growth chart from where failure to thrive can easily be detected (Figures 1 and 2). Other methods like dietary assessments and biochemical techniques are expensive and require substantial training.
2.3.2 Methods of assessing child development

Standardised tests for child development are psychometric measures designed to
inventory individual’s abilities and provide a comparison between the individual
performance on the test to that of the reference or standard. They are designed for
global assessment by providing an inventory of key developmental milestones;
however some also provide the option of assessing an individual’s ability within a
specific developmental domain. In general, standardised assessments comprise formal
tester-administered measures in which a qualified examiner administers the test
adhering to a stringent administration and scoring protocol. The objectivity of these
tests makes them ideal for large-scale use, such as routine follow-up, in which
multiple examiners may administer the test and which comparisons are made between
the results of individuals or groups. The most commonly used standardised
developmental tests at least includes the *Mullen Scales of Early Learning* (MSEL), the
*Battelle Developmental inventory II* (BDI-II), the *Griffiths Mental Developmental
Scales- Revised* (Griffiths Scales), and the *Bayley Scales if Infant Development II*
(BSID-II) (Johnson and Marlow 2006).

2.3.3 Standardisation of assessments methods
Standardisation of anthropometric measurements

To interpret growth measurements appropriately, anthropometric indices are useful. The indices are calculated by combining at least two anthropometric measurements (WHO 1995). The anthropometric indices are used to compare an individual child or a group of children with a reference population, and they can be adjusted for age and sex. In children, the most commonly used anthropometric indices are weight-for-height, height-for-age, and weight-for-age (for wasting, stunting and underweight, respectively) (WHO 1995). The common expressions of anthropometric indices in children are Z-scores, percentiles, or percent of median. These reporting systems are defined by WHO as follows:

- **Z-score** - the deviation of the value for an individual from the median value of the reference population divided by the standard deviation of the reference population.
- **Percentile** - the rank position of an individual on a given reference distribution, stated in terms of what percentage of the group the individual equals or exceeds.
- **Percent of median** - the ratio of a measured value in the individual to the median value of the reference population, expressed as a percentage.

Because of better utilisation characteristics, World Health Organisation (WHO) recommends use of Z-score to standardise weight and height and for assessment of malnutrition (WHO 1995). The Z-score system is preferable because it satisfies all of
the following four characteristics: adhering to reference distribution; working on
linear scale hence permitting summary statistics; using uniform criteria across indices;
and detecting changes at extremes of the distribution; the percentile and the percent of
median, each satisfies only two of those. A Z-score of less than -2 or greater than +2
is considered as evidence of malnutrition; the lower extremity is regarded as
undernutrition and the upper extremity is regarded as overnutrition. Of these,
undernutrition is the common problem for children in low income countries (de Onis

2.3.3.2 Standardisation of developmental scores

During construction, standardised developmental test are subject to rigorous empirical
analyses to assess the psychometric properties of the scale. Firstly, the reference
population is selected to be representative of the population for whom the test is
designed and the sample size has to large enough. Secondly, the developmental test
has to be reliable with high internal consistency, repeatability and inter observer
reliability. Lastly, the test has to be valid with high content validity, construct validity,
concurrent validity and predictive validity. Although some of the standardised tests
available were constructed on a wide ethnic background population none included
children from low income settings. As such when used in such population
standardised developmental tests need to be re-validated.
2.3.4 Reference population

2.3.4.1 Anthropometric reference population

Determination of nutritional status is made by comparing anthropometric measurement to that of a growth reference or a growth standard constructed from reference population. The US National Center for Health Statistics (NCHS)/WHO 1977 international references and the CDC 2000 growth charts have been used until recently (WHO 1983, Kuczmarski 2002). They both have received criticism on either reference population or statistical methods employed in their construction.

The WHO/NCHS growth references were criticised that they do not represent children from low income settings, because the reference covering birth to three years were constructed based on largely formula fed, affluent children of European ancestry from a single community in the United States of America (Kuczmarski et al. 2002). Breast fed children grow rather differently from largely bottle fed children (de Onis and Habicht 1996, de Onis 1997, Dewey 1998, Victora et al. 1998, Garza and de Onis 1999). Additional criticisms have been on centile smoothing procedure and estimation of extreme centiles (Dibley et al. 1987, Kuczmarski et al. 2002); and on poor transition from length to height at 24 months because they were based on two different data sets (Kuczmarski et al. 2002). At the time the NCHS/WHO growth curves were constructed, the statistical methods available were too limited to correctly model the pattern and variability of growth. Likewise, although the CDC 2000 growth charts construction employed LMS methods that fit skewed data adequately and
generate fitted curves that follow closely empirical data, they were also based on restricted population (Cole and Green 1992, Kuczmarski et al. 2002, WHO 2006).

It was with this background that the WHO Child Growth Standards were launched in Geneva in on 27th April 2006 as a standard rather than a reference. Although used interchangeably, a standard define how children should grow, therefore deviations from the pattern it describes are evidence of abnormal growth; on the other hand, a reference does not provide as sound a basis for such value judgements, therefore it is only a tool for grouping and analysing data, and a common basis for comparing population (WHO1995, 2006). The WHO Child Growth Standards were constructed from widely diverse ethnic backgrounds and cultural settings (Brazil, Ghana, India, Norway, Oman and USA) following the WHO Multicentre Growth Reference Study (MGRS). The construction of these growth curves followed a methodical process where initially the existing methods were examined, including type of distributions and smoothing techniques; then flexible software allowing comparison of alternative methods and generation of the curves was selected; and lastly the selected approach was systematically applied to generate models that best fit the data (Borghi 2006, WHO 2006). Like in the development of the CDC 2000 growth charts, LMS procedures were employed as a simplification of the Box-Cox power exponential (Rigby and Stasinopoulos 2004, Borghi 2006) and cubic splines were used for curve smoothing (WHO 2006).
The MGRS is unique in that it was purposely designed to produce a standard by selecting healthy children living under conditions likely to favour the achievement of their full genetic growth potential (WHO 2006). Furthermore, the mothers of the children selected for the construction of these standards engaged in fundamental health promoting practices: exclusive breast feeding for at least 4 months; introduction of complementary feeding by the age of 6 months with continued breast feeding up to 12 months; and the mother not smoking before and after delivery (de Onis et al. 2004b). Only single term birth children in absence of significant morbidity were included (de Onis et al. 2004b).

2.3.4.2 Developmental scores norm-reference population

During standardisation, a test is administered to a large group of children for whom it is designed; this group forms the normative sample or normal population. An individual’s score obtained on the test is essentially a comparison to this normative data and is used to determine how the individual is developing in relation to the norm. Standardised tests thus yield norm-referenced or standardised scores. Standard score typically have a mean score of 100 and standard deviation (SD) of 15. A Z-score of <-2 SD is typically classified as delayed performance. Other norm-referenced score that may be yielded are percentiles and age equivalents. Age equivalents are easy to understand but should be used cautiously as developmental age below chronological age may actually be within normal range of standardised scores.
2.4 Epidemiology of undernutrition and early growth faltering

2.4.1 Age distribution and determinants of early growth failure

Growth faltering in developing countries may start in utero or soon after birth, is usually pronounced between 12 and 18 months and may continue until 40 months, after which the decline often stops (Martorell et al. 1995b, Shrimpton et al. 2001, Maleta et al. 2003a, Victora et al. 2008). Height deficit experienced at 2 or 3 years usually persists with similar magnitude until adulthood (Coley et al. 2006, Victora et al. 2008). Although catch up growth has been reported, it is often in relation to a reference population (Tanner 1963, Cameron 1992, Adair 1999); differences in height between stunted and non-stunted children per population usually remain the same until adulthood. Catch-up growth is largely dependent on maturational delay that prolongs growth period; however, this delay is not common in low income settings (Martorell 1994). In developing countries, reasons for intrauterine growth restriction include poor maternal nutrition and infections; restricted foetal growth especially during the third trimester affects crucial period of brain development (Singh 2005, Grantham-McGregor et al. 2007). Likewise, postnatal growth faltering during the first three years of life is often associated with poor maternal and childhood nutrition and infections (Black et al. 2008).
2.4.2 Micronutrient causes and determinants of growth faltering

In recent years, the significance of micronutrients as a cause of undernutrition has been appreciated more while well known causes of protein energy malnutrition remain an important concern (Allen et al. 2006). Because of the high prevalence worldwide, iron, vitamin A and iodine are considered to be micronutrients of high public health importance; in Africa for example the prevalence of anaemia is estimated at 46%, that of inadequate iodine intake at 43% and that of vitamin A deficiency at 49%. Similar high prevalence of micronutrient deficiencies are estimated to occur in South East Asia (Allen et al. 2006). Other micronutrients likely to contribute substantially to global burden are zinc, folate and vitamin D (Allen et al. 2006). Essential fatty acids are yet another group of micronutrients of high public health important. In recent years, substantial data on their role on neuro-tissue development have been reported. Whatever the cause of undernutrition may be, its consequences on growth and development of the foetus or infants are severe and long lasting; thus, underscoring the need for public health interventions to fight it.

2.4.3 Consequences of undernutrition and growth faltering

Prenatal growth restriction has been associated with poor developments in early childhood and later in life. Low birth weight term infants in Brazil showed lower developmental levels at ages of 6 and 12 months than appropriate birth weight infants
(Grantham-McGregor et al. 1998); in Guatemala they had lower cognitive developments at 2 and 3 years (Villar et al. 1984, Gorman and Pollitt 1992); in Jamaica they had poorer problem solving ability at 7 months and lower development at 15 and 24 months than normal birth weight infants (Gardner et al. 2003, Walker et al. 2004). Effect of intrauterine growth restriction has been reported to continue to adulthood (Strauss 2000) although not in all studies (Li et al. 2004).


Subsequently in adolescent and young adulthood, childhood stunting has been associated with reduced physical performance (Haas et al. 1995) and lower human capital (Victora et al. 2008). Short mothers are more likely to have small children who are likely to grow into short adults themselves (Ramakrishnan et al. 1999, Victora et al. 2008) and low maternal birth weight was associated with lower birth weight in the next generation (Victora et al. 2008).

Childhood undernutrition has also been associated with morbidity later in life, but this is an area of ongoing research. Studies on association between undernutrition and many metabolic diseases (Type 2 diabetes, hypertension and cardiovascular disease) show contradictory results and not many have been done in low income settings (Victora et al. 2008). Foetal undernutrition combined with later quick catch up in infancy appears to be associated with later adult sequelae like glucose intolerance and obesity (Cameron and Demerath 2002). On the contrary, stunting did not predict higher fat deposition in adolescence (Cameron et al. 2005). Results from some studies have shown association between low nutrient intake, mental illnesses and immune
response but not with osteoporosis or cancer. Lung capacity reduction has been seen in low birth weight infants (Victora et al. 2008).

Undernutrition has also been associated with childhood mortality, lethargy, apathy, clinical manifestation of micronutrient deficiency disorders and morbidity from infectious diseases including at least diarrhoea, pneumonia, malaria and measles (Caulfield et al. 2004a, b). These disorders may cause reduced nutrient intake, completing a viscous cycle. What is more shocking is the synergistic effect of mild to moderate malnutrition with the infectious diseases on childhood mortality (Pelletier et al. 1993, Caulfield et al. 2004a, b). In the past few decades 35-50% of world wide under-five year olds’ deaths have been estimated to be directly or indirectly caused by undernutrition (Pelletier et al. 1994, Black et al. 2008). Such serious mortality consequences have been associated with at least stunting, wasting and underweight (Pelletier et al. 1994).

2.5 Epidemiological strategies for promotion of growth and development

Several strategies have been used to promote child growth and development and to prevent childhood undernutrition at different levels which have focused at birth, infants or childhood outcomes. Their target has been to improve the quality and the quantity of general nutrient intake or to reduce morbidity that interacts with nutrition. Some of the notable interventions include health education, micronutrient
supplementation (iron-folate, multiple micronutrient, calcium and zinc vitamin A), food fortification, supplementary and complementary feeding, infectious disease control (deworming, intermittent treatment of malaria, insecticide-treated bed nets), hand washing or hygiene interventions, promotion of breastfeeding, delayed cord clamping and cash transfer.

### 2.5.1 Promotion of general nutrient intake to mothers

Maternal supplementation schemes may be aimed at either building nutrient reserves in preparation for conception or promoting adequate placental transfer of nutrients to the foetus during pregnancy or adequate nutrient transfer through breast milk during lactation period. With necessary improvements, maternal supplementation may be one of the most cost effective methods of tackling undernutrition. Interventions aimed at improving general nutrient intake through maternal intervention so far have largely been found to be effective in promotion of growth and prevention of undernutrition (Bhutta et al. 2008). Maternal supplementary interventions thus far, have focused on provision of micronutrients and general nutrient intake. Notable micronutrients that have been provided to women in low income settings include folate, iron, zinc, iodine, vitamin A and essential fatty acids; details of outcomes of supplementation with these micronutrients will be discussed separately later on. Energy and protein supplementary interventions in pregnancy have been provided together or independently.
In some interventions energy and proteins were balanced and in others protein was high. Prenatal provision of balanced energy and protein intake to mothers were found to reduce small-for-gestation age babies (considered a proxy for intrauterine growth restriction) and to modestly increase birth weight (Kramer 1993, Kramer and Kakuma 2003, Bhutta et al. 2008). Although supplementation in pregnancy was observed to increase birth weight significantly in Gambia, Guatemala and Taiwan (McDonald et al. 1981, Ceesay et al. 1997, Winkvist et al. 1998), other studies elsewhere have failed to show such clearly positive results (Adair and Pollitt 1985, Kramer and Kakuma 2003). Despite small weight difference at birth, later on, especially within in the first 60 postnatal months, energy supplementation during pregnancy was associated with increasingly higher height and weight when compared to non-supplementation (Kusin and Kardjat 1992). This observation may signify the effect of a phenomenon called *programming* (Lucas et al. 1999). Similarly, postnatal supplementation with energy to lactating mother has been shown to have positive effects. It increases breast milk output volume and energy density, especially when supplementation was done to undernourished mothers (Gonzalez-Cossio et al. 1998). On the contrary, provision of protein alone or iso-caloric protein failed to show any positive foetal or birth outcomes. Actually, in some cases data from intervention with high protein supplements were suggestive of harmful birth outcomes (Kramer and Kakuma 2003). These results were suggestive that provision of protein alone, without energy in pregnancy may not have health benefit measurable at birth.

### 2.5.2 Promotion of general nutrient intake to infants
Intervention promoting nutrient intake in infancy aim at promoting exclusive breastfeeding in the first 6 postnatal months which is the ideal food for healthy growth and development at that age. Thereafter, they aim at promoting interventions that promote timely introduction and provision of adequate complementary foods, in conjunction with continued breastfeeding up to 2 years of age or more (Fifty-fourth World Health Assembly 2001, WHO 2003). In undernourished children, supplementary interventions aim at treatment, replacement of lost nutrients and promotion of catch-up growth. Thus, they need to provide for even higher nutrient intake.

2.5.2.1 Promotion of appropriate breastfeeding

In the first 6 months of life, the benefits of exclusive breastfeeding on prevention of childhood morbidity and mortality have been well documented (WHO 2000, Arifeen et al. 2001), thus justifying promotion exclusive breastfeeding up to 6 months of age other than 4 months (Fifty-fourth World Health Assembly 2001). Results from breastfeeding promotion interventions have been encouraging, leading to higher uptake and prevalence, and longer duration of exclusive breastfeeding. These interventions have been associated with reduction in morbidity and mortality in infants and children (WHO 2000, Kramer and Kakuma 2002, Jones et al. 2003, Gau 2004, Britton et al. 2007, Bhutta et al. 2008).

Although 40 % of births in the world still occur at home, the large scale Baby Friendly Hospital Initiative introduced by WHO and UNICEF in 1991 has been
effective in both low and high income countries; the initiative has been associated with improved uptake of breastfeeding by mothers, with increased consumption of breast milk by infants and with reduction in prevalence of childhood symptoms which causes hospital consultation (UNICEF 2007, Walker 2007, Cardoso et al. 2008). Although breast feeding has not been shown to improve weight or length gain more than non breastfeeding infants, exclusively breastfed children have been shown to weigh more than predominantly breastfed infants (Victora et al. 1998). Actually, introduction of complementary feeding before 6 months in low income settings is associated with stunting (Adair and Guilkey 1997). In long term, breastfeeding has also been associated with significantly higher scores for cognitive development (Fewtrell 2007).

2.5.2.2 Promotion of complementary feeding

Supplementary feeding and nutritional education interventions provided together or separately to promote complementary feeding between 6 and 24 months are suggestive of modest effective promotion of growth across different age and ethnic groups, and different food security and socioeconomic backgrounds (Bhutta et al. 2008, Dewey and Adu-Afarwuah 2008). Higher growth impact, however, occurs in food insecure regions (Dewey and Adu-Afarwuah 2008). Most intervention assessing growth promotion efforts used height/length and weight gain as their outcomes, but a few of them also evaluated morbidity and psychomotor development. In some of the studies the largest impact on growth was seen in the younger populations (Schroeder et al. 2002, Rivera et al. 2004). Because the improved growth observed in the intervention groups were often modest, the growth faltering commonly seen in young
children between 6 and 24 months in developing countries is still persistent even with these interventions (Dewey 2001, Shrimpton et al. 2001, Dewey and Adu-Afarwuah 2008). Thus, the impact of these interventions has mostly been to slow the growth faltering but not necessarily stopping it. However, growth and developmental effects from supplementary interventions have been found to be sustainable in many studies and emerging data on long-term follow-up studies suggest that these interventions also promote school performance, individual productivity and economic performance in adulthood (Martorell 1995a, Li et al. 2003a, Stein et al. 2003, Hoddinott et al. 2008).

Caloric supplementary foods were mostly based on cereal and/or legumes fortified with micronutrient, although fortified milk and lipid-based nutrient supplements (LNS) were also used (Dewey and Adu-Afarwuah 2008). For many years there have been concerns of suitability of energy and nutrient density in cereal/legume blend used for the purpose of complementary feeding of 6-24 months old infants (Beaton and Ghassemi 1982, Lutter 2000). Increased energy and nutrient density in porridge made from cereal/legume flour can be achieved by increasing the thickness of gruel or by adding oil. However, thick porridge is considered difficult to feed to infants by most of the mothers. Cheap methods to increase energy density of porridge have not been conclusive either; mixed results were found from 5 studies with efforts to increase the energy density by adding amylase (Dewey and Adu-Afarwuah 2008). On the other hand, increasing nutrient density was associated with reduced breast milk intake (Islam et al. 2006); and edible oils are often too expensive to many poor families in low income countries, thus unlikely to be effective without any
supplementation programmes. In short augmenting nutrient through gruel or porridge intake without advanced technologies is rather difficult.

Except in milk based products or lipid-based nutrient supplements most of the other complementary food provide plant protein from cereal and legumes, especially corn and soy, which are considered to be of lower quality than animal protein. Historically, cereal/soy blends were developed by the U.S. Department of Agriculture (USDA), U.S. Agency for International Development (USAID) and National Institutes of Health and formed the basis of food aid commodities. When introduced by USDA in 1966, they contained non-fat dry milk. As milk availability became difficult in the late 1980s, dairy was removed and thus all protein was derived from plant-based sources (Marchione 2002). Despite being the provider of best legume protein and perhaps appropriate for other age groups, unrefined proteins from soy are difficult for infants and young to digest and contain anti-nutrient factors like phytic acid that limit absorption of mineral micronutrients (Graham et al. 1971, Messina 1999, Hoppe et al. 2008). For many years and using different methods of assessment, including the protein digestibility-corrected amino acid score (PDCAAS) which has been recently adopted by WHO and FAO, unrefined corn and soy protein have been shown to be poorer than that of milk as a source of amino acids (Graham et al. 1971, WHO 2007, Hoppe et al. 2008).

Unlike growth, morbidity results were not universal in complementary interventions. Results on morbidity are mixed with a few reporting reduced morbidity, others
reporting no effect and yet others reporting increased morbidity after the intervention (Dewey and Adu-Afarwuah 2008). Results on developmental outcomes were not conclusive. A few studies reported positive motor development while other showed no difference (Dewey and Adu-Afarwuah 2008).

In recent years, community-based management of severe acute malnutrition with lipid-based nutrient supplement (LNS) called ready-to-use therapeutic food (RUTF) has been shown to be more effective than traditional milk-based management often offered centrally through nutrition rehabilitation units (NRU). Compared to NRU management, the community-based management has been effective in reducing case fatality rates from malnutrition, promoting recovery from undernutrition, promoting weight gain, reducing recurrences and inducing more compliance and acceptability among caregiver and children (Manary et al. 2004, Sandige et al. 2004, Ciliberto et al. 2005, Ndekha et al. 2005, Collins et al. 2006, Bhutta et al. 2008, Dewey and Adu-Afarwuah 2008).

These relatively new products typically contain milk protein, sugar, and a mixture of micronutrients, embedded in a lipid base, need no cooking before use, and can be stored for months even in warm conditions (Briend et al. 1999, Briend 2001). Because they are lipid based, LNS can mask metallic taste of high mineral concentrations; it can be used to deliver micronutrient daily dose ranging from 10 to 100 g. The energy content of LNS is up-to 22 kJ/g (5.4 kcal/g); thus an intake of up to100 g/d can deliver 2200 kJ/d (540 kcal/d). With a dose of 20g/d which is estimated to cost about $0.06,
110 kcal can be consumed (Nestel et al. 2003). It is noteworthy that 110 kcal covers for greater than 10% of daily total energy intake of a child aged 1-2 years.

The LNS used in therapeutic feeding is well accepted by undernourished children, suggesting that similar product could be used as a complementary food supplement (CFS) for prevention purposes and promotion of growth (Nestel et al. 2003). In Malawi, data from dose finding trial suggested that home provision of LNS to moderately underweight 6-17 months old infants improve their nutritional status and results in markedly increased linear growth and improved weight gain (Kuusipalo 2004). In Ghana, these products were shown to promote growth and motor development of infants (Adu-Afarwuah et al. 2007).

### 2.5.3 Promoting micronutrient intake to mothers and infants

There is growing evidence suggesting the nutritional importance of micronutrients. Low nutrient intakes among infant in low income settings are attributable to low intake and low micronutrient density of complementary foods (Kimmons et al. 2005). Thus, some authors have concluded that dietary quality rather than quantity is the key aspect of complementary food diets that needs to be improved (Lutter and Rivera 2003, Allen 2008). One way to improve quality of complementary food is by provision of vitamin and mineral supplements.
Several micronutrient interventions have been implemented either as a single or a multiple micronutrient supplementary intervention or as a single or a multiple food fortification intervention. Because of cost effectiveness as evidenced by lower cost per each disability-adjusted life year (DALY) saved and biological synergism seen with some micronutrient combinations, the multiple micronutrient interventions would be preferable to the single micronutrient interventions in low income settings (Mejia and Arroyave 1982, Homedes 1996, Zimmermann et al. 2004, Horton 2006). Likewise, a centralised fortification would be preferred to the supplementary intervention or community-level fortification interventions (Baltussen et al. 2004, Horton 2006). However, caution should be exercised where the food fortification with a specific micronutrient may pose a health risk by supplementing to subgroups of the population at risk of toxicity or negative interactions (Crane et al. 1995, Smith et al. 2008).

