Effect of Consuming Small-Quantity Lipid-based Nutrient Supplements on Breast Milk, Energy and Nutrient Intake and the Association Between Amount of Breast Milk Intake and Growth and Development of Malawian Rural Children
CHIZA KUMWENDA

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ACADEMIC DISSERTATION
To be presented, with the permission of the Board of the School of Medicine of the University of Tampere, for public discussion in the auditorium F114 of the Arvo building, Lääkärinkatu 1, Tampere, on 10 February 2017, at 9 o’clock.

UNIVERSITY OF TAMPERE
CHIZA KUMWENDA

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Stunting remains a big public health problem in Malawi. Thirty-seven percent of under-five children are stunted in Malawi. While at global level there has been a significant reduction in stunting levels, currently at 27%, Malawi has failed to register significant progress towards reducing stunting. One of the major factors contributing to high levels of stunting in Malawi is suboptimal infant and young child feeding practices, including poor breastfeeding and dietary practices. So far evidence is lacking on the contribution of the amount of breast milk on infant and child growth and development after 6 months of exclusive breastfeeding.

The present study had four aims; to assess the effect of small quantity lipid-based nutrient supplement (SQ-LNS) on breast milk intake, to assess energy and nutrient intake among infants whose complementary foods were supplemented with SQ-LNS and those in the control group, to assess determinants of breast milk intake and finally to assess the relationship between amount of breast milk and growth and development among infants and young children age 9–18