The supplementary interventions, however, may still be preferred where supplementation only targets specific sub-groups of the population, like pregnant women (Werler et al. 1999). Thus far, micronutrient fortification or supplementation programs for folate, iron, zinc, iodine and vitamin A have been widely promoted in low income settings (The World Health Report 2002, Allen et al. 2006); impact from interventions with these micronutrients and those from vitamin D and essential fatty acids intervention are outlined below.
2.5.3.1 Folate

Supplementary folate during peri-conception period has been shown to protect against foetal neural tube defects (NTD) (Rush 1995). Even when food fortification is in place supplementation has been recommended to attain adequate daily intake to prevent NTD (Werler et al. 1999). Folate food fortification has been effective without increasing neurologic damage attributed to vitamin B-12 deficiency masking (Mills et al. 2003). In infants and children, however, folate deficiency is rare and breast milk usually provide adequate intake (Allen 2003).

2.5.3.2 Iron

Iron supplementation and food fortification have been shown to increase haemoglobin concentration in child bearing and pregnant women (Zimmermann et al. 2004, Van Thuy et al. 2005, Bhutta et al. 2008). In childhood, iron supplementation and fortification of complementary food have been associated with haematological, growth and lower morbidity outcomes. Haematological outcomes include reducing prevalence of anaemia, increasing haemoglobin concentration and ferritin concentration in children (Baltussen et al. 2004, Dewey 2007, Bhutta et al. 2008). Lower episodes of diarrhoea and ARI have been reported when iron supplementation has been combined with milk and zinc supplementation (Bhatta et al. 2008). Evidence also shows that iron promotes better cognitive, motor, social-emotional development in supplemented than non-supplemented infants (Lazoff 2007).
2.5.3.3 **Zinc**

Supplementation with zinc has been shown to be effective in increasing zinc intake in children (Sazawal et al. 1996). Among infants and children, several studies have shown that zinc supplementation reduces occurrence of several infections, including diarrhoea, dysentery, acute lower tract infection, and pneumonia (Sazawal et al. 1996, Sazawal et al. 1998, Bhutta 1999). On the other hand impact of zinc fortification of complementary food has not been encouraging. Provision of complementary food supplements fortified with multiple micronutrients, including zinc, however, showed little impact on plasma concentration (Dewey and Adu-Afarwuah 2008), suggesting poor absorption.

2.5.3.4 **Iodine**

Centralised salt iodisation has been shown to be effective in increasing the proportion of houses using iodised salt which has been shown to be associated with increased consumption of iodine in women and children (Melse-Boonstra et al. 1998, Zhao and van der Haar 2004). Health benefits of consuming iodised salt include reduction in occurrence of goitre (Bhutta et al. 2008), increased birth weight (Manson et al. 2002) and increased weight-for-age and length-for-age in children (Manson et al. 2002).

2.5.3.5 **Vitamin D**

Because vitamin D deficiency is mainly a problem above and below latitudes 40°N and 40°S, where the intensity of ultraviolet radiation from sunlight, Vitamin D
deficiency has been considered a problem of industrialised countries, yet insufficient
dietary intake may be as common in low income countries (Zeghoud et al. 1994,
Thacher et al. 1999, Allen 2006). Supplementary and fortification interventions to
lactating mothers or infant and children have been shown to be effective in promoting
vitamin D intake in children (Allen 2006, Wagner et al. 2006). However, in low
income countries children with clinical features of vitamin D deficiency responded
more to calcium supplementation or combined calcium and vitamin D when compared
to those supplemented with vitamin D alone (Thacher et al. 1999).

2.5.3.6 Vitamin A

Centralized food fortification with vitamin A has been shown to contribute
significantly towards intake of the vitamin in the past 3 decades (Guillermo et al.
2008). In a review of effect of vitamin A fortified complementary supplements, more
studies showed significantly higher retinol plasma concentration in infants from the
intervention groups than those from the control groups; however, in those that showed
no difference, investigators attributed the observation to wide spread vitamin
supplementation programmes (Dewey and Adu-Afarwuah 2008). After both
supplementary and fortification interventions, vitamin A has been shown to promote
linear growth, reduce morbidity and mortality, reduce the occurrence of Bitot’s spots,
diarrhoea, respiratory tract infections, measles and their associated mortality (Mejia
1996, Krause et al. 1998). Impact of supplementation with other micronutrients like
iron has been shown to improve when provided together with vitamin A, suggesting
synergistic properties of vitamin A to other micronutrient (Mejia and Guillermo 1982).

2.5.3.7 Essential fatty acids

There has been an increasing evidence indicating that polyunsaturated acids, the essential fatty acids linoleic acid (LA, omega-6) and alpha-linolenic acid (ALA, omega-3), are critical for development and maintenance of structural and functional integrity of the central nervous system and the retina (Uauy et al. 2001, Singh 2005, Innis 2007). These essential fatty acids LA and ALA are precursors of longchain polyunsaturated acids (LCPUFA), including docosahexaenoic acid (DHA, 22:6 n-3); the LCPUFA are one of building elements for membrane, and nervous tissue (Uauy et al. 2001). Supplementary intervention with PUFA or LCPUFA to pregnant or lactating mothers and directly to infants has been shown to increase intake of essential fatty acids (Uauy et al. 2001, Helland et al. 2003, Helland et al. 2006). The impact of the maternal supplementation with essential fatty acids has also been shown to promote growth and development by increasing physical growth (Lauritzen et al. 2005a), visual acuity (Lauritzen et al. 2004b) and intelligence quotient (Helland et al. 2004).

2.5.3.8 Multiple micronutrient supplements

Sprinkles, a vitamin and mineral mix packaged in small sachets containing a daily dose of micronutrients designed to mix with food, have also been tested. Sprinkles
containing iron, vitamin A, zinc and vitamin C have been shown to be effective in treating anaemia and increasing haemoglobin concentration in Ghana (Zlotkin et al. 2003, Adu-Afarwuah 2007).

2.5.4 Prevention and treatment of infections associated with undernutrition

As significant contributors of undernutrition and growth faltering, infections have been targeted as one of the strategies to promote growth. Malaria, diarrhoea, intestinal helminth, and respiratory tract infections which affect a large proportion of under-fives (Snow et al. 2005, Walker 2007) have been major focus for intervention to promote growth in addition to vaccine preventable diseases.

2.5.4.1 Anti malarial interventions

A considerable amount of effort has been devoted anti-malarial interventions in the past few decades. Anti-malarial interventions using insecticide treated bed net (ITN) and regular intermittent presumptive treatment have been shown to be effective in reducing maternal anaemia and increasing the birth weight; in children these interventions have been associated with episodes of clinical malaria, less severe anaemia and fewer hospital admissions (Lengeler 2004, Garner and Gülmezoglu 2006, Gamble et al. 2007, Msyamboza et al. 2007, Bhutta 2008, Meremikwu et al. 2008).
2.5.4.2 *Deworming interventions*

There have been benefits after treating pregnant women, school going children and preschool children albeit lower worm loads occur in preschool children (Stoltzfus et al. 2004). Deworming interventions in pregnancy have been shown to be effective in reducing fall in haemoglobin concentration between first and third trimester and in increasing birth weight (Torlesse and Hodges 2001, Bhutta et al. 2008). Although not an intervention common in the first 12 months, deworming among preschool and school children has been shown to be practical and to promote weight and length gain, and reduce prevalence of anaemia (Stoltzfus et al. 2004, Hall et al. 2008, Alderman et al. 2006, Kirwan et al. 2009, Taylor-Robinson et al. 2009).

2.5.4.3 *Hygiene interventions*

Hygiene interventions have been shown to be effective in reducing episodes of diarrhoea. These include handwashing, water quality treatment, sanitation, and health education (Fewtrell 2005). Improved sanitation resulted in less episode of diarrhoea in Lesotho (Daniels et al. 1991)

2.5.4.4 *Expanded programme on immunization*

Expanded programme on immunization (EPI) introduced by WHO in 1974 has overtime increasingly reported higher coverage, lower morbidity and lower mortality from vaccine preventable diseases (Kim-Farley 1992, Cutts 1998, Arevshatian et al. 2007). High vaccination coverage for measles in low income countries have been
attained in low income countries resulting in reduced morbidity and mortality, although sporadic epidemics have been reported lately (Arevshatian et al. 2007).

2.5.5 General interventions

Although rather different from supplementation and fortification interventions, general approaches aiming at promoting dietary diversification, socioeconomic status and female education are also promising.

2.5.5.1 Dietary diversification

To enhance dietary diversification, agriculture and small-animal production have been promoted and, so far, these interventions have been associated with increase in consumption of animal and economic status of families (Allen 2003b, Bhutta 2008).

2.5.5.2 Caretaker literacy and socioeconomic status

Intervention studies designed to assess causality effect of literacy and socioeconomic status on growth promotion or prevention are rare. Evidence of association between literacy and socioeconomic status mostly come from long-term observations and case control studies. However, higher socioeconomic status and caretaker education (especially maternal education) assessed differently and across different backgrounds
have consistently been associated with better health status and growth in childhood (Fotso and Kaute-Defo 2005, Larrea and Kawachi 2005, Hatt and Water 2006). Improvements in education, purchasing power and access to healthcare facilities overtime have been associated with reduction in undernutrition in Brazil (Monteiro et al. 2009). In rural China rapidly increasing village and household income was associated with increased sanitation, hygiene behaviours and reduced undernutrition (Cangjiang et al. 2009).

2.5.5.3 Health education and behaviour change

Often, nutritional interventions without appropriate behavioural change will have somewhat small effect. To achieve substantial nutritional outcomes, households may have to make substantial changes in their food practices during pregnancy, lactation and weaning periods. Nutritional education to communities and healthcare workers has been shown to reduce underweight (Zaman et al. 2008, Horton 2008, Roy et al. 2008, Monteiro et al. 2009); in pregnant women nutritional counselling has been associated with an increase in haemoglobin concentration and a reduced prevalence of anaemia (Garg and Kashyap 2006).

2.5.6 Concerns of growth promoting strategies

Long term implications of early childhood growth and somewhat negative outcomes of some of the nutrition interventions have raised health concerns. Notably concerns have been raised on breast feeding in HIV infected mother, later age metabolic diseases after supplementary feeding, iron and folate supplementation in malaria
endemic areas, and vitamin A supplementation in pregnancy. Outlined below is a discourse on these concerns.

2.5.6.1 Mother to child HIV transmission

Provision of breastfeeding counselling to HIV positive women is challenging for healthcare workers with background knowledge of mother-to-child HIV transmission through breast milk, especially with some studies suggesting better child survival when infants are fed breast milk substitutes (Mulder 1996, Miotti et al. 1999, Nduati et al. 2000). To the mothers in developing countries HIV brings the difficult choice of balancing between the risk of transmitting HIV to their child through breastfeeding and provision of substantial benefits of breastfeeding. However, overtime evidence weigh more towards child benefits of breastfeeding against the risk of HIV transmission in low income countries where women are left with few options. Most of the trials assessing the benefit of breast feeding substitute provide no evidence of higher negative outcomes among HIV exposed infants who were breastfed to formula fed infants by 7 months of age (Iliff et al. 2005, Thior et al. 2006, Coovadia et al. 2007, Bhutta et al. 2008). Actually, formula feeding was associated with higher mortality in Uganda (Kagaayi et al. 2008).

2.5.6.2 Metabolic syndrome

The hypothesis that intrauterine nutrition and growth can result in foetal adaptation that programme future weight trajectory and project lifetime risk of metabolic
syndrome consequences has been extensively reviewed. Although several studies link birth weight to adult cardiovascular conditions, postnatal upward shift of weight centiles is considered more associated with risk factors of metabolic syndrome (Lucas et al. 1999, Bhargava et al. 2004, Demerath et al. 2007, Victora et al. 2008). Because of this concern, effect of growth promoting interventions on blood lipid, insulin resistance and type 2 diabetes, blood pressure and cardiovascular diseases in adult life are evaluated below. However, very few studies have reported metabolic syndromes as an outcome of early childhood growth promoting intervention in low income countries. While low birth weight is associated with high serum cholesterol values in adults from developed countries, similar associations were not observed in low income countries (Levitt 2000, Stein 2002, Victora et al. 2008). Supplementation in pregnancy and infancy was also not associated with high serum cholesterol levels (Stein et al. 2006).

Insulin resistance and secretion failure are risk factors of type 2 diabetes. Although catch-up growth in infancy and childhood is associated with later insulin resistance (Bhargava et al. 2004, Ong 2004), supplementation to both mothers and infants, and long period of breastfeeding were associated with low fasting plasma glucose in adulthood (Conlisk et al. 2004, Stuebe et al. 2005, Stein et al. 2006). These observations suggest that nutritional interventions promote insulin tolerance and prevent type 2 diabetes. Pooled analysis from low income countries adjusted for adult covariates birth weight, weight for age and body mass index (BMI) at 2 years was inversely associated with adult blood glucose concentration (Victora et al. 2008).
Some authors have reported association of rapid growth in infancy with increase in adult blood pressure or hypertension although weight and height may relate inversely with blood pressure (Cheung et al. 2000, Huxley et al. 2000). Results from low income countries showed that weight and height at 2 years were associated with high blood pressure. However, after adjustment for adult covariates inverse associated was found in some cases (Victora et al. 2008).

In low income countries, obesity (high proportion of fat in the body) is not common. However, because of its relation to other specific metabolic syndrome diseases it is a concern in growth promotion interventions. Birth weight has a bimodal relationship with obesity where higher adult fat proportion is associated with both the low and the high birth weight extremities. Although height, weight and BMI at 2 years were associated with adult BMI, independently linear growth in infancy, body mass index and height at 2 years are associated adult lean mass (Li et al. 2003b, Victora et al. 2008).

Data on cardiovascular disease from low income show that birth weight is inversely associated with prevalence of cardiovascular diseases, albeit few data are available (Stein et al. 1996). Shorter child height and weight are associated with increased risk of cardiovascular disease (Victora et al. 2008).
2.5.6.3 Iron and folate supplementation in malaria endemic areas

Because folate is essential for DNA synthesis and growth of malaria parasite, the parasites acquire folate by de novo synthesis or salvaging preformed folate. Antifolate antimalarial drugs work by inhibiting both the synthesis and the salvaging of the folate from the host (Metz 2007). Hence, folic acid supplements given to pregnant women and children may increase malaria parasites proliferation and may reduce some anti-malarial drug’s effects (Metz 2007). Evidence from Africa suggest that universal supplementation with folate in these areas increases the risk of mortality, the severity of morbidity and treatment failure. (van Hensbroek et al. 1995, Dzinjalamala et al. 2005, Carter et al. 2005, Mulenga et al. 2006, Sazawal et al. 2006). However, targeted folic and iron supplementation combined with treatment of malaria and other infections has been shown to be beneficial to children (Sazawal et al. 2006). There is no evidence suggesting that supplementation in pregnancy may be detrimental either to the mother or to the foetus (Metz 2007). Another concern of universal supplementation with folate is masking of Vitamin B 12 deficiency, albeit rare (Smith et al. 2008).

Evidence on detrimental effect of iron supplementation to young children where malaria is endemic is rather contradictory (Stoltzfus et al. 2007). Some studies and reviews reported an increase in the incidence of clinical malaria, increase in the incidence of other infectious diseases and increase in mortality after universal supplementation (Oppenheimer et al. 2001, Iannotti et al. 2006, Sazawal et al. 2006, Prentice et al. 2007). On the other hand some studies reported no apparent harmful effect or serious adverse event (Oppenheimer 2001, Gera and Sachdev 2002, Verhoef

2.5.6.4 Teratogenicity of vitamin A

Because of teratogenic effects observed in animal studies, those observed after introduction of oral vitamin A analogue isotretinoin for treatment of severe acne in 1983, and some suspicious birth defects reported after high dose exposure in pregnancy, long term high dose vitamin A is better avoided (Rose et al. 1986, Kizer et al. 1990, Rothman et al. 1995). However, low dose vitamin A supplementation may be tolerable even in pregnancy without a risk of teratogenicity and it has been shown to reduce the incidence of maternal mortality by 40% (West et al. 1999, Allen and Haskell 2002, Ross 2002).

2.6 Justification for the present study

Human growth and development is a complex multi-dimensional phenomenon. Hence, there are many causes of growth failure which can be categorised to 2 origins - genetic and environmental. Although the larger part of human growth seems to be determined genetically, environmental factors, especially effective nutrition, are vital for uninterrupted growth. Despite numerous growth promotion efforts, impact on
growth has only been modest thus far, suggesting adaptive genetic down regulation
growth to promote survival in low resources setting. Impact of down regulation of
growth seems to be transferable across several generations, further suggesting that
unwinding this growth faltering process may require interventions that go on to
several generations. However, results of interventions other than growth, especially on
developments, have been encouraging and they may have the effect of accelerating
the attainment of full growth potential in even fewer generations to come.

From the complexity of interacting determinant of growth no single approach seems
feasible to address growth faltering and its associated consequences. However,
adequately addressing complementary feeding for 6 to 24 months infants and nutrition
of pregnant and lactating women are some of the possibly effective ways. The
enormous amount of emerging knowledge on significance of micronutrients in
determining growth, complemented by recent reaffirmation on the need for animal
proteins and adequate energy is vital for development of new dietary strategies for
promotion of effective complementary feeding.

Approaches combining complementary efforts and rigorous morbidity management
seemed to have produced slightly better outcomes, suggesting the significance of
including management of morbidity in new interventions. Social factors, including
background economic status of the families, education levels of the caretakers and
indirect costs like time for food preparation also emerged important. However,
interventions for these are not limited to public health alone rather epidemiologists
need to collaborate with other relevant sectors for such wide approaches.

In the medical principal of “doing no harm”, safety of supplementary effort is a
limiting element to blanket strategies. Cheaper options like centralised fortification of
processed food may not be appropriate as some micronutrients may promote
morbidity to some selected sub-groups. Efficacy of centralised fortification is also
questionable among 6 to 24 months where wide range of complementary food intake
is likely to result in inadequate intake among those consuming small amounts. Again
it may be ethically questionable if such important growth promoting nutrients should
be left to chance purchase in populations living in dire poverty. However, the
potential negative effects of these do not outweigh the potential positive outcomes of
growth promoting efforts, rather, demands careful and evidence based interventions.

While the literature review also emphasises the importance of the age between 6 to 24
months as the window of opportunity to promote growth and to prevent development
of undernutrition or mitigate its effects, the findings also emphasises on the
importance of intervening early within this window. Other than through women’s
nutrition or sanitation and through infection control interventions, the most feasible
intervention to the infants themselves is early complementary feeding from the age of
6 months when breast milk is still very important part of the diet. Therefore, it
remains a public health crucial challenge to identify an effective complementary diet
that provides good quality protein, adequate energy and micronutrients with minimal
risk of displacing breast milk or introducing infections. From the available and identified literature, lipid-based nutrient supplements have most of these properties. It was therefore with this background that the present study and aims were formulated.
3 AIMS

The aim of the present study was to assess the effect of providing lipid-based nutritional supplements (LNS) or corn-soy blend on infant’s or childhood growth and development promotion, and on primary and secondary prevention of undernutrition.

The specific objectives were:

1. To compare growth and recovery from undernutrition among moderately underweight children receiving micronutrient-fortified corn-soy blend (Likuni Phala [LP]) or lipid-based nutrient supplements (LNS) for 12 weeks.

2. To compare growth and incidence of undernutrition in infants receiving long-term dietary supplementation with micronutrient-fortified corn-soy blend (Likuni phala [LP]) or lipid-based nutrient supplements (LNS).

3. To study term growth effect of 12-months supplementation with lipid-based nutrient supplements (LNS) over a subsequent 2-year non intervention period.
4. To compare the effect of a 12-month long complementary food supplementation with lipid-based nutrient supplements (LNS) or corn-soy blend on early childhood development at the age of 18 months
4 METHODS

4.1 Approach to the study

The aim of the study was addressed through two community-based randomised intervention trials conducted in 6 to 18 month old infants. Although the two studies were largely similar, to address specific aims for each study the enrolment criterion and the total time of supplementation were different (Table 1).
Table 1: Comparison of the two trials conducted for the study

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Supplementary feeding</th>
<th>Complementary feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Lungwena</td>
<td>Lungwena</td>
</tr>
<tr>
<td>Enrolment age</td>
<td>6-15 months</td>
<td>6 months</td>
</tr>
<tr>
<td>Trial groups</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Intervention group 1</td>
<td>LNS 50 g/d</td>
<td>LNS 50 g/d</td>
</tr>
<tr>
<td>Intervention group 2</td>
<td>None</td>
<td>LNS 25 g/d</td>
</tr>
<tr>
<td>Control group</td>
<td>LP 71 g/d</td>
<td>LP 71 g/d</td>
</tr>
<tr>
<td>Test dose</td>
<td>LNS 6 g</td>
<td>LNS 6 g</td>
</tr>
<tr>
<td>Primary follow-up</td>
<td>12 weeks</td>
<td>12 months</td>
</tr>
<tr>
<td>Secondary follow-up</td>
<td>None</td>
<td>24 months</td>
</tr>
<tr>
<td>Enrolment nutritional status</td>
<td>WAZ &lt; -2.0</td>
<td>WAZ ≥ -2.0</td>
</tr>
<tr>
<td>Outcome 1</td>
<td>Anthropometry</td>
<td>Anthropometry</td>
</tr>
<tr>
<td>Outcome 2</td>
<td>Haematology</td>
<td>Haematology</td>
</tr>
<tr>
<td>Outcome 3</td>
<td>None</td>
<td>Development outcomes</td>
</tr>
<tr>
<td>Data collectors</td>
<td>Trained</td>
<td>Trained</td>
</tr>
<tr>
<td>Trial registration</td>
<td>NCT00131222</td>
<td>NCT00131209</td>
</tr>
</tbody>
</table>

The supplementary feeding trial was aimed at promoting recovery from moderate underweight; its outcomes were mainly anthropometry and haematological outcomes.

In the other trial which aimed at long-term promotion of complementary feeding in addition to anthropometry and haematological outcomes, developmental outcomes and long-term anthropometric effects were also assessed. Thus, the complementary feeding trial had 3 different sub-studies (Figure 4).
Figure 4: Overall study design for the thesis
4.2 Study setting and subjects

4.2.1 Study Area

The study was conducted in Lungwena area and its neighbouring areas in Mangochi district in the Southern part of Malawi, a low income country in Africa (Appendix 12.1). The area is located on the south eastern shore of Lake Malawi was approximately 20km long and 5km wide. The people were mostly from the Yao tribe and predominantly spoke Chiyao language. Staple food was maize that was grown once a year in rainy season between December and April. Lungwena Health Centre provided free curative and primary health services, including family planning, antenatal and delivery services, growth monitoring, vaccinations, distribution of ITN and treatment of diseases. Those requiring further management were referred to nearby hospitals.

5 Study subjects

The study participants were eligible infants from the study area or it neighbouring villages. In the complementary feeding trial infants turning 6 months were recruited to participate in a 12-month intervention study. On the other hand, the recruitment age
range in the supplementary feeding trial was wider; moderately underweight infants aged 6 and less than 15 months were enrolled to the 12 weeks intervention.

5.1 Nutritional interventions

Control groups in both trials received 71g corn–soy blend called Likuni Phala (LP) in Malawi which was cooked into thin porridge. In both trials there was an intervention group that received iso-energetic lipid-based nutrient supplement (FS50-50 g/d) to the LP. The complementary feeding trial had a second intervention group that received half the dose of lipid-based nutrient supplement (FS25-25 g/d). Likuni phala was purchased from a local producer (Rab Processors, Limbe, Malawi). Lipid-based nutrient supplement was produced at a Malawian non-governmental organization, Project Peanut Butter (Blantyre, Malawi) or Nutriset (Malaunay, France), from peanut paste, milk powder, vegetable oil, sugar, and premade micronutrient mixture (Nutriset, Malaunay, France). All supplements were fortified with micronutrients, but the level of fortification varied between the products (Table 2).

Table 2. Energy and nutrient content of a daily ration of each food supplement used in this trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>LP: 71, FS50: 50, FS25: 25</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>LP: 282, FS50: 256, FS25: 127</td>
</tr>
<tr>
<td>Variable</td>
<td>LP</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>10.3</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>NA</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.1</td>
</tr>
<tr>
<td>Retinol (µg RE)</td>
<td>138</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>43</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>3</td>
</tr>
<tr>
<td>Pantothenic acid (mg)</td>
<td>NA</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.3</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>0.9</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>48</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>NA</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>71</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>NA</td>
</tr>
<tr>
<td>Iodine (µg)</td>
<td>NA</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>5</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>NA</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>NA</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>3.6</td>
</tr>
</tbody>
</table>
5.2 Data collection

5.2.1 Enrolment

For both trials, inclusion criteria included acceptable age, residence in the study area, and an informed consent from at least one authorised guardian. To assess recovery from moderate underweight only those with WAZ < -2.0 were enrolled to the supplementary feeding trial. Exclusion criteria were presence of oedema, history of peanut allergy, severe illness warranting hospitalisation on the enrolment day, concurrent participation in another clinical trial, or any symptoms of food intolerance within 30 minutes after the ingestion of a test dose of lipid-based nutrient supplement, one of the food supplements used in the trial.

The enrolment procedures were similar in both trials. To identify potential participants, trained health surveillance assistants contacted all families who were known to live in the area and have a baby of approximately right age. After initial screening process in the village, infants were invited to an enrolment session at the health centre, where further screening for eligibility took place and guardians were given detailed information on the trial. Before enrolment, a guardian signed a written consent form for trial participation.
For group allocation, blocked randomisation was used in both studies. The guardians to eligible infants picked one from a set of identically appearing opaque envelopes, each containing a paper indicating an identification number and randomly assigned allocation to one of the intervention groups. The randomization lists and envelopes were made by people not involved in the trial implementations and the codes were not disclosed to the researchers or those assessing the outcomes until all data had been entered into a database.

5.2.2 Follow-up

The intervention and follow-up periods were different in the 2 trials and the complementary feeding trial and a long-term postintervention follow-up. However, in both studies participant were visited at home every week when data on use supplements provided was collected together with data on morbidity and other possible adverse events. At each and every other third visit in the supplementary and complementary trials additional food supplements were delivered to the homes. As a way of checking food use within the family, empty food container were collected back to the health centre every week. At regular intervals (every 6 weeks and 4 months in the supplementary and the complementary feeding trials, respectively), the participants underwent a physical examination and anthropometric assessment at the health centre. Haematological tests were limited to enrolment and final health visits only except those for malaria which were done whenever a participant presented with fever at the health centre.
5.2.3 Anthropometric measurements

Anthropometric measurements collected in both trials were weight, length/height, head circumference, MUAC (Appendix 12.2). Weight was measured from lightly naked infants with electronic infant weighing scale (SECA 834, Chasmors Ltd, UK) and recorded to the nearest 10g. Length (at \( \leq 24 \) months of age) and height (at \( > 24 \) months) were measured to the nearest 1 mm with a high quality length board (Kiddimetre, Raven Equipment Ltd, Essex, UK) and a high quality a stadiometer (Harpenden stadiometer; Child Growth Foundation, London, United Kingdom). Mid-upper-arm and head circumferences were measured with nonstretchable plastic tapes (Lasso-o tape, Harlow Printing Limited, South Shields, United Kingdom).

Anthropometric indices (WAZ, LAZ, and WLZ) were calculated with Epi-Info 3.3.2 software (Center for Disease Control and Prevention, Atlanta, GA), based on the CDC 2000 growth reference (Kuczmarski et al. 2000).

5.2.4 Haematological tests

A 2 ml venous blood sample was drawn and serum separated by centrifugation at the beginning and end of follow-up. Haemoglobin concentration was measured from a fresh blood drop with Hemo-Cue® cuevettes and reader (HemoCue AB, Angelholm, Sweden). In the complementary feeding trial only, serum ferritin concentration was analysed from frozen sera with commercial test kits according to the manufacturer’s instructions (Ramco Laboratories, Stafford, Texas, USA).
Thick and thin blood smears were made, stained with Giemsa, and screened microscopically for malaria parasites from symptomatic participants at enrolment. Screening for human immunodeficiency virus infection was done from fresh blood with two antibody rapid tests and positive results were confirmed with DNA amplification technology, according to the manufacturers’ instructions (Determine, Abbot Laboratories, Abbot Park, USA, Uni-Gold, Trinity Biotech plc, Bray, Ireland, Amplicor HIV-1 Monitor Test Version 1.5, Hoffmann-La Roche, Ltd, Basel, Switzerland).

5.2.5 Developmental assessments

Development was assessed in the complementary feeding trial only. The assessments was conducted using the Griffith’s developmental assessment tool for 0-2 year-olds (Griffiths & Huntley 1996). This was adapted by removing some items considered inappropriate in the rural Malawian setting. Local research assistants were trained and certified in assessment and use of the Griffiths by a qualified trainer. The participant’s raw score for the general scale and each subscale were expressed as the sum of all the items scored (Cheung et al. 2008). The sub- and general raw scores were used to find developmental quotients from the Griffith Manual (Griffith & Huntley 1996). The mental age column in the Griffith Scale was used to read the mental age corresponding to attained the subscale raw score, and general mental age was calculated as the mean of all subscale mental ages.
5.2.6 Training and quality control

All data collection was implemented according to standard operating procedures (SOP) developed before commencing the study. All research assistants for the trials were trained on the use of these SOPs as well as the use of questionnaires and food hygiene. The performance of field data collectors were daily monitored by an experienced senior research assistant and the author.

All measurements were conducted by the author or one other colleague. Reliability on anthropometric measurements was assessed for all measurements against an experienced anthropometry measurement expert before the commencement of the trial. Standardization measurements were collected from eight infants. Technical error of measurement (TEM) for all people involved in collecting anthropometric data and coefficient of reliability for length, MUAC and head circumference were calculated from standardisation measurements collected from 8 infants not participating in the study.
5.3 Statistical approach

5.3.1 Sample size calculation

Sample sizes were calculated from expected values for one of the primary outcome (weight gain) and predicted equal standard deviation in comparison groups based on an earlier preliminary dose-finding trial (Kuusipalo et al. 2006). The sample sizes calculation was set to provide the trial with 80% power and 95% confidence. Finally, the sample sizes were adjusted to allow for approximately 5% attrition estimated from previous studies (Kuusipalo et al. 2006).

5.3.2 Data management and analysis

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

Statistical analysis was carried out using Stata 9.0 (StataCorp, College Station, USA) on intention-to-treat basis. Infants with no anthropometric data after enrolment were
only included in the comparison of baseline characteristics. The analyses on anthropometric measures used data from young children who were available at analyses time point, while infants and young children with at least one measurement after enrolment were used to analyze incidence of stunting using survival analysis method to deal with censoring.

For continuous and categorical outcomes, the intervention groups were compared with analysis of variance (ANOVA) or t-test and Fisher’s exact test, respectively. Survival analysis was used to determine cumulative probability of severe or moderate stunting among different groups and the difference were tested by the log-rank test. An event was considered to have happened at mid-point between the time the event was detected and the previous measurement. Individuals with severe or moderate-to-severe stunting already at enrolment were excluded from survival analyses concerning the incidence of that outcome.

For comparison of continuous baseline and final anthropometric and haematological group values paired t-test was used, where as categorical baseline and final variables were compared using sign-test. For the studies of compliance using visits as units of analysis, the Huber-White robust standard error was used to allow for correlated data (multiple visits per child).
5.4 Ethical compliance

Both trials were performed according to the International Conference on Harmonisation/Good Clinical Practice guidelines, and they adhered to the principles of the Declaration of Helsinki. Before the onset of enrolment, both the trials protocols were reviewed and approved by University of Malawi, College of Medicine Research and Ethics Committee and the ethical committee of Pirkanmaa Hospital District in Finland. The details of the protocols were published at the clinical trial registry of the National Library of Medicine NCT00131222 and NCT00131209.
6 RESULTS

The main results of the 2 trials will be presented separately but those for the 3 sub-studies in the complementary feeding trial will be presented rather integrated. The results on supplementary feeding to underweight infants and children will be presented first, followed by main result from the complementary feeding trial. Pooled results on compliance to trial supplements, morbidity and serious adverse event (SAE) will be presented together for the 2 trials.

6.1 Supplementary feeding to moderately underweight infants (study I)

6.1.1 Enrolment and Follow-up

Of the 1657 initially screened infants and children for the study on management of moderate underweight, 367 were too old (aged > 14.99 months), 1010 were above anthropometric cut-off for further evaluation (WAZ > - 1.80). Among the rest, 30 were not brought to the enrolment session, 72 were not underweight (WAZ > - 2.00), and parents of 2 infants declined participation after receiving full information of the trial. The remaining 176 infants and children were randomised into two intervention groups. Two infants had their enrolment day rescheduled because they were too ill on the first pre-planned enrolment day. There was no difference in the mean weight for
age Z-score between the enrolled children and those who were eligible but not enrolled ($P = 0.34$).

Three participants died and another 3 were lost to follow-up before the end of the intervention. The assumed causes of death were anaemia and respiratory tract infection and respiratory tract infection. From the lost participants, one moved away and two were unavailable for final measurements although they had participated in all home visits and received and apparently eaten the intervention food. Comparison of baseline WAZ between participants who completed the follow-up and those who died or were lost did not suggest that they came from different populations ($P = 0.15$ and $P = 0.70$; respectively). The losses to follow-up were also not significantly different between the intervention groups ($P = 0.61$ for deaths and $P = 0.68$ for total loss to follow-up; Fisher’s exact test). Sensitivity analysis to assess robustness of the results after loss to follow-up produced similar standardised weight gain.

### 6.1.2 Background data and compliance to the interventions

At enrolment, participants in the LP group were on average five days younger, 100 g lighter and 0.6 cm shorter than those in the FS group. The prevalence (number of participants) with underweight (WAZ < -2) was 100% (86) in the LP and 99% (89) in the FS group. Comparable figures for stunting (LAZ < -2) were 69% (59) vs. 64% (33) and those for wasting (WLZ < -2) 16% (14) vs. 13% (12), in the LP and FS groups.
groups, respectively. Eight of the participants had a positive HIV-antibody test at enrolment, but only five of them were truly HIV infected, as evidenced by a positive polymerase chain reaction (PCR) test (Table 2 in original publication 1).

### 6.1.3 Growth and recovery from undernutrition during supplementary feeding

After 12 weeks intervention, mean gain in weight was 50 g (95% CI -80 to 174 g) higher in the FS50 group than the LP group. Comparable change for length was 0.2 cm (95% CI: -0.5 to 0.2 cm) lower in the FS group. Correspondingly, mean weight-for-age *Z*-score (WAZ) and weight-for-length *Z*-score (WLZ) increased more and mean length-for-age *Z*-score (LAZ) fell more in the FS50 group than the LP group, but none of these differences reached statistical significance. Average changes in mean mid upper arm circumference, head circumference, and blood haemoglobin concentration were also quite similar in the two groups. All 95% CIs were consistent with only relatively small between-group-differences to either direction.

Because of the limited outcome difference between the groups, we compared the variation in weight gain to the predictions used for sample size calculation. The observed standard deviation for weight gain was 0.38 kg in FS50 group and 0.46 kg in LP group, compared to estimated SD of 0.39 kg in both groups. Whilst the observed variation is slightly higher than expected, the 95% CI of the SD of the whole sample and that of the two intervention groups included the expected value of 0.39 (Jacknife method), suggesting that a marked between-group difference would have been identified with the current sample and trial design if it existed in the population.
During the 12 week intervention, approximately 20% of the initially underweight individuals, 85% of the initially wasted, and 10% of the initially stunted children recovered from their condition, with little difference between the two intervention groups. Correspondingly, prevalence of undernutrition at the end of follow-up (the function of enrolment prevalence, incidence of new cases and recovery during the intervention) was not significantly different across the groups. However, the prevalence of underweight (WAZ < -2.00) was reduced significantly in both groups ($P < 0.001$ for LP and 0.001 for FS50, sign test). A similarly reduced trend was noticed for the prevalence wasting ($P < 0.001$ for LP and 0.07 for FS50, sign test), and low mid-upper arm circumference ($P = 0.02$ for LP and $< 0.001$ for FS50, sign test). The prevalence of stunting ($P = 0.27$ for LP and $> 0.99$ for FS50, sign test) and anaemia ($P = 0.69$ for LP and 0.04 for FS50, sign test) were not markedly changed during the intervention.

At the end of intervention, the mean WAZ increased by 0.22 (95% CI: 0.07 to 0.37) $Z$-score units in the LP group and by 0.28 (95% CI: 0.18 to 0.40) $Z$-score units in the FS50 group (Figure 5a). Increase for mean weight-for-length was 0.39 (95% CI: 0.20 to 0.57) $Z$-score units and 0.52 (95% CI: 0.38 to 0.65) $Z$-score units for LP and FS50 groups, respectively (Figure 5b). No similar shift was observed for length for age $Z$-score units (Figure 5c).
Figure 5: Anthropometric population shift after supplementary feeding

6.2 Supplementation of complementary food to health infants (Study II)

6.2.1 Enrolment and follow-up

Of the 303 initially screened infants, 65 were too old (aged > 6.99 months), 2 were too young (aged < 5.5 months) and 2 were too ill on the day of screening or enrolment. Of the remaining 234 infants, 49 were not brought to the enrolment session (3 infants died, 16 infants moved away, parents of 7 infants were not interested and
we could not get any explanations from parents of 23 infants), and parents of 3 infants declined participation after receiving full information of the trial. The remaining 182 infants were randomized into three intervention groups.

During the 12-month follow-up, 10 infants died and 4 were otherwise lost to follow-up before age 18 months. The success rate of following up to age 18 months was not significantly different between intervention groups ($P = 0.47$; Fisher’s exact test). Only 6 participants had no anthropometric data at all after enrolment and there was no difference in this between intervention group ($P = 0.70$; Fisher’s exact test). The assumed causes for the 10 deaths were infections (diarrhoea, malaria, meningitis, and respiratory tract infection) and injuries (drowning, poisoning). Of the 176 from whom we collected anthropometric measurements 5 did not show-up for developmental assessment. Thus 163 infants participated in the developmental assessment study. Like for anthropometric measurements, no statistically significant differences were observed in proportions of success to follow-up, deaths and dropouts across the trial groups ($P = 0.24, 0.77$ and $0.13$, respectively). Reasons for not showing up were not established.

To assess sustainability of intervention effects after, we continued the follow-up for another 24 months after the intervention. During the 24-month post-intervention follow-up, there were six deaths and 13 further losses to follow-up, leaving 149 participants, who completed the 36 month assessment. Like in during the intervention period, there was no statistically significant difference in the proportion of deaths and
dropouts at 36 months follow-up between the food intervention groups ($P = 0.72$ and 0.26, respectively).

6.2.2 Background data

At enrolment in the complementary feeding trial, mean anthropometric measurements were comparable in the LP and FS50 groups, whereas infants in the FS25 group were on average 250 and 380 g heavier than the FS50 and LP groups respectively. They were also 0.3 and 0.7 cm longer than infants in the other 2 groups, respectively. No participant was severely wasted at beginning; the prevalence (number of infants) of severe underweight in the LP, FS50, and FS25, groups was 2% (1), 3% (2), and 0% (0), and that of severe stunting 3% (2), 7% (4), and 2% (1), respectively. Nine of the participants had a positive human immunodeficiency virus (HIV) antibody test at enrolment, but only 1 of them was truly HIV infected, as evidenced by a PCR test.

6.2.3 Growth during complementary feeding and incidence of undernutrition

Of the 176 participants with anthropometric data, mean gains in weight and length were 100 g (95% CI -143 to 343 g and 0.8 cm (95% CI: -0.1 to 1.7 cm) higher in the FS50 than the LP group. Correspondingly, mean decreases in weight-for-age $Z$-score (WAZ) or length-for-age $Z$-score (LAZ) were smaller among the FS50 than the FS25
or LP infants and there was also a smaller drop in blood haemoglobin concentration in the FS50 group. None of the differences, however, reached statistical significance.

There was an interaction between intervention group (FS50 vs. LP) and baseline LAZ, both using length gain ($P = 0.04$) or weight gain ($P < 0.01$) as an outcome. Among subjects with baseline LAZ below median in this trial (-1.035), the mean gain in length was 1.9 (95% CI: -0.3 to 3.5) cm or 0.40 (95% CI: -0.15 to 0.95) Z-score units bigger in the FS50 than in the LP group. Comparable differences in weight gain were 404 (95% CI: -74 to 735) g or 0.43 (95% CI: 0.07 to 0.80) Z-score units. Among subjects with baseline LAZ above median, the difference in length was -0.4 (95% CI: -1.3 to 0.5) cm or -0.09 (95% CI: -0.58 to 0.39) Z-score units and that in weight was -254 (95% CI: -604 to 96) g or -0.25 (95% CI -0.64 to 0.14) Z-score units. There was no significant interaction between intervention and baseline WAZ ($P = 0.14$).

As secondary end-points, we looked at the proportion of subjects who developed severe or moderate-to-severe underweight, stunting and wasting during the follow-up. The proportion of subjects who developed other forms of undernutrition did not differ markedly between the intervention groups, but severe stunting occurred significantly less frequently in FS50 and FS25 than in LP group. After enrolment, no infant in FS50 group, 4% in FS25, and 13% of LP infants developed severe stunting ($P = 0.01$; Fisher’s exact test). The 95% CI for the difference between FS50 and LP was 4 to 21%.
Cumulative incidence of stunting during the 12-month period was also calculated based on survival analysis methods that dealt with censoring differently from the analysis confirmed that severe stunting developed less often and later in the FS50 and FS25 than in the LP group (0%, 4% and 14% respectively; \( P = 0.01 \), log-rank test). The cumulative incidence of moderate-to-severe stunting was similar in the three groups, but infants in the FS50 group developed the condition on average somewhat later. However, the latter result was not statistically significant (\( P = 0.66 \), log-rank test).

### 6.2.4 Developmental outcomes during complementary feeding (Study III)

The background anthropometric characteristics at developmental assessment and changes between enrolment and developmental assessment were mostly similar. The age at developmental assessment was similar across the groups. The proportion of boys and mean WAZ, LAZ and WLZ were lowest in LP group and highest in FS25. Like in the main growth analysis, changes in anthropometric indices among participants in developmental assessment during the intervention period did not differ significantly between the groups (\( P = 0.80, 0.70 \) and 0.67 for WAZ, LAZ and WLZ, respectively).

The Griffith scores were not different across the groups. The mean (SD) general raw Griffiths scores were 227 (9.0), 226 (11) and 226 (10) for the LP, FS50, and FS25
groups, respectively \((P = 0.90)\). Correspondingly, mean general quotients were 101 (7), 100 (9) and 100(8) \((P = 0.88)\) and mean (SD) mental ages for each group were 17.9 (1.3), 17.9 (1.3) and 17.9 (1.2) months \((P > 0.99)\). Similar comparisons of the trial groups on all the developmental subscales showed no statistically significant differences. There was no statistically significant differences between mean chronological age and general mental age in each of the groups or when all the groups were pooled together; pooled mean difference was 0.01 (95% CI: -0.19 to 0.21).

Exploratory analysis through multiple regression suggested that only the capacity of mothers to write and the participant’s length-for-age Z-score at 18 months of age were significantly associated with the general developmental score \((P < 0.05)\).

### 6.2.5 Post intervention growth after complementary feeding (Study IV)

In the post-intervention follow-up, 4-7% of the participants in each group developed severe stunting, bringing the cumulative 3-year incidence (number of cases) of severe stunting to 20% (11) in LP, 4% (2) in FS50, and 10% (6) in FS25 groups \((P = 0.03, \text{ log-rank test})\). The point estimate for the difference between LP and FS50 was 16% (95% CI: 5% to 26%) and number needed to treat to prevent one case of severe stunting was 6 (95% CI: 4 to 20). Cumulative incidence of moderate-to-severe stunting was similar in the three groups, although infants in the FS50 group tended to develop the condition at on the average somewhat later age \((P = 0.50, \text{ log-rank test})\).
Among the 149 participants who completed the follow-up, mean 3-year gains in weight, height, mid upper arm and head circumferences were highest in the FS50 group and lowest in the FS25 groups. Average values for anthropometric indexes weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) fell in all groups, but the reductions were smallest among the FS50 and largest in the FS25 groups. However, inter-group differences reached statistical significance among unselected children only for WAZ-change. Compared to children in the FS50 group, those getting LP had on average 0.33 (95% CI: -0.39 to 0.69) Z-score units and those getting FS25 0.46 (95% CI: 0.10 to 0.82) Z-score units larger reduction in their WAZ during the follow-up. Similarly, those getting LP had 0.10 (95% CI: -0.23 to 0.43) Z-score units and those getting FS25 0.34 (95% CI: 0.02 to 0.67) Z-score units larger reduction in their HAZ during the follow-up. Adjusting the analyses for baseline weight resulted in similar findings to the unadjusted analyses (data not shown).

Because of our earlier observation showing that there was a strong interaction between child’s length at 6 months of age and his/her weight and length gains during the intervention, we conducted stratified analyses on these outcomes also during the post-intervention follow-up. Largest anthropometric gains were seen in the FS50 group and smallest in the FS25 group, both among children with baseline height below the population median as well as those above it. However, the absolute differences in weight and length were bigger and they more often reached statistical significance in the initially shorter participants. Among these infants, those getting FS50 had on average 0.61 (95% CI: -0.15 to 1.37) kg higher weight gain or 0.53 (95%
CI: 0.07 to 0.99) Z-score unit smaller reduction in WAZ than LP children and 0.91
(95% CI: 0.09 to 1.73) kg higher gain or 0.81 (95% CI: 0.31 to 1.30) Z-score unit
smaller reduction in WAZ than FS25 children. Differences in height gains showed a
similar pattern but did not reach statistical significance.

The differences in height-for-age (HAZ) between LP and FS groups started to develop
after 4 months (when children were 10 months old) and reached a peak 4-8 months
later (at 14-18 months of age). In contrast, differences in weight-for-age or weight for
height increased gradually during the 12-month intervention and continued to grow
wider during the 2-year post-intervention follow-up. The patterns were similar among
participants with baseline height above or below median but absolute values were
more pronounced among those who were shorter at the beginning.

6.3 Haematological changes (Study I and II)

In the supplementary feeding trial, the mean gain in haemoglobin concentration after
12 weeks supplementation was 3.8 g/L in the FS50 group compared to 1.5 g/L in the
LP group; these shifted the group means to 94.5 and 94.8 g/L, respectively. Compared
to enrolment measurements, in all the 3 trial groups, the mean haemoglobin
concentration declined marginally after one year provision of complementary food
supplements the 3 groups. The smallest decline occurred in the LNS groups; <1 g/L, 4
g/L and 7 g/L in the FS50, FS25 and LP groups, respectively. Correspondingly after
one year of complementary intervention, all the three groups had groups had mean
haemoglobin concentration of 106 g/L, 109 g/L and 107 g/L respectively; at most 4g below the cut-off for anaemia in children (110 g/L).

6.4 Compliance to the interventions supplements (Study I and II)

All mothers reported that their children readily ate the provided supplement and diversion of any portion to someone else than the intended beneficiary was reported in less than 1% food delivery interviews, for all the food groups in both trials. Within trial comparisons showed no difference across the groups. From the home visits during which trial products were checked, the percentage of visits found with leftovers ranged from 3% to 10%. Between groups the proportion of leftovers in the supplementary feeding trial were 4.9% and 6.5% in the LP and FS50 groups, respectively ($P = 0.18$). The difference was significant in the complementary feeding trial found to be 2.8%, 9.8%, and 5.6% in the LP, FS50 and FS25 groups, respectively ($P < 0.001$).

6.5 Morbidity and serious adverse events (Study I and II)

All the food products (LNS and LP) were well tolerated by the participants in both trials. None of the eligible participants who received the 6g test dose was allergic to LNS. In addition to the 13 deaths, 6 participants were hospitalised and recorded as
having experienced a serious adverse event (SAE) during the follow-up. In both trials the SAEs were not significantly different between the trial groups. Both the study physician and the data monitoring boards for the trials considered all deaths and SAE unlikely related to the trial interventions.
7 DISCUSSION

The present study was planned to compare the health benefits (promotion of growth and development, primary prevention of undernutrition and secondary prevention of severe acute undernutrition) associated with supplementary provision of either lipid-based nutrient supplement or iso-energetic multiple micronutrient fortified corn-soy blend. The complementary feeding trial tested the effect on primary prevention of undernutrition and promotion of growth and development; on the other hand, the supplementary feeding trial tested the effect on secondary prevention of undernutrition. This chapter presents a discourse on the observed health benefits including growth and developmental outcomes of this study.

7.1 Strength and weaknesses for the study

Several factors contributed to the strength of this study: the study design, population based recruitment, high enrolment rate, high compliance to the study with minimal attrition and minimisation of measurement bias. Group allocation in both trials was random ensuring that the distribution of known and unknown confounding variables was similar in each of the comparison trial groups. The randomisation was perceived successful in both trials based on similarity of baseline group characteristics in each of the trials and hoping that distribution of unknown confounders was also equally successful. The recruitment to the trials was population based in which all guardians
to potential participants in the catchment area were directly invited to participate. Turn up was high further minimising the risk of selection bias. The sample sizes were calculated to provide both trials power of 80% and confidence of 95%; and to mitigate effects of attrition, an additional 5% of the initially calculated sample size was added to each. After enrolment, adherence to the trials were high with few deaths and losses to follow-up across the trial groups. Measurement bias was minimised by training of the data collection team. Reliability indices for the two people who took anthropometric measurements were high. All instruments and laboratory equipment were calibrated regularly, ensuring minimal variability of outcomes.

The trial implementations were highly controlled. All food supplements were delivered at home at appropriated interval for each study. Food use was verified by collecting back empty containers; both nutritional supplements were well accepted with greater 90% consumption reported from all the trial groups. Disease symptoms were monitored every week, and infants found ill referred for treatment at the nearest health centre and where necessary the cost of treatment were met by the study. Mothers were encouraged to continue breast feeding and all infants were still breastfeeding by the end in the interventions.
However, despite the above strengths there are a few limitations on how far we can make an inference from this study. Because in both trials for ethical reasons, there were no non-supplemented control groups, thus conclusions drawn from the study are mainly on comparison of health benefits after provision of lipid-based nutrient supplements or corn-soy blend. Although used widely and for many years in food aid, efficacy and effectiveness of corn-soy blends to vulnerable population has been questioned (Marchione 2002, Hoppe et al. 2008); and furthermore data are scarce. In a review on complementary food supplements it was found that in the past several decades only 2 relatively new studies compared corn-soy blends to no food supplements (Dewey and Adu-Afarwuah 2008). Initially supplied as corn-soy-milk (CSM) because they contained non-fat milk, scarcity of milk in 1980s led to adaptation to corn-soy blend (CSB) without field trials (Marchione 2002). Despite this, we know that the CSB control group in our study provided a reasonable range of protein, with the 2 crops (corn and soy) complementing each others amino acids deficits. However, the protein quality only provided up to 65% PDCAAS. As plant source they also carry anti-nutrients such as phytic acids which may limit absorption of other minerals like zinc and iron. However, compared to no supplementation results from efficacy studies conducted in Malawi, Ghana and South Africa suggest modest growth promotion effects after supplementation with corn-soy blends (Kuusipalo et al. 2006, Lartey et al. 1999, Oelofse at al. 2003); thus we expected the corn-soy blend to have had some growth promotion effect.
Another potential weakness is lack of dietary data during the post-intervention period in the complementary feeding trial. However, we assume that food consumption patterns were distributed equally between the groups because of the randomisation.

It is with this background of the study’s strengths and weakness that conclusion from the findings of the study are drawn. Despite the shortfall on how far the results of this study can be extrapolated, the study design and implementation allow us to make firm conclusions on the comparison between effect of LNS and CSB on promotion of growth and development, and on prevention and treatment of moderate undernutrition.

7.2 Growth promotion and management of undernutrition

7.2.1 Enrolment growth status

At enrolment age (6 months) in the complementary feeding trial as expected, mean weight-for-age and weight for length were consistently normal compared to reference population (Shrimpton 2001). However, length-for-age was rather low with all groups being mildly stunted. Corresponding enrolment indices for the supplementary feeding trial were determined by inclusion and exclusion criteria. Thus as expected the mean weight-for-age \(Z\text{-score} \) were \(< -2\) units and weight-for-length \(Z\text{-score} \) \(> -2\). All wasted
infants were referred for to the health centre for management of acute malnutrition in accordance with the Ministry of Health (Malawi) policy on management of infant malnutrition. Both groups in the supplementary feeding trial were, however, also found to be stunted at enrolment, suggesting that the infants may have been underweight because to stunting rather than wasting.

7.2.2 Promotion of growth

At the end of both interventions, compared to the LP group, mean gain in weight were higher in the group of infants receiving iso-energetic LNS, albeit not statistically significant. Similar gains for mean length were only observed in the complementary feeding trial. There was interaction between baseline length-for-age Z-score versus LNS for weight and length gains in the complementary feeding trial. Significantly higher weight and length gains occurred in those below baseline median length-for-age Z-score than in those above median; suggesting stronger effect of LNS in those already undernourished at the start of trial. Twenty-four months after the supplementary intervention stopped weight and length differences were at least the same or larger as seen after 4 to 12 months and somewhat larger in the LNS groups than in the LP group. These observations were similar either in absolute weight and length values or standardised values and more marked in those with baseline length-for-age Z-score less than median (Figure 3 in original publication III); and they support the notion that the critical time to achieve maximum effect from promotion of
complementary feeding is as early as possible but of course not earlier than 6 months of age (Bhandari 2001, Dewey 2001).

Compared to the LP group, the different upward direction taken by the length trend of the FS50 group as early as 4 month into intervention period is consistent with the idea that FS50 supplementation somehow affected the timing of initiating the childhood growth phase or acceleration in the infants’ linear growth velocity in of the ICP growth model (Karlberg et al. 1994). Such acceleration occurs at an average age of 9 months in industrialized countries, but often much later in low-income settings (Liu et al. 2000, Xu et al. 2002). Each month of delay in childhood growth-spurt is associated with on average approximately 0.5 cm deficits by 5 years in length gain (Liu et al. 2000, Xu et al. 2002).

Factors inducing the childhood growth-spurt in growing infants are unknown at present, but dietary intake of cow’s milk may play a role in the process (Hoppe et al. 2006). Theoretically, it is thus possible, that the observed linear growth differences in our trial were due to the cow milk protein fraction in FS50 (10 g in the 50 g daily dose), an increased intake of which might have led to on average of 4-month earlier induction of the childhood growth-spurt in the intervention compared to the control group. This suggestion, whilst still quite hypothetical, receives some support from the fact that children grew less in the group that received a lower dose of FS (FS25), with same micronutrient content but only half the amount of milk. Furthermore, another recent complementary feeding trial in Malawi, in which same age children were
supplemented with soy-containing modification of LNS (no milk powder) did not
document any length gain acceleration (Lin et al. 2008). Although corn and soy
complement each others limiting deficient amino acids (amino acid tryptophan for
corn and amino acids methionine and cysteine for soy), the PDCAAS of corn soy
blend is only up to 65%. Milk powder and peanut on the other hand have a PDCASS
of 95% each (WHO 2007, Hoppe et al. 2008). Other possible explanations to the
linear growth variation during intervention period include the different concentration
and bioavailability of other micronutrients, e.g. zinc or essential fatty acids, in the
FS50 and corn-soy blend (Brown et al. 2002 Adu-Afarwuah et al. 2007).

Contrary to the linear growth, weight differences between the groups grew larger
especially after the intervention. Whilst part of this may be attributed to the timing of
IC-spurt, other variables are likely to play a role, as well. Possible explanations
include the impact of FS50 supplementation on the children’s general health and
susceptibility to infections, or appetite. Alternatively, the guardians could have
continued providing nutritious snacks to children who had earlier received
snack-like FS50 but not to those, who had received LP. Whilst biologically plausible
explanations, however, we have no data to support any of these possibilities. Another
hypothetical explanation for this is programming; however, it has been suggested that
this phenomenon occurs in utero (Lucas et al. 1999)

Another notable point we would like to highlight from our results is the comparison of
outcomes among children receiving the higher (50 g/d) and the lower (25 g/d) dose of
FS. As the price for such (50 g) a dose (approx 0.2 USD / day) would be relatively high for the rural families in Malawi, we wanted to see if half of the dose would produce the same growth outcomes but more inexpensively. Unfortunately, this was not the case, children in the lower-dose group had both higher incidence of severe stunting and lower mean weight and height gains during the intervention and especially thereafter. A comparable phenomenon has been earlier observed in a Malawian dose-finding trial, where underweight infants were given different doses of LNS for 12 weeks (Kuusipalo et al. 2006). Because a smaller (20 g/d) dose of a similar supplement (Nutributter) yielded positive results on linear growth and motor development among 6-12 month old infants in Ghana (Adu-Afarwuah et al. 2007), the larger dose may thus be more appropriate in the Malawian setting. The apparent discrepancy may be explained by the much higher degree of stunting in the Malawian setting or the fact that the higher dose was not tested in the Ghanaian setting (Adu-Afarwuah et al. 2007).

In the supplementary feeding trial, considering the length of intervention period and timing of observations it is not surprising that no change was observed in length gain. This does not necessarily contradict observations from the complementary feeding trial but rather point toward unsynchronised timing of intervention and anthropometric measurements. Length gain acceleration has often been shown to follow weight gain increase with a certain lag period approximately 3 months in rural Malawi (Maleta et al. 2003b). Mean gain in head circumference and mid-upper arm circumference were almost comparable among the groups in each of the trials.
In the supplementary feeding trial, gains for mean weight-for-age and weight-for-length \( Z \)-score were higher in the LNS group than the LP groups, but not significantly so. There was a somewhat decrease or no change in length-for-age \( Z \)-score. Correspondingly, there was good proportion that recovered from wasting and underweight but not as much on stunting. These observations suggest that either both supplementation schemes were effective in promoting weight gain or it is an effect from a confounding variable equally distributed in the trial groups such as seasonality; or from regression to the mean phenomenon. A comparison to earlier studies in the same area suggest that unsupplemented underweight children of this age would have a rather lower weight gain velocity (Kuusipalo et al. 2006) and that seasonal effect on weight gain would be markedly lower than the approximately 0.3 \( Z \)-score unit increase observed in this trial (Maleta et al. 2003b). These increases in standardised weight, however, are comparable to those observed after other supplementary feeding intervention to similar target groups (Simondon et al. 1996, Larney et al. 1999, Becket et al. 2000, Bhandari et al. 2001, Oelofse et al. 2003, Adu-Afarwuah et al. 2007). These results on recovery from undernutrition supports findings from other studies and programmes suggesting that LNS promotes recovery from moderate wasting and underweight (Patel et al. 2005, Defourny et al. 2007, Matilsky et al. 2009); thus acting as a secondary prevention of severe acute undernutrition.
7.2.4 Prevention of undernutrition

Although the long-term LNS and LP supplementation failed to prevent plummeting of mean weight-for-age, length-for-age and weight-for length Z-scores which are common in infants aged 6 to 24 months in developing countries (Shrimpton 2001), the decline was slowest in participants in the LNS group, and markedly so among those with baseline length for age Z-score at enrolment (Figure 4, in original article III). The corresponding incidence of stunting was lower in the group provided with LNS than those provided with iso-energetic LP or half the dose of LNS. During the intervention period no case of severe stunting occurred in the group receiving high dose LNS, 4% in group receiving half the dose of LNS and 14% in the group receiving LP. Postintervention assessment showed that incidence of severe stunting continued to be significantly lower in the LNS groups than the LP groups, suggesting that the observed results were sustainable (4 %, 10% and 20% for the FS50, FS25 and LP groups, respectively). These observations were confirmed in a survival analysis that dealt with censoring differently.

Despite the trends of weight-for-age and weight-for-length Z-score being similar to that length-for-age Z-score, incidence of underweight and wasting were similar in all the trial groups. As weight responds more to daily body composition, temporary body composition changes may have influenced the incidence of underweight and wasting while not having the same impact on much more stable measure like height and the trends.
After a rather high quality implementation of a supplementary trial we only managed to mitigate the decline but not necessarily stop the pattern of declining anthropometric indices commonly seen between 6 and 24 months in developing countries (Shrimpton 2001). The possible explanation for this could be inadequate energy and nutrient intake, disease burden, or predetermined down regulation programming occurring before the intervention period. All infants were breastfeeding up to 18 months therefore they received an estimated of 413, 379 and 346 kcal/d for ages 6-8, 9-11 and 12-23 months (WHO 1998, Lutter 2003). With the LP and FS50 providing 282 and 256 kcal/d and the daily energy needs for the 3 age-ranges being 615, 686 and 894 kcal/d, it means the breast milk plus the LP or FS50 provided over 100%, 93% and 70% for the these age ranges (WHO 1998, Lutter 2003). These infants were also on traditional household complementary foods, thus it unlikely that they were deficient of energy during the intervention. Within the same group of participant there was no evidence of breast milk intake displacement at for the first month after starting the provision of the supplements (Galpin et al. 2006), although it has been reported elsewhere (Bhandari et al. 2001). The distribution of days with fever or reported diarrhoea or respiratory tract infections were equally distributed across the study groups. The mean number of days with diarrhoea was 5 and the total mean days with fever was 11 days per person per year. All morbidity cases were rigorously identified through weekly home visits and mother were encouraged to go for medical care if participants were found ill. It is therefore again unlikely that infections either directly or through competing for nutrients explain the growth faltering. All the supplements were fortified with multiple micronutrients suggesting that micronutrient deficiency is also unlikely. The presence of anti-nutrients in corn soy blend, however, may explain
the relative difference between LP and FS50 but not the general decline in all groups. Of course, all groups could have been receiving anti-nutrients from their traditional complementary food stuff. Theoretically, the growth response to the supplementation may have been constrained by predetermined prenatal *programming* or intergenerational effect of maternal stunting (Dewey 2001). The explanation for the persistent growth faltering despite the intervention is difficult to establish from this study; thus, these results call further research to evaluate this question further.

7.3 Promotion of recovery from and prevention of anaemia

At enrolment, infant in the supplementary feeding trial were anaemic with the mean group haemoglobin concentration of 93 g/L and 91 g/L for the LP and FS50 groups. Those in the complementary feeding trial were 114 g/L, 106 g/L and 113 g/L for the LP, FS50 and FS25 groups. Haemoglobin of less than 110 g/L defines anaemia in this age group (Allen et al. 2006). In the supplementary feeding trial, the mean gain in haemoglobin concentration was 3.8 g/L in the FS50 group compared to 1.5 g/L in the LP group. Provision of micronutrient fortified supplements to anaemic population to induce induces population recovery from anaemia. However, the provision period may have not been long enough to reach the cut of point. All haemoglobin concentration declined modestly after one year provision of complementary food supplements the 3 groups. The smallest decline occurred in the LNS groups; <1 g/L, 4 g/L and 7 g/L in the FS50, FS25 and LP groups. All the groups had mean haemoglobin concentration greater or equal to 106 g / L, reasonably close 110 g/L, the cut-off for anaemia. This finding suggests that all supplementary schemes may have
had an effect on haemoglobin concentration. However, without non-supplemented control group this interpretation needs to be considered cautiously as these finding may be coming an equally distributed confounding factor.

7.4 Developmental outcomes after complementary feeding

Developmental assessment was conducted in the complementary feeding trial only. In all its 3 trial groups the developmental findings at the end of intervention were equal. These results are similar to those observed in other studies where food supplements fortified with multiple micronutrients produced comparable developmental outcomes (Black et al. 2004, Adu-Afarwuah et al. 2007). After a six-month supplementary intervention from the age of 6 months, a trial in Ghana showed no significant differences between micronutrient fortified LNS and micronutrient powder or micronutrient tablets in promoting motor development (Adu-Afarwuah et al. 2007). Another trial in Bangladesh showed no significant difference in psychomotor development when a group receiving multiple micronutrients was compared to a group receiving combined iron and zinc supplements (Black et al. 2004). However, all micronutrient fortified supplements in Ghana, and multiple micronutrient or combined iron and zinc supplements in Bangladesh promoted motor development more than non-supplementation or placebo groups, respectively (Adu-Afarwuah et al. 2007, Black et al. 2004). Several other studies have also shown that micronutrient fortification of supplements (Moffat et al. 1994, Harahap et al. 2000, Olney et al. 2006,) or unfortified supplementation (Husaini et al. 1991) promotes neurobehavioral development more than non supplementation in infants.
The 3 food supplements used in this study had different levels of essential fatty acids: The corn soy blend, FS50 and FS 25 provided an additional 0.3, 0.8 and 0.4 g of alpha linolenic acid (ALA) per day and 2.7, 7.3 and 3.6 g of linolenic acid (LA), respectively. A differential impact on development was plausible as essential fatty acids are needed for brain growth (Helland et al. 2003, Innis 2008 and 2009). It is possible, however, that with the additional essential fatty acids provided by breast milk, the overall difference of intake was not sufficient to have an influence on development quotient. The recommended adequate intake of LA and ALA was 4.6 and 0.5 g/d (Food and Nutrition Board 2005), and breast milk was estimated to provide at least 4.4 and 0.4g/d given the minimum intake was 830 g/d (Galpin et al. 2006).

Although with lack of a non-supplemented control group it is difficult to explain what were the independent effects of the supplementation schemes, the fact that the participants attained mean general mental ages comparable to their own chronological age is, however, consistent with the possibility that all the schemes were effective. This possibility is further supported by the notion that the observed mental ages were similar to those from the reference population of the Griffiths Scales (Black et al. 2004) and those attained by British children of similar age in a different study (44). Because the study area had a high prevalence of stunting, malaria is endemic and income-level was low (UNICEF 2008, Maleta et al. 2003c), it was unlikely that the participants from these living conditions would attain higher developmental outcomes when compared to reference population from a high socioeconomic setting (Olney et
al. 2007, Siegel et al. 2005, Cheung et al. 2001, Grantham-McGregor et al. 2007, Kariger et al. 2005). In a study mentioned earlier, the Ghana trial, using a somewhat similar approach to ours, a difference in motor development was noticed in an analysis that compared no intervention to one with lipid-based nutrient supplements but not between two different supplement types (Adu-Afarwuah et al. 2007).

The adaptation method of the locomotor and personal social subscales for use in rural Malawian community resulted in lower possible scores than would typically be in those subscales. Therefore, the attained raw score were likely to be lower than could normally be attained. No specific correction was performed on the raw score or the corresponding mental ages in these subscales. Thus, the lower mental ages than chronological ages attained in these specific subscales are likely or partly explained by the adaptation of the Griffith tools. The lower mental ages in these subscales, however, should not have affected our group comparison as the adaptation equally affected all the groups.
The present study was planned to compare the health benefits (promotion of growth and development, primary prevention of undernutrition and secondary prevention of severe acute undernutrition) associated with supplementary provision of either lipid-based nutrient supplement or iso-energetic multiple micronutrient fortified corn-soy blend. After the implementation the results show that

1. Supplementary provision with lipid-based nutrient supplements and corn-soy blend to moderately undernourished 6 to 15 months old infants have similar impact on weight and length gain in low income settings. The results are also consistent with the notion that both interventions provided for 12 weeks only promote recovery from wasting and underweight but not stunting.

2. Compared to iso-energetic corn-soy blend, 12 months supplementary provision with lipid-based nutrient supplements to otherwise healthy 6 months old infants induce earlier accelerated childhood growth phase of the ICP growth model, with subsequent higher length and weight gains in the next 36 months. Provision of LNS at half the energy and the milk dose does not induce similar growth response when supplemented to initially mildly stunted population.
3. Long-term provision with lipid-based nutrient supplements but not with corn-soy blend protects against the incidence of severe stunting. However, neither supplementation with LNS or CSB arrests the growth faltering commonly seen between 6 and 24 months completely.

4. Provision of supplementary food fortified with multiple micronutrients, including iron to mildly anaemic population of infants induces population recovery; and when provided to non-anaemic infants for a long period they have the potential to protect the population from gross manifestation of anaemia.

5. Developmental status of groups of infants after one provision of micronutrient fortified complementary food supplements is similar.
This study shows that provision of infants with high dose lipid-based nutrient supplements (LNS) achieves better growth rates than provision of low dose LNS or corn-soy blends, especially among those already mildly stunted and prevent development of stunting. Developmentally, their psychometric outcomes are not any worse than reference population or any better than those in the CSB control group. This may be one of possible solution to prevent development of undernutrition in low in come countries, especially stunting which is associated with negative human capital development. Failure to achieve completely normal growth rates during the challenging period of infant growth with such a well implemented study, apparently calls for more comprehensive approach such as maternal interventions as well.

The most effective public health strategy would be that which can be taken up by the communities with minimal support from donor funds. The estimated ex-factory price of LNS when made in Malawi is U.S. $ 4.00 / kg, corresponding to a daily cost of U.S. $ 0.20 for a 50 g dose. Unsubsidized, this price is presumably unaffordable to many families in rural Malawi or in other low income countries. Therefore, this strategy is unlikely to be taken up by the communities without some kind of donor or social support. Considering long-term effect of growth promotion intervention which have been linked with better human capital it may still be cost effective approach despite the high cost. Potential means for increased affordability include amendments
to the spread recipe (e.g. cheaper protein source) and social marketing.

Hypothetically, another way may be to vary dose based on prevalence of stunting at 6 months. In Ghana where prevalence of stunting at 6 months was lower, a 20 g daily dose of LNS was found to be effective in promoting at least growth (Adu-Afarwuah 2007). Before implementing such a titre strategy aimed at lowering implementation cost, this hypothesis need to be confirmed by clinical trials.

For populations where the focus is prevention of anaemia or promotion of development only cheaper strategies of providing multiple micronutrients like the Sprinkles need to be evaluated. Fortified corn-soy blend may be an equally good option for promotion haemoglobin concentration and secondary prevention of severe acute undernutrition.

Therefore, the findings of this study justify the need of several studies to find ways to achieve full infant growth potential and to address the limited inference level of this study.

1. An integrated intervention combining this approach with a trial arms that promote reduction of prevalence of stunting at 6 months is justified. Growth faltering seems to be programmed in utero or in early infancy. Such kind of trial would target promoting pregnancy outcome or quality of breast milk
2. Interventions aimed at reducing cost of LNS are justified. Substituting milk protein with soy protein extract is one option. Similarly, improving the quality of corn soy blend by adding milk or using soy extracts is another option.

3. Trials using different doses of LNS doses titrated against baselines prevalence of stunting at 6 months are justified.

4. To firmly make inferences on these finding trials with non-supplemented control arms are warranted.
The study was carried out at the Department of International Health, Tampere University Medical School and at the Department of Community Health, College of Medicine, University of Malawi between 2004 and 2009.

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12 APPENDICES

12.1 Geographical and demographic profile of Malawi

Malawi has a total area of 94,276 km$^2$ and is located mostly on the western side of Lake Malawi and both eastern and western sides of Shire river that drain water form Lake Malawi. The lake itself is the south most lake within the Great African Rift Valley. Malawi has a total population of 12.8 million with annual growth rate 2.1%. The population of under fives was 2,340,000. The crude birth and death rates were 43 and 21 per 1000 population, respectively. The life expectancy at birth was at 40 and the total fertility rate is at 5.9. The population living in urban areas was 17% and mean growth rate of urban population was 4.6%. Maternal mortality ratio was reported at 980 per 100,000. The latest adjusted maternal mortality ratio for under-reporting and misclassification was at 1800 per 100,000 live births in 2000 and its corresponding lifetime risk of maternal death was 1 in 7.

12.1.1 Health indicators

Up to 73% of the population was drinking water from improved sources and 61% used sanitation facilities. Immunisation coverage was high; DPT 1 and DPT3 were 99 and 93% respectively, polio was 94%, measles was 82%, hepB3 and Hib3 were at
93%. Twenty percent of under-fives slept under bed net and similar age group sleeping under treated bed nets were 15%. Among the under fives with fever receiving anti malarial drugs was 28% and those with diarrhoea receiving Oral Rehydration Salts (ORS) was 51%. The estimated adult HIV prevalence rate was 14.1%.

12.1.2 Nutritional indicators

In Malawi 16% infants were born with low birth weight. Prevalence of exclusive breast feeding for less than 6 months was 56%, but 89% were breast fed combined with complementary feeding between 6 and 9 months and 73% of children aged 20 to 23 months were still breast fed. Among under-fives, 22% and 5% were moderate-to-severe and severe underweight, respectively. Correspondingly, 5% and 48% were moderate-to-severe wasting and stunting, respectively. Vitamin A supplementation coverage was at among 6-59 mo old was at 57% and 49% of households consumed iodised salt.

12.1.3 Economic indicators

Malawi is one of the low income countries with it economy relying on agriculture exports. At the time of the study gross national income (GNI) was at 160 US$ per
GDP per capita mean growth rate was at 1.0%. The mean annual rate of inflation was at 29% and 42% of the population lived on less than 1 US$ per day. Of the total annual budget 7% was spent on health, 12% on education and 5% on defence. The net official development assistance (ODA) inflow was at 476 million US$ that contributed to 23% of the GNI. The countries debt service as percentage of exports of goods and services was at 6%.

12.2 Anthropometric measurements

12.2.1 Height

Height (stature) is uni-dimensional measurement that indicates the length of long bone of the lower limb and bones in the vertebra column. Length is measured in supine position up to 24 months using a fixed board then standing after height is measured in standing position using a stadiometer. During growth period height is dependent on age and sex. For several reasons, including malformations, deformations, and congenital disorders, an individual may become dysmorphic resulting in abnormal stature. In the absence of dysmorphism extreme variations of normality, prenatal problems, malnutrition, psychological deprivation, chronic diseases, treatments and endocrines disorders may be responsible for short stature.
12.2.2 Weight

Weight, a measure of body mass, is dependent on height and body composition (total body water, muscle mass, total lean body mass, fat) in addition to age and sex. Because of its dependency on body composition which may vary within a short period of time, weight changes may be observed within as short period as a day. Weight is measured using scales of preferred accuracy with minimum clothing or appropriately adjusted for if clothes are on.

12.2.3 Head Circumference

Head circumference is a proxy to measuring the brain size. Out of normal range head circumference measurements help in diagnosing brain abnormalities like hydrocephalous or microcephaly. Head circumference is measured using non-stretching tape measures.

12.3 Mid-Upper Arm Circumference

Mid-upper arm circumference is a proxy of measuring the amount of fat layer in the arm. It is a simpler way of assessing wasting or weight for height. Arm circumference
grows very fast in the first year, thereafter it remains the same up to 60 months; making MUAC an important to assess wasting. MUAC is measured using non-stretching tape measures.
Supplementary feeding with fortified spread among moderately underweight 6–18-month-old rural Malawian children

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Abstract

We aimed to analyse growth and recovery from undernutrition among moderately underweight ambulatory children receiving micronutrient-fortified maize–soy flour (Likuni Phala, LP) or ready-to-use fortified spread (FS) supplementary diet. One hundred and seventy-six 6–18-month-old individuals were randomized to receive 500 g LP or 350 g FS weekly for 12 weeks. Baseline and end of intervention measurements were used to calculate anthropometric gains and recovery from underweight, wasting and stunting. Mean weight-for-age increased by 0.22 (95% CI 0.07–0.37) and 0.28 (0.18–0.40) Z-score units in the LP and FS groups respectively. Comparable increase for mean weight-for-length was 0.39 (0.20–0.57) and 0.52 (0.38–0.65) Z-score units. Recovery from underweight and wasting was 20% and 93% in LP group and 16% and 75% in FS group. Few individuals recovered from stunting and mean length-for-age was not markedly changed. There were no statistically significant differences between the outcomes in the two intervention groups. In a poor food-security setting, underweight infants and children receiving supplementary feeding for 12 weeks with ready-to-use FS or maize–soy flour porridge show similar recovery from moderate wasting and underweight. Neither intervention, if limited to a 12-week duration, appears to have significant impact on the process of linear growth or stunting.

Keywords: fortified spread, infants and young children, randomized controlled trial, supplementary feeding, moderately underweight, undernutrition.
Introduction

In Malawi, like most low-income countries, childhood malnutrition is very common. Between 22% and 48% of Malawian children are undernourished by the age of 18 months (National Statistical Office & ORC Macro 2001). The peak incidence is between 6 and 18 months of age and moderate underweight is the most common presentation (National Statistical Office & ORC Macro 2001; Maleta et al. 2003a). Apart from causing acute morbidity and adverse long-term sequelae, malnutrition is estimated to contribute to approximately half of the worldwide deaths in children under 5 years of age (Pelletier et al. 1995; Caulfield et al. 2004).

The epidemiology of undernutrition in Malawi necessitates emphasis on prevention or early home-based management of children who have developed signs of malnutrition but who do not yet require hospitalization. There are, however, no easy options for such approaches as infection control has not proven widely successful and not many indigenous foods are rich in all the nutrients or available throughout the year (ACC/SCN 2001). Furthermore, widespread poverty in many communities reduces the possibility to purchase commercially available complementary foods. Thus, there is a need to develop and test new and inexpensive food supplements that could be easily used at the community level.

Cereal and legume mixtures that resemble the indigenous diet and are prepared in a manner similar to staple food have often been recommended for supplementary feeding of moderately undernourished children (Maleta et al. 2004). One such item is porridge made of vitamin and mineral-enriched maize–soy flour [Likuni Phala (LP)], but data on its actual impact to the children’s growth and nutritional status are scarce. Another option are micronutrient-fortified, high-energy spreads (ready-to-use therapeutic foods, RUTF) that have proven very beneficial for severely malnourished children both in famine and non-famine situations (Briend et al. 1999; Briend 2001; Collins 2001; Diop et al. 2003; Manary et al. 2004; Sandige et al. 2004; Ciliberto et al. 2005, 2006; Ndekha et al. 2005). Similar products have recently been given also to less severely wasted children (Patel et al. 2005; Defourny et al. 2007), and recent data from a preliminary dose-finding trial in Malawi suggested that they might improve growth also among moderately underweight young individuals (Kuusipalo et al. 2006). Thus far, however, the effects of fortified spreads (FS) and maize–soy flour have not been compared in this target group with a randomized controlled trial.

To more comprehensively analyse the efficacy of FS and maize–soy flour in promoting growth and recovery from malnutrition among moderately underweight 6–18-month-old children, we conducted a randomized controlled, single-blind trial where moderately underweight children were provided for 12 weeks with weekly supplementary rations of either maize–soy flour or FS. The present communication reports mean growth, recovery from malnutrition and change in blood haemoglobin concentration among 6–18-month-old children getting the two supplements.

Methods

Study area and timing

The study was conducted in Lungwena, Mangochi district of Malawi, South-Eastern Africa. In Lungwena, exclusive breastfeeding for 6 months is almost non-existent and infant diet is typically complemented with thin maize porridge (‘phala’, 10% dry weight of maize flour) as early as from 2 to 6 months of age. Underweight and stunting are very common: in a recent prospective cohort study of 813 newborns, underweight [weight-for-age Z-score (WAZ) < −2] and stunting [length-for-age Z-score (LAZ) < −2] prevalence was 10% and 50% by 6 months of age and 40% and almost 80% by 18 months of age respectively (Maleta et al. 2003a). The climate in the area has one rainy season between December and March during which the staple food maize is grown. Enrolment to the trial was performed during the growing season when food levels were at the lowest, i.e. in March 2005. The 12-week follow-up of the last participant ended in July 2005.
Eligibility criteria, enrolment and randomization of the trial participants

Inclusion criteria for the trial included age of at least 6 months but less than 15 months, low WAZ (WAZ < −2.0), assumed residence in the study area throughout the follow-up period and signed informed consent from at least one authorized guardian. Exclusion criteria were severe wasting, weight-for-length Z-score (WLZ < −3.0), presence of oedema, history of peanut allergy, severe illness warranting hospitalization on the enrolment day, concurrent participation in another clinical trial or any symptoms of food intolerance within 30 min after the ingestion of a 6-g test dose of FS, one of the food supplements used in the trial (given to all potential participants to exclude the possibility of peanut allergy).

For enrolment, trained health surveillance assistants from a local health centre contacted families, who were registered to live in the study area and known to have a baby of approximately right age. Infants and children, whose ages were appropriate and verified from an under-five-clinic card, were weighed in their homes and those who were moderately underweight or near the cut-off point for it (WAZ < −1.8) were invited for further screening at the health centre. At the enrolment session, guardians were given detailed information on the trial contents and the infants and children were fully assessed for eligibility.

For group allocation, consenting guardians of eligible participants were shown and asked to pick one from a set of 10 identical opaque envelopes containing information on the group allocation of the participant. Because one set had to be finished before using the next, for each block the first guardian chose one from a total of 10, the second chose one from a total of 9 and the 10th picked the last envelope. The blocked randomization list and envelopes were created by people not directly involved in trial implementation and the code was not broken until all data had been collected and entered into a database. The actual randomization was done with a tailor-made computer program, using random number and rank functions of a Microsoft Excel spreadsheet.

Table 1. Energy and nutrient content of the daily ration of the food supplement used in the trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group</th>
<th>FS group</th>
<th>Recommended nutrient intake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>71 g</td>
<td>50 g</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>282</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>10.3</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>n/a</td>
<td>13.8</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.1</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Retinol (µg RE)</td>
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<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>43</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Panthothenic acid (mg)</td>
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<td>2</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
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<td>0.5</td>
<td>0.5</td>
</tr>
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<td>Thiamin (mg)</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
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<td>Vitamin B6 (mg)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
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<td>0.9</td>
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</tr>
<tr>
<td>Vitamin C (mg)</td>
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<td>30</td>
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<tr>
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</tr>
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<tr>
<td>Selenium (µg)</td>
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<tr>
<td>Zinc (mg)</td>
<td>3.6</td>
<td>8.4</td>
<td>8.4</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; n/a, information not available; * FAO & WHO 2002.

Interventions and follow-up

Trial participants received one of two interventions: An average of 71 g day^{-1} of micronutrient-fortified maize–soy flour (LP) or an average 50 g of micronutrient FS. The supplements were home-delivered to the participants at weekly intervals (either 500 g LP or 350 g FS at each food delivery). LP was packed in bags of 500 g and was purchased from a local producer (Rab Processors, Limbe, Malawi). Fortified spread was produced and packed in 50 g daily dose packets by Nutriset (Malaunay, France). Ingredients of LP were maize flour, soy flour and micronutrients and those of FS were peanut butter, milk, vegetable oil, sugar and micronutrients (Table 1).

FS could be eaten as such, whereas the maize-soy flour (LP) required cooking into porridge before consumption. The guardians were provided with spoons and advised to daily offer their infants or children either a packet of FS (FS group) or porridge containing 12 spoonfuls of maize-soy flour.
(LP group). All mothers were encouraged to continue breastfeeding on demand and to feed their children only as much of the food supplement as the child wanted to consume at a time (i.e. using responsive feeding practices and recognizing signs of satiety).

The participants were visited weekly at their homes to collect information on supplement use and possible adverse events. Empty food containers were collected every 3 weeks. At 6 and 12 weeks after enrolment, the participants were invited to a local health centre where they underwent physical examination, anthropometric assessment and laboratory tests.

**Measurement of outcome variables**

The primary outcome of the trial was weight gain during the 12-week follow-up. Secondary outcomes comprised length gain, mean change in anthropometric indices WAZ, LAZ and WLZ, recovery from moderate underweight, stunting or wasting, change in mid upper arm circumference (MUAC) and change in blood haemoglobin concentration.

Weight was measured from naked children with an electronic children weighing scale (SECA 834, Chasmos Ltd, London, UK) and recorded to the nearest 10 g. Length was measured to the nearest 1 mm with a high quality length board (Kiddimetre, Raven Equipment Ltd, Essex, UK). MUAC and head circumference were measured to the nearest 1 mm with non-stretchable plastic tapes (Lasso-o tape, Harlow Printing Limited, South Shields, Tyne and Wear, UK). All measurements were done in triplicate by one author (JP), whose measurement reliability was assessed at the start of the study and who was blinded of the participant study allocation from enrolment to the end of follow-up. All instruments were calibrated every day before the measurements. Anthropometric indices (WAZ, LAZ and WLZ) were calculated with Epi-Info 3.3.2 software (CDC, Atlanta, USA), based on the CDC 2000 growth reference (Kuczmarski et al. 2002), which was the latest available reference material at the time of enrolment to the trial.

A 2 ml venous blood sample was drawn and serum was separated by centrifugation at the beginning and end of follow-up. Haemoglobin concentration was measured from a fresh blood drop with Hemo-Cue® cuvettes and reader (HemoCue AB, Angelholm, Sweden).

All participants were tested for malaria parasites and human immunodeficiency virus (HIV) infection at the beginning of the trial. Thick and thin blood smears were made, stained with Giemsa and screened microscopically for malaria parasites from all participants. Screening for HIV-infection was done from fresh blood with two antibody rapid tests, according to the manufacturers’ instructions (Determine, Abbott Laboratories, Abbot Park, IL, USA and Uni-Gold, Trinity Biotech plc, Bray, Ireland). Dried filter paper blood samples from participants with a positive result in either of the rapid tests were further tested with DNA amplification technology, according to the manufacturers’ instructions (Amplicor HIV-1 Monitor Test Version 1.5, Hoffmann-La Roche, Ltd, Basel, Switzerland).

**Training and quality control**

All data collection was implemented according to standard operating procedures (SOP) developed before commencing the study. The four research assistants for the trial were trained on the use of these SOPs as well as the use of questionnaires and food hygiene. The performance of data collectors were daily monitored by one author (JP) and a senior research assistant (MB).

The reliability of anthropometric measurements was assessed for one authors (JP) and one research assistant (MB) before the commencement of the trial. In standardization measurements from eight infants, the technical error of measurement for JP was 0.41 cm, 0.13 cm and 0.35 cm for length, MUAC and head circumference respectively (WHO, Multicentre Growth Reference Study Group 2006). Compared with the expert (MB), the observer (JP) overestimated MUAC by 0.35 cm and underestimated head circumference and length on average by 0.35 cm and by 0.37 cm respectively. Coefficient of reliability was 0.98, 0.89 and 0.96 for length, MUAC and head circumference respectively (Marks et al. 1989).
Sample size calculation

Sample size was calculated from expected values for the primary outcome (weight gain) based on an earlier preliminary dose-finding trial (Kuusipalo et al. 2006). Assuming a standard deviation (SD) of 0.39 kg for changes in weight in both groups during intervention period and a mean gain difference of 0.17 kg between the intervention group (FS) and the control group (LP), a sample size of 84 infants per group was required to provide the trial with 80% power and 95% confidence. To allow for approximately 5% attrition, the target enrolment was 88 infants per group.

Data management and analysis

Raw data were initially recorded on paper forms and their coherence was checked daily by a senior research assistant. Summary data were transcribed to paper case report forms and then double-entered into a tailor-made Microsoft Access 2003 database. The two entries were electronically compared; all extreme and otherwise susceptible values were confirmed or corrected.

Statistical analysis was carried out using Stata 9.2 (StataCorp, College Station, TX, USA). For the statistical analysis, the study participants were classified into two groups according to the trial allocation. Participants with no anthropometric data at the final measurement were excluded from outcome analyses but included in the comparison of baseline characteristics, and all those with at least one follow-up measurement were included in the sensitivity analysis.

For continuous and categorical outcomes, the two intervention groups were compared with t-test and Fisher’s exact test respectively. For comparison of continuous baseline and final anthropometric and haematological group values, paired t-test was used, whereas categorical baseline and final variables were compared using sign test. For the studies of compliance using visits as units of analysis, the Huber–White robust standard error was used to allow for correlated data (multiple visits per child). Confidence interval for SD was calculated using the Jacknife method.

Recovery from underweight was defined as WAZ ≤ −2.0 at enrolment and WAZ > −2.0 at the end follow-up. Recovery from wasting (WLZ ≤ −2.0 at enrolment) and stunting (LAZ ≤ −2.0 at enrolment) were defined accordingly as WLZ > −2.0 and LAZ > −2.0 at completion of the trial respectively.

Ethics, study registration and participant safety

The trial was performed according to International Conference of Harmonization–Good Clinical Practice (ICH-GCP) guidelines and it adhered to the principles of Helsinki declaration and regulatory guidelines in Malawi. Before the onset of enrolment, the trial protocol was reviewed and approved by the College of Medicine Research and Ethics Committee (University of Malawi) 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, Bethesda, MD, USA (http://www.clinicaltrials.gov, trial identification is NCT00131222).

A data safety and monitoring board (DSMB) continuously monitored the safety of the trial. All suspected serious adverse events (SAE) were reported to the DSMB for assessment. SAE was defined as any untoward medical occurrence that either resulted in death or was life-threatening, or required inpatient hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity or other serious medical condition.

Results

Of the 1657 initially screened infants and children, 367 were too old (>14.99 months), 1010 were above anthropometric cut-off for further evaluation (WAZ > −1.80) and two were too ill on the day of screening. Among the rest, 30 were not brought to the enrolment session, 72 were not underweight (WAZ > −2.00) and 2 declined participation after receiving full information of the trial. The remaining 176 infants and children were randomized into two intervention groups (Fig. 1). There was no difference in the mean WAZ between the enrolled children and those who were eligible but not enrolled (P = 0.34). None of the participants showed any adverse reaction to the 6-g test dose of FS.
Table 2 shows the baseline characteristics and anthropometric measurements of the participants by intervention group. At enrolment, participants in the LP group were on average 5 days younger, 100 g lighter and 0.6 cm shorter than those in the FS group (Table 2). The prevalence (number of participants) with underweight (WAZ < -2) was 100% (86) in the LP and 99% (89) in the FS group. Comparable figures for stunting (LAZ < -2) were 69% (59) vs. 64% (33) and those for wasting (WLZ < -2) were 16% (14) vs. 13% (12), in the LP and FS groups respectively. Eight of the participants had a positive HIV-antibody test at enrolment, but only five of them were truly HIV-infected, as evidenced by a positive Polymerase Chain Reaction (PCR) test.

During the 12-week follow-up, three participants died and three were lost to follow-up (Fig. 1). The assumed causes of death were anaemia and respiratory tract infection in the LP group and respiratory tract infection in the FS group. From the lost participants, one moved away and two were unavailable for final measurements although they had participated in all home visits and received and apparently eaten the intervention food. Comparison of baseline WAZ between participants who completed the follow-up and those who died or were lost did not suggest that they come from different populations (P = 0.15 and P = 0.70 respectively). The losses to follow-up were also not significantly different between the intervention groups (P = 0.61 for deaths and P = 0.68 for total loss to follow-up; Fisher’s exact test).

All mothers reported that their children readily ate the provided supplement and diversion of any portion to someone else than the intended beneficiary was reported only at 2/2065 (0.10%) food delivery interviews, both in LP and FS groups. From the weekly home visits during which trial products were checked, the percentage of visits with leftovers found were 4.9% and 6.5% in the LP and the FS groups respectively (P = 0.18).

Table 2. Background characteristics of the participants at enrolment

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group</th>
<th>FS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>86</td>
<td>90</td>
</tr>
<tr>
<td>Mean (SD) age (months)</td>
<td>11.58 (2.77)</td>
<td>11.73 (2.46)</td>
</tr>
<tr>
<td>Proportion of boys</td>
<td>0.4986 (57.0%)</td>
<td>0.4490 (49.0%)</td>
</tr>
<tr>
<td>Mean (SD) weight (kg)</td>
<td>7.02 (1.05)</td>
<td>7.12 (0.82)</td>
</tr>
<tr>
<td>Mean (SD) length (cm)</td>
<td>67.3 (4.5)</td>
<td>67.9 (3.8)</td>
</tr>
<tr>
<td>Mean (SD) mid-upper arm circumference (cm)</td>
<td>12.9 (1.0)</td>
<td>13.0 (0.87)</td>
</tr>
<tr>
<td>Mean (SD) head circumference (cm)</td>
<td>44.3 (1.9)</td>
<td>44.4 (1.6)</td>
</tr>
<tr>
<td>Mean (SD) weight-for-age Z-score</td>
<td>-3.09 (0.85)</td>
<td>-2.95 (0.71)</td>
</tr>
<tr>
<td>Mean (SD) length-for-age Z-score</td>
<td>-2.45 (0.93)</td>
<td>-2.26 (0.88)</td>
</tr>
<tr>
<td>Mean (SD) weight-for-length Z-score</td>
<td>-1.20 (0.83)</td>
<td>-1.21 (0.69)</td>
</tr>
<tr>
<td>Mean (SD) blood haemoglobin concentration (g L⁻¹)</td>
<td>93 (15)</td>
<td>91 (17)</td>
</tr>
<tr>
<td>Proportion with PCR confirmed HIV infection</td>
<td>4/82 (4.9%)</td>
<td>1/82 (1.2%)</td>
</tr>
<tr>
<td>Proportion with peripheral blood malaria parasitaemia</td>
<td>10/86 (11.6%)</td>
<td>10/89 (11.2%)</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; SD, standard deviation; PCR, Polymerase Chain Reaction; HIV, human immunodeficiency virus.

Table 3 shows the main outcome data for continuous variables. Among the 170 participants followed up for 12 weeks, mean gain in weight was 50 g [95% confidence interval (CI) 18–80] slightly higher in the FS group than the LP group. Comparable change for height was 1.5 mm (95% CI 1.1–1.9 mm) lower in the FS group. Correspondingly, mean WAZ and WLZ increased more and mean LAZ fell more in the FS group than the LP group. None of these differences reached statistical significance. Average changes in mean MUAC, head circumference and blood haemoglobin concentration were also quite similar in the two groups (Table 3). As shown, all 95% CIs were consistent with only relatively small between-group differences to either direction. Because of the limited outcome difference between the groups, we compared the variation in weight gain to the predictions used for sample size calculation. The observed standard deviation for weight gain was 0.38 kg in FS group and 0.46 kg in LP group, compared with estimated SD of 0.39 kg in both groups. While the observed variation is slightly different in either direction than expected, the 95% CI of the SDs of the whole sample and that of the two intervention groups included the expected value of 0.39 (Jacknife method), suggesting that a marked between-group difference would have been identified with the current sample size and trial design if it existed in the population.

Table 4 documents recovery from various forms of nutrient deficiency. During the 12-week intervention, approximately 20% of the initially underweight individuals, 85% of the initially wasted, and 10% of the initially stunted children recovered from their condition, with little difference between the two intervention groups.

Table 5 demonstrates the prevalence of nutrient deficiency at the end of follow-up, i.e. the function of enrolment prevalence, incidence of new cases and recovery during the intervention. Again, no statistically significant group-level differences were observed (Table 5).

In a comparison between enrolment status (Table 2) and end of intervention (Tables 3, 5), the mean WAZ increased by 0.22 (95% CI 0.07–0.37) Z-score units in the LP group and by 0.28 (95% CI 0.18–0.40) Z-score units in the FS group. Increase for mean weight-for-length was 0.39 (95% CI 0.20–0.57) and 0.52 (95% CI 0.38–0.65) Z-score units for LP and FS groups respectively. The prevalence of underweight (WAZ < −2.00) was reduced significantly in both groups (P < 0.001 for LP and P = 0.001 for FS, sign test). A similarly reduced trend was noticed for the prevalence wasting (P < 0.001 for LP and P = 0.07 for FS, sign test), and low MUAC (P = 0.02 for LP and P < 0.001 for FS, sign test). The prevalence of stunting (P = 0.27 for LP and P > 0.99 for FS, sign test) and anaemia (P = 0.69 for LP and P = 0.04 for FS, sign test) were not markedly changed during the intervention.

Sensitivity analysis to assess robustness of the results after loss to follow-up produced similar standardized weight gain as those reported above: 0.30 Z-score (95% CI 0.19–0.41) and 0.22 Z-score (95% CI 0.19–0.41) for FS and LP groups respectively.

There was no significant interaction between intervention and baseline WAZ, WLZ or LAZ, either using weight gain (P = 0.26 for WAZ, P = 0.93 for WLZ and P = 0.25 for LAZ) or length gain (P = 0.47 for WAZ, P = 0.19 for WLZ and P = 0.10 for LAZ) as an outcome.

Morbidity was similar in both groups (Table 6). All interventions were well tolerated by the participants.
Table 3. Anthropometric and haematological outcomes among underweight infants receiving a 12-week dietary supplementation with either FS or maize–soy flour

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group (Mean (SD))</th>
<th>FS group (Mean (SD))</th>
<th>Difference in mean changes (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight (kg)</td>
<td>0.84 (0.46)</td>
<td>0.89 (0.38)</td>
<td>0.05 (–0.80 to 0.17)</td>
</tr>
<tr>
<td>Change in length (cm)</td>
<td>2.65 (1.1)</td>
<td>2.50 (1.3)</td>
<td>−0.14 (–0.51 to 0.22)</td>
</tr>
<tr>
<td>Change in mid-upper arm circumference (cm)</td>
<td>0.3 (0.8)</td>
<td>0.4 (0.8)</td>
<td>0.1 (–0.1 to 0.3)</td>
</tr>
<tr>
<td>Change in head circumference (cm)</td>
<td>0.5 (0.6)</td>
<td>0.4 (0.5)</td>
<td>−0.1 (–0.3 to 0.1)</td>
</tr>
<tr>
<td>Change in weight-for-age Z-score</td>
<td>0.22 (0.69)</td>
<td>0.29 (0.51)</td>
<td>0.07 (–0.11 to 0.26)</td>
</tr>
<tr>
<td>Change in length-for-age Z-score</td>
<td>−0.08 (0.41)</td>
<td>−0.13 (0.42)</td>
<td>−0.05 (–0.17 to 0.08)</td>
</tr>
<tr>
<td>Change in weight-for-length Z-score</td>
<td>0.39 (0.85)</td>
<td>0.52 (0.63)</td>
<td>0.13 (–0.09 to 0.36)</td>
</tr>
<tr>
<td>Change in blood haemoglobin concentration (g L⁻¹)</td>
<td>1.5 (16.1)</td>
<td>3.8 (16.9)</td>
<td>2.3 (–2.7 to 7.3)</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; CI, confidence interval; SD, standard deviation.

Table 4. Recovery from moderate malnutrition among participants in maize–soy flour and FS groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group</th>
<th>FS group</th>
<th>Absolute risk difference (95% CI)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-for-age Z-score ≥ –2</td>
<td>17/84 (20.2)</td>
<td>14/88 (15.9)</td>
<td>−4.3 (–15.8 to 7.2)</td>
<td>0.8 (0.4 to 1.5)</td>
</tr>
<tr>
<td>Weight-for-length Z-score ≥ –2</td>
<td>13/14 (92.9)</td>
<td>9/12 (75.0)</td>
<td>17.9 (–45.8 to 10.1)</td>
<td>0.8 (0.6 to 1.2)</td>
</tr>
<tr>
<td>Length-for-age Z-score ≥ –2</td>
<td>5/57 (8.8)</td>
<td>7/57 (12.3)</td>
<td>3.5 (–7.7 to 14.8)</td>
<td>1.4 (0.5 to 4.2)</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; CI, confidence interval.

Table 5. Prevalence of malnutrition with various criteria at final measurement among participants in maize–soy flour and FS groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group (n = 84)</th>
<th>FS group (n = 86)</th>
<th>Difference of proportions (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-for-age Z-score &lt; –2</td>
<td>68 (81.0)</td>
<td>74 (86.1)</td>
<td>5.1 (–6.1 to 16.2)</td>
</tr>
<tr>
<td>Weight-for-length Z-score &lt; –2</td>
<td>4 (4.8)</td>
<td>5 (5.8)</td>
<td>1.0 (–5.7 to 7.8)</td>
</tr>
<tr>
<td>Length-for-age Z-score &lt; –2</td>
<td>62 (73)</td>
<td>54 (62.8)</td>
<td>−1.10 (–24.9 to 28.7)</td>
</tr>
<tr>
<td>Mid-upper arm circumference &lt; 12.5 cm</td>
<td>17 (20.2)</td>
<td>7 (8.1)</td>
<td>−12.1 (–22.5 to 17.4)</td>
</tr>
<tr>
<td>Blood haemoglobin &lt; 100 g L⁻¹</td>
<td>48 (57.8)</td>
<td>51 (60.0)</td>
<td>2.2 (–12.7 to 17.0)</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; CI, confidence interval; *n for blood haemoglobin was 83 and 85 for LP and FS respectively.

Table 6. Occurrence of morbidity among participants in maize–soy flour and FS groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP group (n = 1020)</th>
<th>FS group (n = 1063)</th>
<th>Difference of proportions (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home visits fever was reported</td>
<td>36 (3.6)</td>
<td>54 (5.1)</td>
<td>1.6 (–0.5 to 3.6)</td>
</tr>
<tr>
<td>Home visits diarrhoea was reported</td>
<td>74 (7.3)</td>
<td>79 (7.4)</td>
<td>0.2 (–2.7 to 3.1)</td>
</tr>
<tr>
<td>Home visits ARI was reported</td>
<td>93 (9.1)</td>
<td>95 (8.9)</td>
<td>−0.2 (–2.7 to 2.2)</td>
</tr>
<tr>
<td>Home visits other illnesses were reported</td>
<td>78 (7.7)</td>
<td>63 (5.9)</td>
<td>−1.7 (–4.3 to 0.9)</td>
</tr>
</tbody>
</table>

LP, Likuni Phala (maize–soy flour); FS, fortified spread; CI, confidence interval; ARI, acute respiratory tract infection.
Besides the three deaths, three participants were hospitalized and recorded as having experienced an SAE during the follow-up. The LP and FS groups shared four and two of these SAEs ($P = 0.44$; Fisher’s exact test). Both the study physician and the DSMB considered all deaths and SAE unlikely related to the trial interventions.

**Discussion**

The present trial was carried out to test the growth-promoting effects of a micronutrient-fortified, energy dense ready-to-use spread (FS), when used as a supplementary food among 6–18-month-old underweight infants and children in rural Malawi. Enrolment rate for the trial was high, group allocation was random, trial groups were rather similar at enrolment, follow-up was identical for all groups, compliance with the intervention appeared good, loss to follow-up was infrequent and balance between the groups and people measuring the outcomes were blinded to the group allocation. Additionally, because calculation of anthropometric indices (WAZ, LAZ and WLZ) standardizes for gender, baseline differences of gender proportions between the trial groups did not affect the interpretation of the main results. Hence, the observed results are likely to be unbiased and thus representative of the population from which the sample was drawn.

In our sample, participants receiving FS for 12 weeks gained on average slightly more weight but less length than those receiving an iso-energetic portion of maize–soy flour (LP). Mean changes in MUAC and blood haemoglobin concentration were also higher in the FS group but head circumference increased more in the LP group. However, all differences between the groups were marginal and the 95% CIs for point estimates excluded large difference in population. Although the higher end of the 95% CI for the difference in mean weight gain just overlapped with the predefined cut-off for clinical significance, the data do not therefore support our initial hypothesis that weekly home provision of FS to 6–15 months underweight children in rural Malawi would be noticeably better in alleviating their being underweight or promoting catch-up growth over a 12-week period than similar supplementation scheme with maize–soy flour. Rather, our results are consistent with the two interventions having a similar or only slightly different impact on these outcomes.

Children in both intervention groups showed good recovery from wasting, some recovery from being underweight and no change in their stunting status. For ethical reasons, however, we did not include an unsupplemented control group in our trial design. This omission, while allowing a comparison between the two interventions, limits our possibility to make conclusions on their effect as compared with young children receiving no intervention. It is thus possible that the observed recovery from wasting or being underweight, and the increase in mean weight-for-age or weight-for-length result from a confounder like a seasonal effect on growth or regression to the mean phenomenon. A comparison to earlier studies in the same area suggest, however, that unsupplemented underweight children of this age would have a somewhat lower weight and length gain velocity (Kuusipalo et al. 2006) and that seasonal effect on weight gain would be markedly lower than the approximately 0.3 Z-score unit increase observed in this trial (Maleta et al. 2003b). Rather, the increase in relative weight is comparable to observations with other supplementary feeding interventions in similar target groups (Simondon et al. 1996; Lartey et al. 1999; Becket et al. 2000; Bhandari et al. 2001; Oelofse et al. 2003; Adu-Afarwuah et al. 2007).

Micronutrient FS were initially designed to be used as a rehabilitation food for malnourished children (Briend et al. 1999). In a succession of studies, therapeutic versions of the spreads (RUTF) were shown to induce recovery, first in a hospital setting, then as outpatients in efficacy trials and finally in programmatic settings (Collins 2001; Diop el et al. 2003; Manary et al. 2004; Sandige et al. 2004; Ciliberto et al. 2005, 2006; Ndekha et al. 2005). Recently, there has been a major interest to expand the use of spreads to treatment of less severe conditions or primary prevention malnutrition. The results from our study support the findings of Patel et al. (2005) in Malawi and those of Medicins Sans Frontieres in Niger (Defourny et al. 2007), suggesting that FS can also efficiently promote recovery from...
moderate wasting, thus acting as a secondary prevention of severe malnutrition.

In contrast to recovery from wasting and associated increase in weight-for-age, linear growth and the process of stunting seems to be very little affected by a 12-week supplementary feeding with FS or LP, as evidenced by a decreasing LAZ and a very low recovery rate from stunting in both groups in our trial. This is not surprising, as length gain acceleration has often been shown to follow weight gain increase with a certain lag period, e.g. by 3 months in rural Malawi (Walker & Golden 1988; Costello 1989; Heikens et al. 1989; Maleta et al. 2003b). Hence, only a longer intervention might have the potential for the primary prevention of stunting or rehabilitation of moderately underweight children in an area, where low weight is usually a function of inadequate linear growth. First results from such an approach, i.e. 6–12-month supplementation of complementary feeding with 20–50 g FS have been promising, documenting increased length gain and prevention of severe stunting among rural infants in Ghana and Malawi (Adu-Afarwuah et al. 2007; Phuka et al. 2008). Possible nutrients in FS that could account for improved linear growth in longer interventions include, e.g. essential fatty acids, zinc or components of cow milk (Brown et al. 2002; Hoppe et al. 2006; Adu-Afarwuah et al. 2007).

Taken together, our results suggest that 12-week-long supplementary feeding with FS and LP porridge have similar impacts on the weight and length gain of underweight infants in a low-income setting like Malawi. Although the results are consistent with the idea that both interventions promote recovery from wasting or moderate underweight, the lack of no-food control group hinders any firm conclusions on the independent effect of either intervention. This, and the possible effect of seasonality on the growth outcomes, should be addressed in further controlled trials.

Acknowledgements

We are grateful to the people of Lungwena, the staff at the Lungwena Training Health Centre and our research assistants for their positive attitude, support and help in all stages of the study, and to Laszlo Csonka for designing the collection tools and data entry programs. André Briend is currently a staff member of the World Health Organization (WHO). André Briend alone is responsible for the views expressed in this publication and they do not necessarily represent the decisions or stated policy of the WHO.

Source of funding

The trial was funded by grants from the Academy of Finland (grants 200720 and 109796), Foundation for Paediatric Research in Finland and Medical Research Fund of Tampere University Hospital. The micronutrient mixture used in the production of FS was provided free of charge by Nutriset, Inc. (Malaunay, France). John Phuka and Chrissie Thakwalakwa are receiving personal stipends from Nestlé Foundation.

Conflict of interest statement

André Briend was a consultant to Nutriset until December 2003 and the company has also financially supported the planning of another research project by the same study team through Per Ashorn and the University of Tampere after the completion of this trial. Other authors declare no conflict of interest. The funders of trial had no role in the implementation, analysis or reporting.

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Complementary Feeding With Fortified Spread and Incidence of Severe Stunting in 6- to 18-Month-Old Rural Malawians

John C. Phuka, MBBS; Kenneth Maleta, PhD; Chrissie Thakwalakwa, BEd; Yin Bun Cheung, PhD; André Briend, PhD; Mark J. Manary, MD; Per Ashorn, PhD

Objective: To compare growth and incidence of malnutrition in infants receiving long-term dietary supplementation with ready-to-use fortified spread (FS) or micronutrient-fortified maize-soy flour (likuni phala [LP]).

Design: Randomized, controlled, single-blind trial.

Setting: Rural Malawi.

Participants: A total of 182 six-month-old infants.

Intervention: Participants were randomized to receive 1 year of daily supplementation with 71 g of LP (282 kcal), 50 g of FS (FS50) (256 kcal), or 25 g of FS (FS25) (127 kcal).

Outcome Measures: Weight and length gains and the incidences of severe stunting, underweight, and wasting.

Results: Mean weight and length gains in the LP, FS50, and FS25 groups were 2.37, 2.47, and 2.37 kg (P = .66) and 12.7, 13.5, and 13.2 cm (P = .23), respectively. In the same groups, the cumulative 12-month incidence of severe stunting was 13.3%, 0.0%, and 3.5% (P = .01), of severe underweight was 15.0%, 22.5%, and 16.9% (P = .71), and of severe wasting was 1.8%, 1.9%, and 1.8% (P > .99). Compared with LP-supplemented infants, those given FS50 gained a mean of 100 g more weight and 0.8 cm more length. There was a significant interaction between baseline length and intervention (P = .04); in children with below-median length at enrollment, those given FS50 gained a mean of 1.9 cm more than individuals receiving LP.

Conclusion: One-year-long complementary feeding with FS does not have a significantly larger effect than LP on mean weight gain in all infants, but it is likely to boost linear growth in the most disadvantaged individuals and, hence, decrease the incidence of severe stunting.

Trial Registration: clinicaltrials.gov Identifier: NCT00131209.

Arch Pediatr Adolesc Med. 2008;162(7):619-626

The high incidence and serious consequences of childhood undernutrition in sub-Saharan Africa and some parts of southern Asia necessitate emphasis on early prevention, but there are no easy options for it.1-4 Infection control has not proved to be widely successful, few indigenous foods are rich in all-important nutrients or available throughout the year, and poverty limits possibilities for purchasing commercially available nutritious foods.5 In Malawi, thin porridge made of micronutrient-enriched maize and soy flour is often promoted as the main complementary food for infants and young children. However, it has low calorie density, and it resembles the staple food in the area, which may result in displacement of habitual foods from the beneficiary’s diet or diversion of the complementary food to other family members.6 Recently, Briend and his collaborators7,8 developed the concept of highly nutrient- and calorie-dense spreads, which are simple to produce, need no cooking before use, and can be stored for months even in warm conditions. The best-known formulation of such spreads is called ready-to-use therapeutic food.9,10 Several clinical trials6,11-15 in Malawi have shown that ready-to-use therapeutic food is safe and effective in the rehabilitation of severely malnourished children and that a modification of it interferes with habitual diet less than a porridge supplement and has a positive effect on 3-to-4-year-old children who are less severely underweight and experience less stunting. Recent data from a preliminary dose-finding trial16 suggested that home provision of fortified spread (FS) to

Author Affiliations are listed at the end of this article.
### METHODS

#### STUDY AREA AND TIMING

This study was conducted between October 11, 2004, and December 19, 2005, in Lungwena, a rural Malawian community with a high prevalence of early childhood stunting and underweight. The staple food, maize, was grown during the single rainy season between December and March. Exclusive breastfeeding for infants was almost nonexistent, and the infant diet was typically complemented with thin maize porridge already from 2 to 6 months of age.

### MEASUREMENT OF OUTCOME VARIABLES

The primary outcome was weight gain during 12-month follow-up. Secondary outcomes included length gain; mean change in head circumference in the amount of food base given (50 vs 25 g/d). The difference between the FS50 and FS25 supplements was in the amount of food base given (50 vs 25 g/d).

#### ELIGIBILITY CRITERIA, ENROLLMENT, AND RANDOMIZATION

The inclusion criteria included age 5.50 to 6.99 months, residence in the study area, and informed consent from at least 1 authorized guardian. The exclusion criteria were low weight for length (<2.0), presence of edema, history of peanut allergy, severe illness warranting hospitalization on the enrollment day, concurrent participation in another clinical trial, and any symptoms of food intolerance within 30 minutes after ingesting a 6-g test dose of FS, 1 of the food supplements used in the trial.

For enrollment, trained health surveillance assistants contacted all the families known to live in the area and known to have an infant of approximately the right age. Infants were invited to an enrollment 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.

For group allocation, guardians chose 1 envelope from a set of identical-appearing opaque envelopes, each containing a piece of paper indicating an identification number and randomly assigned allocation to 1 of the 3 interventions. The randomization list and envelopes were made by individuals 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 into a database.

#### INTERVENTIONS AND FOLLOW-UP

There were 3 intervention schemes. Infants in the control group were provided with a mean of 71 g/d of LP. Participants in the other 2 groups received a mean of either 50 or 25 g/d of micronutrient-fortified spread (FS50 or FS25, respectively). The supplements were home delivered at 3-week intervals (at each food delivery, three 500-g bags of LP, four 262-g jars of FS50, or two 262-g jars of FS25 were given).

Likuni phala was purchased from a local producer (Rab Processors, Blantyre, Malawi). Fortified spread was produced at a Malawian nongovernmental organization, Project Peanut Butter (Blantyre), from peanut paste, milk powder, vegetable oil, sugar, and premade micronutrient mixture (Nutriset Inc, Maluany, France). All the supplements were fortified with micronutrients, but the level of fortification varied between the products. The difference between the FS50 and FS25 supplementation was in the amount of food base given (50 vs 25 g/d). The micronutrient content, however, was adjusted so that children in both FS groups received similar daily micronutrient doses. Table 1 provides the calories and nutrient contents of a daily ration of each supplementation scheme.

Both FS50 and FS25 could be eaten as such, whereas the LP required cooking into porridge before consumption. Guardians were provided with spoons and were advised to daily offer their infants porridge containing 12 spoonfuls of LP, 8 spoonfuls of FS50, or 4 spoonfuls of FS25, divided into 2 to 3 daily doses. 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 weekly at their homes to collect information on supplement use and possible adverse events. Empty food containers were collected every 3 weeks. At 17, 34, and 52 weeks after enrollment, the participants underwent a physical examination, an anthropometric assessment, and laboratory tests.

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### Table 1. Caloric and Nutrient Contents of a Daily Ration of Each Food Supplement Used in This Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP</th>
<th>FS50</th>
<th>FS25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>282</td>
<td>256</td>
<td>127</td>
</tr>
<tr>
<td>Caloric intakes, kcal</td>
<td>10.3</td>
<td>7.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>13.8</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.1</td>
<td>16.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Retinol, µg RE</td>
<td>138</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Folate, µg</td>
<td>43</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Niacin, mg</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pantothenic acid, mg</td>
<td>NA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B₆, mg</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</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>48</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>NA</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>71</td>
<td>366</td>
<td>283</td>
</tr>
<tr>
<td>Copper, mg</td>
<td>NA</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Iodine, µg</td>
<td>NA</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>5</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>NA</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Selenium, µg</td>
<td>NA</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>3.6</td>
<td>8.4</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Abbreviations: FS25, fortified spread, 25 g/d; FS50, fortified spread, 50 g/d; LP, likuni phala; NA, not available; RE, retinol equivalents.
the anthropometric indexes of weight-for-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) z scores; the incidence of severe (WAZ, LAZ, and WLZ <-3) or moderate to severe (WAZ, LAZ, and WLZ <-2) underweight, stunting, or wasting; change in head or middle upper arm circumference; and change in blood hemoglobin and serum ferritin concentrations.

Un clothed infants were weighed using an electronic infant weighing scale (SECA 834, Chasmos 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). Middle upper arm circumference and head circumference were measured using nonstretchable plastic tape measures (Lasso-o Tape; Harlow Printing Ltd, South Shields, Tyne & Wear, England). Anthropometric indexes (WAZ, LAZ, and WLZ) were calculated using Epi Info 3.3.2 software (Centers for Disease Control and Prevention, Atlanta, Georgia), based on the Centers for Disease Control and Prevention 2000 growth reference.18

A 2-mL venous blood sample was collected, and serum was separated by means of centrifugation at the beginning and end of follow-up. Hemoglobin concentration was measured from a fresh blood drop using cuvettes and a reader (HemoCue AB, Angelholm, Sweden). Serum ferritin concentration was analyzed from frozen serum samples using commercial test kits according to the manufacturer’s instructions (Ramco Laboratories, Stafford, Texas).

Thick and thin blood smears were stained with Giemsa and screened microscopically for malaria parasites from symptomatic participants at enrollment. Screening for human immunodeficiency virus infection was performed from fresh blood using 2 antibody rapid tests, and positive results were confirmed using DNA amplification technology according to the manufacturers’ instructions (Determine; Abbott Laboratories, Abbott Park, Illinois; Uni-Gold; Trinity Biotech Plc, Bray, Ireland; and Amplicor HIV-1 Monitor Test Version 1.5; Hoffmann-La Roche Ltd, Basel, Switzerland).

DATA MANAGEMENT AND ANALYSIS

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 2 entries were electronically compared, and extreme or otherwise suspicious values were confirmed or corrected.

Statistical analysis was performed using Stata 9.0 (Stata Corp, College Station, Texas) on an intention-to-treat basis. Infants with no anthropometric data after enrollment were excluded from outcome analyses but were included in the comparison of baseline characteristics. The main analyses on anthropometric measures used data from all 176 analyzable children using the last values carried forward (and back-transform WAZ and LAZ at 18 months to kilograms and centimeters for metric presentation) for the 8 children who dropped out early or using the survival analysis method to deal with censoring. Sensitivity analysis limited to the 168 children with complete data gave similar findings (details not shown).

For continuous and categorical outcomes, the 3 intervention groups were compared using analysis of variance and the Fisher exact test, respectively. Survival analysis was used to determine the cumulative probability of severe or moderate malnutrition in different groups, and the differences were tested by means of the log-rank test. An event was considered to have happened at the midpoint between the time the event was detected and the previous measurement. Individuals with a particular form of malnutrition already at enrollment were excluded from survival analyses concerning the incidence of that outcome. For the studies of compliance using visits as the unit of analysis, the Huber-White robust standard error was used to allow for correlated data (multiple visits per child).

ETHICS, STUDY REGISTRATION, AND PARTICIPANT SAFETY

This trial was performed according to International Conference on Harmonisation/Good Clinical Practice guidelines, and it adhered to the principles of the Declaration of Helsinki and regulatory guidelines in Malawi. Before the onset of enrollment, the trial protocol was reviewed and approved by the University of Malawi College of Medicine and the ethical committee of Chikankata Hospital District (Finland). Key details of the protocol were published in 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, defined as any untoward medical occurrence that either resulted in death or was life threatening or required inpatient hospitalization or prolongation of existing hospitalization or resulted in persistent or significant disability or incapacity or other serious medical conditions.

RESULTS

Of the 303 initially screened infants, 65 were too old (aged >6.99 months), 2 were too young (aged <5.5 months), and 2 were too ill on the day of screening or enrollment. Of the remaining 234 infants, 49 were not brought to the enrollment session (3 died, 16 moved away, 7 parents were not interested, and 23 parents gave no explanation) and 3 parents declined participation after receiving full information about the trial. The remaining 182 infants were randomized into 3 intervention groups (Figure 1). None of the eligible participants who received the 6-g test dose were allergic to FS.

Table 2 provides the baseline characteristics of the participants by intervention group. At enrollment, the mean anthropometric measurements were comparable in the LP and FS50 groups, whereas infants in the FS25 group were a mean of 250 and 380 g heavier than those in the FS50 and LP groups, respectively (Table 2). Infants in the FS25 group were also 0.3 and 0.7 cm longer than infants in the other 2 groups, respectively. No participant experienced severe wasting at the beginning. The prevalence of severe underweight in the LP, FS50, and FS25 groups was 1.6% (n=1), 3.3% (n=2), and 0.0%, respectively; and that of severe stunting was 3.3% (n=2), 6.6% (n=4), and 1.7% (n=1), respectively. Nine participants had a positive human immunodeficiency virus antibody test result at enrollment, but only 1 of them was truly human immunodeficiency virus infected, as evidenced by a positive polymerase chain reaction test result.

During the 12-month follow-up, 10 infants died, 2 were not located at the end of follow-up, and 2 were unavailable for follow-up before the age of 18 months (Figure 1). The success rate of following up to age 18 months was not significantly different among intervention groups (P=.47, Fisher exact test). Only 6 participants had anthropometric data at all after enrollment, and there was no difference in this among intervention groups (P=.70, Fisher exact test). The assumed causes of the 10 deaths were di-
arrhea, malaria, and drowning in the LP group; diarrhea, meningitis, malaria, and poisoning in the FS50 group; and respiratory tract infection and malaria in the FS25 group.

All mothers reported that their infants readily ate the provided supplement, and the diversion of any portion to someone other than the intended beneficiary was reported at only 4 of 8864 food delivery interviews (0.05%), 3 in the LP group and 1 in the FS50 group. From the 3-week home visits during which leftover trial products were checked, the percentages of visits with leftovers found were 2.8%, 9.8%, and 5.6% in the LP, FS50, and FS25 groups, respectively (P < .001).

Of the 176 participants with anthropometric data, mean gains in weight and length were 100 g (95% confidence interval [CI], −143 to 343 g) and 0.8 cm (95% CI, −0.1 to 1.7 cm), respectively, higher in the FS50 group than in the LP group. Correspondingly, mean decreases in WAZ and LAZ were smaller in FS50 infants than in FS25 or

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**Table 2. Baseline Characteristics of the 182 Participants at Enrollment**

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP (n=61)</th>
<th>FS50 (n=61)</th>
<th>FS25 (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, No./total No. (%)</td>
<td>24/61 (39.3)</td>
<td>33/61 (54.1)</td>
<td>34/60 (56.7)</td>
</tr>
<tr>
<td>PCR-confirmed HIV infection, No./total No. (%)</td>
<td>0/55</td>
<td>0/55</td>
<td>1/55 (1.8)</td>
</tr>
<tr>
<td>HIV antibodies, No./total No. (%)</td>
<td>3/55 (5.5)</td>
<td>2/55 (3.6)</td>
<td>4/55 (7.3)</td>
</tr>
<tr>
<td>Clinical malaria, No./total No. (%)</td>
<td>1/61 (1.6)</td>
<td>1/61 (1.6)</td>
<td>2/60 (3.3)</td>
</tr>
<tr>
<td>Children aged &lt;5 y per participant household, mean (SD)</td>
<td>2 (0.9)</td>
<td>2 (0.8)</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Age, mean (SD), mo</td>
<td>5.91 (0.41)</td>
<td>5.93 (0.44)</td>
<td>5.89 (0.36)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>6.92 (0.93)</td>
<td>7.05 (0.90)</td>
<td>7.30 (0.92)</td>
</tr>
<tr>
<td>Length, mean (SD), cm</td>
<td>62.8 (2.1)</td>
<td>63.2 (2.6)</td>
<td>63.5 (2.4)</td>
</tr>
<tr>
<td>Middle upper arm circumference, mean (SD), cm</td>
<td>13.4 (0.9)</td>
<td>13.5 (1.1)</td>
<td>13.9 (1.1)</td>
</tr>
<tr>
<td>Head circumference, mean (SD), cm</td>
<td>43.0 (1.5)</td>
<td>43.1 (1.7)</td>
<td>43.2 (1.3)</td>
</tr>
<tr>
<td>Weight-for-age z score, mean (SD)</td>
<td>−0.65 (1.07)</td>
<td>−0.62 (1.04)</td>
<td>−0.33 (0.94)</td>
</tr>
<tr>
<td>Length-for-age z score, mean (SD)</td>
<td>−1.20 (0.82)</td>
<td>−1.20 (1.01)</td>
<td>−1.00 (0.77)</td>
</tr>
<tr>
<td>Weight-for-length z score, mean (SD)</td>
<td>0.48 (1.08)</td>
<td>0.55 (0.90)</td>
<td>0.75 (0.86)</td>
</tr>
<tr>
<td>Blood hemoglobin concentration, mean (SD), g/dL</td>
<td>11.4 (1.6)</td>
<td>10.6 (1.7)</td>
<td>11.3 (1.5)</td>
</tr>
<tr>
<td>Serum ferritin concentration, mean (SD), ng/mL</td>
<td>56.9 (73.6)</td>
<td>45.7 (37.4)</td>
<td>67.2 (74.4)</td>
</tr>
</tbody>
</table>

Abbreviations: FS25, fortified spread, 25 g/d; FS50, fortified spread, 50 g/d; HIV, human immunodeficiency virus; LP, likuni phala; PCR, polymerase chain reaction.

SI conversion factors: To convert ferritin to picomoles per liter, multiply by 2.247; hemoglobin to grams per liter, multiply by 10.

*Reported fever and observed peripheral blood malaria parasitemia.
LP infants, and there was also a smaller decrease in blood hemoglobin concentration in the FS50 group. None of the differences, however, reached statistical significance (Table 3).

There was an interaction between intervention group (FS50 vs LP) and baseline LAZ using length gain (P=.04) or weight gain (P=.002) as an outcome. In infants with baseline LAZ below the median in this trial (−1.04), the mean change in middle upper arm circumference, mean (SD), cm was 1.1 (0.9) in the LP group, 1.0 (1.1) in the FS50 group, and 1.0 (0.8) in the FS25 group. Comparable differences in 

Table 3. Outcome Changes in Infants Receiving Different Doses of FS and LP During Up to 12 Months of Follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP</th>
<th>FS50</th>
<th>FS25</th>
<th>P Value^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight, mean (SD), kg</td>
<td>2.37 (0.60)</td>
<td>2.47 (0.77)</td>
<td>2.37 (0.61)</td>
<td>.66</td>
</tr>
<tr>
<td>Change in length, mean (SD), cm</td>
<td>12.7 (1.7)</td>
<td>13.5 (2.9)</td>
<td>13.2 (2.9)</td>
<td>.23</td>
</tr>
<tr>
<td>Change in middle upper arm circumference, mean (SD), cm</td>
<td>1.1 (0.9)</td>
<td>1.0 (1.1)</td>
<td>1.0 (0.8)</td>
<td>.64</td>
</tr>
<tr>
<td>Change in head circumference, mean (SD), cm</td>
<td>3.7 (0.5)</td>
<td>3.7 (0.8)</td>
<td>3.7 (0.6)</td>
<td>.96</td>
</tr>
<tr>
<td>Change in weight-for-age z score, mean (SD), cm</td>
<td>−1.29 (0.63)</td>
<td>−1.18 (0.90)</td>
<td>−1.32 (0.65)</td>
<td>.53</td>
</tr>
<tr>
<td>Change in length-for-age z score, mean (SD), cm</td>
<td>−0.74 (0.95)</td>
<td>−0.59 (1.22)</td>
<td>−0.64 (0.86)</td>
<td>.71</td>
</tr>
<tr>
<td>Change in weight-for-length z score, mean (SD), cm</td>
<td>−0.98 (0.83)</td>
<td>−1.05 (0.86)</td>
<td>−1.13 (0.75)</td>
<td>.62</td>
</tr>
<tr>
<td>Change in blood hemoglobin concentration, mean (SD), g/dl</td>
<td>−0.68 (2.06)</td>
<td>−0.04 (2.21)</td>
<td>−0.38 (1.82)</td>
<td>.28</td>
</tr>
<tr>
<td>Change in serum ferritin concentration, mean (SD), ng/mL</td>
<td>16.2 (116.4)</td>
<td>11.5 (61.2)</td>
<td>−0.7 (172.4)</td>
<td>.83</td>
</tr>
</tbody>
</table>

Abbreviations: FS25, fortified spread, 25 g/d; FS50, fortified spread, 50 g/d; LAZ, length-for-age z score; LP, likuni phala; WAZ, weight-for-age z score.

^a Obtained by analysis of variance.

Table 4. Proportion of Participants Developing Various Forms of Undernutrition During Trial Follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>LP</th>
<th>FS50</th>
<th>FS25</th>
<th>P Value^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever developed severe stunting (LAZ &lt;-3), No./total No. (%)</td>
<td>7/56 (12.5)</td>
<td>0/56</td>
<td>2/58 (3.5)</td>
<td>.008</td>
</tr>
<tr>
<td>Ever developed moderate to severe stunting (LAZ &lt;-2), No./total No. (%)</td>
<td>11/49 (22.5)</td>
<td>10/51 (19.6)</td>
<td>15/55 (27.3)</td>
<td>.64</td>
</tr>
<tr>
<td>Ever developed severe underweight (WAZ &lt;-3), No./total No. (%)</td>
<td>8/57 (14.0)</td>
<td>11/58 (19.0)</td>
<td>9/58 (15.5)</td>
<td>.81</td>
</tr>
<tr>
<td>Ever developed severe wasting (WHZ &lt;-3), No./total No. (%)</td>
<td>23/53 (43.4)</td>
<td>20/56 (35.7)</td>
<td>20/57 (35.1)</td>
<td>.62</td>
</tr>
<tr>
<td>Ever developed moderate to severe wasting (WHZ &lt;-2), No./total No. (%)</td>
<td>1/58 (1.7)</td>
<td>1/60 (1.7)</td>
<td>1/58 (1.7)</td>
<td>&gt;.99</td>
</tr>
</tbody>
</table>

Abbreviations: FS25, fortified spread, 25 g/d; FS50, fortified spread, 50 g/d; LAZ, length-for-age z score; LP, likuni phala; WAZ, weight-for-age z score; WHZ, weight-for-height z score.

^a Obtained by Fisher exact test.

As secondary end points, we looked at the proportion of infants who developed severe or moderate to severe underweight, stunting, and wasting during follow-up. The proportion of infants who developed other forms of malnutrition did not differ markedly among the intervention groups, but severe stunting occurred significantly less frequently in the FS50 and FS25 groups than in the LP group (Table 4). After enrollment, no infants in the FS50 group, 3.5% in the FS25 group, and 12.5% in the LP group developed severe stunting (P=.008, Fisher exact test). The 95% CI for the difference between the FS50 and LP groups was 3.8% to 21.2%.

The cumulative incidence of stunting during the 12-month period was also calculated based on survival analysis methods that dealt with censoring differently from the analysis given in Table 4. This approach confirmed that severe stunting developed less often and later in the FS50 and FS25 groups than in the LP group (0.0%, 3.5%, and 13.3%, respectively; P=.01, log-rank test) (Figure 2A). The cumulative incidence of moderate to severe stunting was similar in the 3 groups, but infants in the FS50 group developed the condition on average somewhat later. However, the latter result was not significant (P=.66, log-rank test) (Figure 2B). Calculated from the absolute risk reduction, the number of infants needed to be supplied for 1 year with FS50 to prevent 1 case of severe stunting was 8 (95% CI, 5-26).

The proportion of those developing severe and moderate to severe underweight and severe and moderate to severe wasting did not differ significantly between the trial groups (Table 4). The cumulative incidence of severe underweight was 15.0%, 22.5%, and 16.9% for the LP, FS50, and FS25 groups, respectively (P=.71); the cumulative incidence of severe wasting was 1.8%, 1.9%, and 1.8% for the same groups, respectively (P>.99).
All the interventions were well tolerated by the participants. In addition to the 10 deaths (4, 4, and 2 in the LP, FS50, and FS25 groups, respectively) (Figure 1), 3 other participants (1 in each intervention group) were hospitalized and recorded as having experienced a serious adverse event during follow-up. The LP, FS50, and FS25 groups shared 5, 5, and 3 of these serious adverse events, respectively ($P = .82$, Fisher exact test). Three of the serious adverse events were considered to be unrelated, and 10 were probably unrelated to the trial interventions.

**COMMENT**

The present trial was performed to test the growth-promoting effects of 2 micronutrient-fortified, calorie-dense, ready-to-use spreads (FS50 and FS25) when used as complementary foods for 6- to 18-month-old infants in rural Malawi. The enrollment rate for the trial was high, group allocation was random, follow-up was identical for all groups, loss to follow-up and unavailability for follow-up were infrequent, and the people measuring the outcomes were masked to group allocation. Hence, the observed results are likely to be unbiased and, thus, representative of the population from which the sample was drawn.

In this sample, infants receiving FS50 or FS25 for 1 year gained on average slightly more weight and length than those receiving an approximately isoenergetic portion of LP. However, the mean differences between the FS50 and LP groups were modest (100 g and 0.8 cm), and statistical hypothesis testing could not exclude the possibility of random finding. Therefore, the data do not support the initial hypothesis that 1 year of complementary provision of FS to all infants older than 6 months in rural Malawi would be noticeably better than LP in promoting their mean weight or length gain by 18 months of age. Instead, the results seem to be mostly in line with the modest findings from other dietary intervention trials\(^\text{20-24}\) for infants in similar settings, documenting a 0- to 0.6-SD higher mean weight (SD, 0-400 g) or length (SD, 0-1 cm) gains in the intervention groups than in unsupplemented controls.

In contrast to the mean gains, the present study demonstrated a marked and statistically significant difference in the incidence of severe stunting between the FS50 and LP groups. Also, a stratified analysis suggested an interaction between initial height and intervention group, as demonstrated by bigger between-group differences in length and weight gain in infants with at least mild stunting at enrollment. Caution must be exercised when interpreting these results because the incidence of severe stunting was only one of several secondary outcomes of the trial and the interaction finding came from a post hoc exploratory analysis. The results are, however, biologically plausible, and there was a trend toward a dose response. Therefore, we believe that the results in this sample appropriately represent the larger population. Furthermore, the results are consistent with those of an earlier trial from West Africa\(^\text{25}\) in which supplementation of children with FS who experienced stunting induced marked catch-up growth among them. These data suggest that a dietary intervention with FS can boost linear growth and reduce the incidence of the severest forms of stunting, especially when directed to initially disadvantaged infants. If the whole unselected infant group needs to be targeted, the intervention should probably also address the other major risk factors for growth failure (infections and inappropriate child care).\(^\text{26}\)

In the present sample, 8 infants needed supplementation for 1 year with FS50 to prevent 1 case of severe stunting. It is difficult to assess the public health impact resulting from such an intervention and the potential reduction in severe stunting. Stunting is associated with various adverse sequelae, such as developmental delay,
lower work capacity, worse economic status as an adult, and delivery problems. However, it is not known whether these outcomes are particularly associated with any linear growth failure or only its most severe forms. Such associations and the possibility of preventing various health consequences with an FS intervention need to be addressed in later trials. Such studies are further justified by recent evidence from a Ghanaian trial, suggesting that infant motor development can be markedly enhanced in a low-income setting by a 6-month-long provision of Nutributter, another lipid-based nutrient supplement.

Although we collected information on compliance, it came from parental recollections, which are often unreliable in dietary interventions. Hence, we cannot rule out the possibility of food sharing and its potential effect on the results, especially because the 2 supplement types differed in many ways from each other. In fact, earlier studies from the same research site suggest that LP and FS are shared within families, but this occurs more often with LP. In this respect, this trial, thus, looked at effectiveness (ie, infant outcomes after the provision of different food supplements to the family), rather than assessing growth under ideal conditions in which participants truly ate all the supplements that were intended for them.

Another major limitation of the trial is the lack of a nonsupplemented control group; therefore, we can make solid conclusions only on the relative value of FS and LP supplementation but not on their independent effect. In a previous study conducted 10 years earlier in the same area, the differences in the 50th percentile weight and length between 6 and 18 months of age were 2.3 kg and 11.9 cm, respectively, in unsupplemented infants (ie, 0.1 kg and 0.8 cm less than mean gains for the LP control group in the present trial). However, the different anthropometric methods used in the earlier trial and its historical nature limit its use for conclusive comparisons.

The ex-factory price of FS, when made in Malawi, is approximately US $4 per kilogram, corresponding to a daily cost of US $0.20 for a 50-g dose. Unsubsidized, this price is presumably unaffordable to many families in rural Malawi. The present results with FS25, and those from a recent Ghanaian trial using a daily dose of 20 g, suggest, however, that a lower and markedly cheaper dose may be as efficient in growth and development promotion as the FS50 dose used in the present trial. Other potential means for increased affordability include amendments to the FS recipe (eg, a cheaper protein source) and social marketing, both of which are being tested in the sub-Saharan African setting.

Taken together, these results suggest that 1-year-long complementary feeding with FS does not have a markedly larger effect than LP supplementation on mean weight gain in all infants, but it seems to boost linear growth in the most disadvantaged individuals and, hence, decrease the incidence of severe stunting in a population in which it is otherwise common. A larger and longer trial, however, is needed to confirm the finding and to look at the effect on outcomes other than growth, to analyze whether LP supplementation has some health impact, to see whether the growth effect persists beyond the age of 18 months, and to guide policy decisions on the use of spreads or corn–soy flour in malnutrition prevention in Malawi and similar settings. Further studies on the possible mechanisms of the growth-promoting effect of FS are also warranted.

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Author Contributions: Dr Phuka had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Phuka, Maleta, Thakwalakwa, Briend, Manary, and Ashorn. Acquisition of data: Phuka, Maleta, and Thakwalakwa. Analysis and interpretation of data: Phuka, Maleta, Thakwalakwa, Cheung, Manary, and Ashorn. Drafting of the manuscript: Phuka, Maleta, Thakwalakwa, Briend, and Ashorn. Critical revision of the manuscript for important intellectual content: Phuka, Maleta, Thakwalakwa, Cheung, Manary, and Ashorn. Statistical analysis: Phuka, Cheung, Briend, and Ashorn. Obtained funding: Ashorn. Administrative, technical, or material support: Phuka, Maleta, Thakwalakwa, Manary, and Ashorn. Study supervision: Maleta, Manary, and Ashorn.

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Postintervention growth of Malawian children who received 12-mo dietary supplementation with a lipid-based nutrient supplement or maize-soy flour¹–⁴

John C Phuka, Kenneth Malata, Chrissie Thakwalakwa, Yin Bun Cheung, André Briend, Mark J Manary, and Per Ashorn

ABSTRACT

Background: Therapeutic feeding with micronutrient-fortified lipid-based nutrient supplements (LNSs) has proven useful in the rehabilitation of severely malnourished children. We recently reported that complementary feeding of 6–18-mo-old infants with an LNS known as FS50 was associated with improved linear growth and a reduction in the incidence of severe stunting during the supplementation period.

Objective: Our objective was to assess whether a reduction in stunting seen with 12-mo LNS supplementation was sustained over a subsequent 2-y nonintervention period.

Design: One hundred eighty-two 6-mo-old healthy rural Malawian infants were randomly assigned to receive daily supplementation for 12 mo with 71 g of maize-soy flour1–4 (likuni phala LP; control group, 282 kcal) or either 50 g of FS50 (264 kcal; main intervention group), or 25 g of FS25 (130 kcal). Main outcome measures were incidence of severe stunting and mean weight-for-age, length-for-age, and weight-for-length during a 36-mo follow-up period.

Results: The cumulative 36-mo incidence of severe stunting was 19.6% in LP, 3.6% in FS50, and 10.3% in FS25 groups (P = 0.03). Mean weight-for-age changes were −1.09, −0.76, and −1.22 (P = 0.04); mean length-for-age changes were −0.47, −0.37, and −0.71 (P = 0.10); and mean weight-for-length changes were −1.52, −1.18, and −1.48 (P = 0.27). All differences were more marked among individuals with baseline length-for-age below the median. Differences in length developed during the intervention at age 10–18 mo, whereas weight differences continued to increase after the intervention.

Conclusions: Twelve-month-long complementary feeding with 50 g/d FS50 is likely to have a positive and sustained impact on the incidence of severe stunting in rural Malawi. Half-dose intervention may not have the same effect. This trial was registered at clinicaltrials.gov as NCT00131209.  Am J Clin Nutr 2009;89:382–90.

INTRODUCTION

Stunting, or statural growth failure, affects ≈170 million children <5 y of age, with a prevalence of 40% in southern Asia and 50% in sub-Saharan Africa (1–5). The condition is associated with many long-term consequences, such as poor cognitive or school performance, delayed motor development, impaired physical performance, reduced income in adulthood, obstetric emergencies, and lower birth weight in offspring (4, 6–15). Because of these adverse sequelae and the persistence of stunting after the age of 2 y (16, 17), prevention strategies are a global health priority. To date, however, few interventions have proven successful in markedly promoting sustainable linear growth of children <2 y old in low-resource settings (18).

In recent years, the treatment of children with severe acute malnutrition has drastically changed through the use of lipid-based nutrient supplements (LNSs) (19–25). These products typically contain milk protein, sugar, and a mixture of micronutrients, embedded in a lipid base (26). Because of their positive impact on severely malnourished individuals, ease of production and use, excellent storage properties, and flexibility of the fortified micronutrient composition, modified LNSs have also been considered a potential means for the prevention of stunting. Actual research data on this possibility are still scarce, but 3 recent clinical trials from sub-Saharan Africa suggest that provision of small daily doses of complementary LNS to 6–18-mo-old infants can promote their linear growth and yield other health benefits (27–29). To date, however, no longer term results have been published from trials using LNSs to prevent stunting.

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² AB is a staff member of the World Health Organization. The author alone is responsible for the views expressed in this publication, which do not necessarily represent the decisions or the stated policy of the World Health Organization. The funders of this trial had no role in its implementation, analysis, or reporting.

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We recently reported findings from a randomized trial in Malawi, which showed a 0% incidence of severe stunting [length-for-age z score (LAZ) ≤ −3] between 6 and 18 mo of age among infants receiving daily dietary supplementation with 50 g LNS for 1 y, compared with 13% among those receiving an isoenergetic dose of maize-soy flour and 4% among those receiving 25 g/d LNS (29). In the current article, we extend the follow-up of the same children to the subsequent 2-y period with no further dietary intervention. The new analysis focused on the incidence of stunting as well as mean length and weight gain between 6 and 42 mo of age.

SUBJECTS AND METHODS

Study area and timing

The study was conducted between 11 October 2004 and 8 January 2008 in Lungwena, a rural Malawian community with a high prevalence of early childhood stunting and underweight children (1, 30). The staple food, maize, was grown during a single rainy season between December and March. Exclusive breastfeeding for babies was almost nonexistent and infant diet was typically complemented with thin maize porridge from 2 to 6 mo of age.

Eligibility criteria, enrollment, and randomization of the trial participants

Inclusion criteria included age of 5.50–6.99 mo, residence in the study area, and an informed consent from at least one authorized guardian. Exclusion criteria were low weight-for-length z score (WLZ; ≤−2.0), presence of edema, history of peanut allergy, severe illness warranting hospitalization on the enrollment day, concurrent participation in another clinical trial, or any symptoms of food intolerance ≤30 min after the ingestion of a 6-g test dose of the peanut-based LNS used as a trial intervention.

For enrollment, trained health surveillance assistants contacted all families who were known to live in the area with a child between 5 and 7 mo of age. Infants were invited to an enrollment session where they were screened for eligibility, and guardians were given detailed information on the trial contents.

For group allocation, guardians picked one from a set of identically appearing opaque envelopes, each containing a paper indicating an identification number and a randomly assigned allocation to 1 of the 3 interventions. The randomization list and envelopes were made by persons not involved in trial implementation, and those assessing the outcomes were blinded throughout the study.

Interventions and follow-up

There were 3 intervention schemes given for 12 mo from the age of 6 mo, after which the participants were monitored for another 24 mo without additional intervention. Infants in the control group were provided with an average of 71 g/d of micronutrient-fortified maize and soy flour, locally called likuni phala (LP). Participants in the other 2 groups received, on average, either 50 or 25 g/d of an LNS known as fortified spread (FS; FS50 or FS25). The supplements were home delivered at 3 weekly intervals (at each food delivery, three 500-g bags of LP, four 262-g jars of FS50, or two 262-g jars of FS25 were provided).

LP was purchased from a local producer (Rab Processors, Limbe, Malawi). LNS was produced at a Malawian nongovernmental organization (Project Peanut Butter, Blantyre, Malawi) from peanut paste, milk powder, vegetable oil, sugar, and pre-made micronutrient mixture (Nutriset, Malaunay, France). All supplements were fortified with micronutrients, but the level of fortification varied between the products. The difference between the FS50 and FS25 supplementation was the amount of food base given (50 compared with 25 g/d). The micronutrient content, however, was adjusted so that children in both FS50 and FS25 groups received similar daily micronutrient doses. The energy and nutrient contents of a daily ration of each supplementation scheme are shown in Table 1.

FS50 and FS25 could be eaten raw, whereas the maize-soy flour (LP) required cooking into porridge before consumption. The guardians were provided with spoons and advised to daily offer their infants porridge containing 12 spoonfuls of LP, 8 spoonfuls of FS50, or 4 spoonfuls of FS25, which were divided into 2–3 daily doses. All mothers were encouraged to continue breastfeeding on demand and to feed their infants only as much of the food supplement as the infant wanted to consume at a feeding.

During the intervention, the participants were visited weekly at their homes to collect information on supplement use and possible adverse events during the intervention period. Empty food containers were collected every 3 wk. At 4, 8, 12, 18, and 36 mo after enrollment, the participants visited the research office at a nearby health center and underwent a physical examination and anthropometric assessment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP</td>
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<tr>
<td>Weight (g)</td>
<td>71</td>
</tr>
<tr>
<td>Energy (kcal)</td>
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<tr>
<td>Protein (g)</td>
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<tr>
<td>Carbohydrate (g)</td>
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<tr>
<td>Fat (g)</td>
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<td>Retinol (μg RE)</td>
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<tr>
<td>Folate (μg)</td>
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</tr>
<tr>
<td>Niacin (mg)</td>
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<tr>
<td>Pantothentic acid (mg)</td>
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</tr>
<tr>
<td>Riboflavin (mg)</td>
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</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.1</td>
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<tr>
<td>Vitamin B-6 (mg)</td>
<td>0.3</td>
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<tr>
<td>Vitamin B-12 (μg)</td>
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<tr>
<td>Vitamin C (mg)</td>
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<tr>
<td>Vitamin D (μg)</td>
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</tr>
<tr>
<td>Calcium (mg)</td>
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</tr>
<tr>
<td>Copper (mg)</td>
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</tr>
<tr>
<td>Iodine (μg)</td>
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</tr>
<tr>
<td>Iron (mg)</td>
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</tr>
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<td>Magnesium (mg)</td>
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<tr>
<td>Selenium (μg)</td>
<td>NA</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>3.6</td>
</tr>
</tbody>
</table>

[1] FS25, fortified spread 25 g/d; FS50, fortified spread 50 g/d; LP, likuni phala; NA, not available; RE, retinol equivalents.
Measurement of outcome variables

The primary outcome for the initial trial was weight gain. Secondary outcomes included the following: mean changes in length, head circumference, and mid-upper-arm circumference; the anthropometric indexes weight-for-age z score (WAZ), length- and height-for-age z score (LAZ/HAZ), and WLZ; and the incidence of underweight children, wasting, and stunting. In this secondary phase, we focused the analyses on the incidence of severe stunting (LAZ/HAZ < −3 z score units) or moderate-to-severe stunting (LAZ/HAZ < −2 z score units) and changes in the mean anthropometric indexes.

Weight was measured with naked infants by using an electronic infant weighing scale (SECA 834; Chasmons Ltd, London, United Kingdom) and recorded to the nearest 10 g. Length (at ≤24 mo of age) and height (at >24 mo) were measured to the nearest 1 mm with a high-quality length board (Kiddimetre; Raven Equipment Ltd, Essex, United Kingdom) and a high-quality stadiometer (Harpenden stadiometer; Child Growth Foundation, London, United Kingdom). Mid-upper-arm and head circumferences were measured with nonstretchable plastic tapes (Lasso-o tape; Harlow Printing Ltd, South Shields, United Kingdom). Anthropometric indexes (WAZ, LAZ, and WLZ) were calculated with Epi-Info 3.3.2 software (Centers for Disease Control and Prevention, Atlanta, GA) with the use of the the CDC 2000 growth reference charts (31).

Sample size calculation

Sample size was calculated from expected weight gain at the end of the initial 12-mo intervention period. Assuming a SD of 1.40 kg in all intervention groups and a difference of 0.75 kg between the means in the main intervention group (FS50) and the control group (LP), a sample size of 55 infants/group was calculated to provide the trial with 80% power and 95% confidence. To allow for ≈10% attrition, the target enrollment was 60 infants/group.

Data management and analysis

Data were recorded on paper forms, transcribed to paper case report forms, and entered twice into a custom Microsoft Access 2003 database program (Microsoft Corp, Redmond, WA). The double entries were electronically compared, and extreme values were confirmed or corrected.

Statistical analysis was performed by using Stata 9.0 (StataCorp, College Station, TX) on an intention-to-treat basis. Infants with no anthropometric data after enrollment were included only in the comparison of baseline characteristics. The analyses of anthropometric measures used data from young children who were available at 42 mo of age; data from infants and young children with at least one measurement after enrollment were analyzed for incidence of stunting with the use of the survival analysis method to control for censoring.

For continuous and categorical outcomes, the 3 intervention groups were compared by using analysis of variance (ANOVA) and Fisher’s exact test, respectively. Post hoc pairwise comparisons for statistically significant intergroup differences were analyzed using the Tukey wholly significant difference test. Survival analysis was used to determine the cumulative probability of severe or moderate stunting among different groups, and the differences were tested by the log-rank test. An event was considered to have occurred at midpoint when it occurred between the time the event was detected and the previous measurement. Individuals with severe or moderate-to-severe stunting at enrollment were excluded from survival analyses on the incidence of that outcome. Effect sizes were calculated by dividing the difference in mean z scores between FS50 and either FS25 or LP groups by the pooled SDs of the 2 means.

Ethics, study registration, and participant safety

The trial was performed according to International Conference of Harmonisation—Good Clinical Practice guidelines (ICH-GCP), and it adhered to the principles of the Declaration of Helsinki and the regulatory guidelines in Malawi. Before the onset of enrollment, the trial protocol was reviewed and approved by the College of Medicine Research and Ethics Committee (University of Malawi) and the Ethical Committee of Pirkannaa Hospital District (Finland). Key details of the protocol were published at the clinical trial registry of the National Library of Medicine, Bethesda, MD. Before enrollment and again between the initial 12-mo intervention and the subsequent 24-mo follow-up, participant guardians were briefed about the study, and they signed a written consent form for trial participation.

A data safety and monitoring board continuously monitored the incidence of suspected serious adverse events during the intervention period, defined as any untoward medical occurrence that resulted in death, was life threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in a persistent or significant disability or incapacity or other serious medical condition.

RESULTS

Of the 303 initially screened infants, 182 were enrolled in the complementary feeding trial. Of these, 10 died and 4 were lost to follow-up during the 12-mo food-supplementation period; thus, 168 participated in the postintervention follow-up. During the 24-mo postintervention follow-up, there were 6 deaths and 13 further losses to follow-up, which left 149 participants who completed the 36-mo assessment (Figure 1). There was no statistically significant difference in the proportion of deaths and number of dropouts between the food intervention groups (P = 0.72 and 0.26, respectively).

Selected baseline characteristics of the participants by intervention group are shown in Table 2. At enrollment, mean anthropometric measurements were comparable in the LP and FS50 groups, whereas infants in the FS25 group were, on average, slightly heavier and taller (Table 2). The prevalences (number of infants) of severe stunting and severe underweight at enrollment in the LP, FS50, and FS25, groups, respectively, were as follows: severe stunting: 3.3% (n = 2), 6.6% (n = 4), and 1.7% (n = 1); severe underweight: 1.6% (n = 1), 3.3% (n = 2), and 0.0% (n = 0).

Figure 2 describes a time-to-event analysis of stunting in different intervention groups. By the end of the 12-mo intervention period, 13.3% (n = 7), 0.0% (n = 0), and 3.5% (n = 2) of the participants in the LP, FS50, and FS25 groups, respectively, had developed severe stunting (29). In the postintervention follow-up, 4–7% of the participants in each group...
developed the condition, which increased the cumulative 3-y incidence (number of cases) of severe stunting to 19.6% (n = 11) in LP, 3.6% (n = 2) in FS50, and 10.3% (n = 6) in FS25 groups (P = 0.03, log-rank test; Figure 2A). The point estimate (95% CI) for the difference between the LP and FS50 groups was 16% (5–26%), and the number needed to treat or prevent one case of severe stunting was 6 (4–20). The cumulative incidence of moderate-to-severe stunting was more similar in the 3 groups, although infants in the FS50 group tended to develop the condition on average somewhat later (P = 0.50, log-rank test; Figure 2B).

Among the 149 participants who completed the follow-up, mean 3-y gains in weight, height, and mid-upper-arm and head circumferences were highest in the FS50 group and lowest in the FS25 group. Average values for the anthropometric indexes WAZ, HAZ, and weight-for-height z score (WHZ) decreased in all groups, but the reductions were smallest among the FS50 group and largest in the FS25 group. However, intergroup differences reached statistical significance among unselected children only for WAZ change (Table 3). During the follow-up period, those who received LP or FS25, on average, had larger reductions in their WAZ compared with FS50 children: 0.33 (95% CI: −0.39, 0.69) 0.46 (95% CI: 0.10, 0.82) z score units for LP and FS25 groups, respectively. Similarly, those who received LP or FS25 had larger reductions in their HAZ during the follow-up: 0.10 (95% CI: 0.02, 0.67) 0.34 (95% CI: 0.02, 0.67) z score units for LP and FS25 groups.

TABLE 2
Baseline characteristics of the 182 participants at enrollment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP (n = 61)</td>
</tr>
<tr>
<td>Male sex</td>
<td>24/61 (39.3)(^2)</td>
</tr>
<tr>
<td>PCR-confirmed HIV infection</td>
<td>0/55 (0.0)</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>5.91 ± 0.41(^4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6.92 ± 0.93</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>62.8 ± 2.1</td>
</tr>
<tr>
<td>Mid-upper-arm circumference (cm)</td>
<td>13.4 ± 0.9</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>43.0 ± 1.5</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>−0.65 ± 1.07</td>
</tr>
<tr>
<td>Length-for-age z score</td>
<td>−1.20 ± 0.82</td>
</tr>
<tr>
<td>Weight-for-length z score</td>
<td>0.48 ± 1.08</td>
</tr>
</tbody>
</table>

\(^4\) FS25, fortified spread 25 g/d; FS50, fortified spread 50 g/d; LP, likuni phala; PCR, polymerase chain reaction.

\(^2\) n/total n; percentage in parentheses (all such values).

\(^4\) Mean ± SD (all such values).
respectively. Adjusting the analyses for baseline weight resulted in findings similar to the unadjusted analyses (data not shown).

Because of our earlier observation that there was a strong interaction between a child’s length at 6 mo of age and his or her weight and length gains during the intervention ($P = 0.002$ and $P = 0.04$, respectively) (29), we also conducted stratified analyses on these outcomes during the postintervention follow-up. As shown in Table 3, the largest and smallest anthropometric gains were seen in the FS50 and FS25 groups, respectively, both among children with baseline height below the population median and those above it. However, the absolute differences in weight and length were bigger, and they more often reached statistical significance in the initially shorter participants (Table 3). Among these infants, those who received FS50 had, on average, a 0.61 kg (95% CI: $-0.15, 1.37$ kg) higher weight gain or a 0.53 (95% CI: 0.07, 0.99) $z$ score unit smaller reduction in WAZ than did LP children and a 0.91 kg (95% CI: 0.09, 1.73 kg) higher gain or a 0.81 (95% CI: 0.31, 1.30) $z$ score unit smaller reduction in WAZ than did FS25 children. Differences in height gains showed a similar pattern but did not reach statistical significance (Table 3).

**FIGURE 2.** Cumulative incidence functions of (A) severe stunting [length- and height-for-age $z$ score (LAZ/HAZ) $< -3$ $z$ score units] and (B) moderate-to-severe stunting (LAZ/HAZ $< -3$ $z$ score units) among children in the LP, FS25, and FS50 groups. Throughout the follow-up, all surviving participants were used as the denominator for the incidence. Supplementation was provided for the first 12 mo only. LP, likuni phala; FS50, fortified spread 50 g/d; FS25, fortified spread 25 g/d.

### Table 3
Outcome changes during 36 mo of follow-up in young children who received different doses of fortified spread (FS) and likuni phala (LP)$^1$

<table>
<thead>
<tr>
<th>Variable</th>
<th>All participants</th>
<th>Baseline LAZ $&lt; $ median</th>
<th>Baseline LAZ $\geq$ median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP group ($n = 50$)</td>
<td>FS50 group ($n = 46$)</td>
<td>FS25 group ($n = 53$)</td>
</tr>
<tr>
<td>Change in weight (kg)</td>
<td>5.61 $\pm$ 1.12$^2$</td>
<td>6.00 $\pm$ 1.31</td>
<td>5.58 $\pm$ 1.08</td>
</tr>
<tr>
<td>Change in height (cm)</td>
<td>28.4 $\pm$ 2.7</td>
<td>28.6 $\pm$ 2.8</td>
<td>27.9 $\pm$ 3.1</td>
</tr>
<tr>
<td>Change in mid-upper-arm circumference (cm)</td>
<td>1.5 $\pm$ 1.1</td>
<td>1.4 $\pm$ 1.3</td>
<td>1.0 $\pm$ 1.1</td>
</tr>
<tr>
<td>Change in head circumference (cm)</td>
<td>5.6 $\pm$ 0.7</td>
<td>5.8 $\pm$ 0.9</td>
<td>5.6 $\pm$ 0.8</td>
</tr>
<tr>
<td>Change in weight-for-age $z$ score</td>
<td>$-1.09$ $\pm$ 0.90</td>
<td>$-0.76$ $\pm$ 0.99</td>
<td>$-1.22$ $\pm$ 0.84</td>
</tr>
<tr>
<td>Change in height-for-age $z$ score</td>
<td>$-0.47$ $\pm$ 0.76</td>
<td>$-0.37$ $\pm$ 0.83</td>
<td>$-0.71$ $\pm$ 0.87</td>
</tr>
<tr>
<td>Change in weight-for-length $z$ score</td>
<td>$-1.52$ $\pm$ 1.20</td>
<td>$-1.18$ $\pm$ 1.28</td>
<td>$-1.48$ $\pm$ 0.92</td>
</tr>
</tbody>
</table>

$^1$ FS25, 25 g/d; FS50, 50 g/d; LAZ, length-for-age $z$ score.

$^2$ Obtained by ANOVA.

$^3$ Mean $\pm$ SD (all such values).
To demonstrate the timing of growth failure in the 3 intervention groups, we plotted the changes of children’s mean anthropometric indexes as a function of the trial duration (Figure 3). As shown, the difference in HAZ between LP and FS groups started to develop after 4 mo (when children were 10 mo old) and reached a peak 4–8 mo later (at 14–18 mo of age). In contrast, differences in WAZ or WHZ increased gradually during the 12-mo intervention and continued to grow wider during the 2-y postintervention follow-up (Figure 3). The patterns were similar among participants with baseline height above or below the median, but absolute values were more pronounced among those who were shorter at the beginning (data for individuals with higher baseline HAZ not shown). The corresponding attained weight or height by age and intervention group, both among all children and only those with baseline LAZ below the median, is shown in Figure 4.

DISCUSSION

We previously reported findings from a randomized controlled trial that reported a lower incidence of severe linear growth failure (stunting) among infants whose diet was supplemented for 1 y with a specific formula (FS50) of micronutrient-fortified LNS rather than an iso-energetic ration of maize and soy flour (LP) (29). In the present study, we analyzed the duration of the effect by extending the growth follow-up for an additional 2 y without providing further food supplements. After the intervention, severe stunting continued to occur slightly more often in the control group, so that over the entire 3-y follow-up, its incidence was almost 20% in the LP group but only 4% in the FS50 group. Hypothesis testing suggested that the observed difference was unlikely to be caused by chance alone. Selection and implementation bias was avoided by procedures such as population-based enrollment, use of a control group, randomized group allocation and blinding of outcome assessors, strict control of dropouts, and the use of standardized outcome variables. The observed results are thus consistent with the idea that 1-y supplementation of infant diet with 50 g/d FS50 is associated in rural Malawi with a reduced incidence of severe stunting during a period that covers the intervention and subsequent 2 y.

Similar to our earlier results (29), the growth difference between the intervention and the control group was largest when measured with dichotomous and more severe outcomes or among those individuals who were somehow already disadvantaged (mildly stunted) at enrollment. Differences in mean length or height developed mostly during the intervention (at 10–18 mo of age), whereas weight differences became more pronounced later,

FIGURE 3. Development of mean cumulative anthropometric changes in all participants and in those with baseline length-for-age z score (LAZ) below median. WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score; LP, likuni phala; FS50, fortified spread 50 g/d; FS25, fortified spread 25 g/d.
in the second and third year of life. Although no conclusive explanation can be given to this phenomenon, the results are consistent with the idea that FS50 supplementation somehow affected the timing of acceleration in the infants’ linear growth velocity (32). Such acceleration, known as the infancy-to-childhood growth spurt (IC spurt), occurs at an average age of 9 mo in industrialized countries but often much later in low-income settings (17, 33). Each month of delay in IC spurt is associated, on average, with 0.5-cm deficit in length gain by 5 y (17, 33).

FIGURE 4. Attained weight and length in all participants and those with baseline length-for-age z score (LAZ) below median during the follow-up. LP: likuni phala; FS50, fortified spread 50 g/d; FS25, fortified spread 25 g/d.

Factors that induce the IC spurt in infants are unknown at present, but dietary intake of cow milk may play a role in the process (34). Theoretically, it is thus possible that the observed linear growth differences in our trial were due to the cow milk protein fraction in FS50 (10 g in the 50-g/d dose), an increased intake of which might have led, on average, to a 4 mo earlier induction of IC spurt in the intervention compared with the control group. This suggestion, while still hypothetical, receives some support from the fact that children grew less in the group that received a lower dose of FS (FS25), which had the same micronutrient content but only half the amount of milk. Furthermore, another recent complementary feeding trial in Malawi, in which the same-age children were supplemented with a soy-containing modification of LNS (no milk powder), did not document any length-gain acceleration (35). Other possible explanations of the linear growth variation during the intervention period include the different concentration and bioavailability of other nutrients, eg, zinc or essential fatty acids, in the FS50 and maize-soy rations (27, 36).

Contrary to the findings on linear growth, weight differences between the groups grew larger, especially after the intervention. Whereas part of this finding may be attributed to the timing of the IC spurt, other variables are likely to play a role as well. Possible explanations include the impact of FS50 supplementation on the children’s general health and susceptibility to infections or on appetite. Alternatively, the guardians may have continued to provide nutritious snacks to children who had earlier received FS50 but not to those who had received LP. Although these are biologically plausible explanations, we have no data to support any of these possibilities.

A third important point from our results is the comparison of outcomes among children who received the higher (50 g/d) and the lower (25 g/d) doses of FS. The higher dose was designed to provide approximately the recommended amount of energy from supplement foods for 6-mo-old infants without affecting the breast
milk intake of the recipients (37, 38). Because the price for such a dose (~$0.20/d) would be relatively high for the rural families in Malawi, we wanted to see if half of the dose would produce the same growth outcomes but more inexpensively. Unfortunately, this was not the case; children in the lower-dose group had both a higher incidence of severe stunting and lower mean weight and height gains during the intervention and especially thereafter. A comparable phenomenon was earlier observed in a Malawian dose-finding trial, in which underweight infants were given different doses of LNS for 12 wk (39). Although a smaller (20 g/d) dose of a similar supplement (Nutributter; Nutriset, Malaunay, France) yielded positive results on linear growth and motor development among 6–12-mo-old infants in Ghana (27), the larger dose may thus be more appropriate in the Malawian setting. The apparent discrepancy may be explained by the much higher degree of stunting in the Malawian setting or the fact that the higher dose was not tested in the Ghanaian setting (27, 29).

The 2 main limitations of our trial were the lack of comprehensive data on the participants’ dietary intakes during or after the intervention and the lack of a nonsupplemented control group. Breast milk intakes were comparable in all trial groups before the intervention and 4 wk after its onset, but subsequent dietary intakes are unknown (40). Furthermore, because of the lack of an unsupplemented control group, we can make solid conclusions only on the relative value of the FS50 and maize-soy flour (LP) supplementations but not on their independent effect. In this comparison, the effect size on linear growth was comparable with other complementary-feeding trials that made comparisons with unsupplemented control children (18). However, in our setting, even the control intervention (LP supplementation) may have had some impact on the growth outcomes, as suggested by better outcomes in the control group than in the half-dose LNS group. If compared with no-food control children, the growth-promoting effect of LNS might thus prove even more favorable than what was observed in this sample. Further trials should investigate this possibility.

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The authors’ responsibilities were as follows—JCP, KM, CT, AB, MJM, and PA: designed the trial; JCP and CT: responsible for data collection; YBC: analyzed the data; JCP, KM, and PA: supervised the planning of another research project by the same study team responsible for its integrity and the accuracy of the data analysis. AB was responsible for the planning of another research project by the same study team responsible for its integrity and the accuracy of the data analysis. AB was responsible for the planning of another research project by the same study team responsible for its integrity and the accuracy of the data analysis. The authors declared no conflict of interest.

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supplements is well accepted and has positive effects on infant iron status in Ghana. Am J Clin Nutr 2008;87:929–38.


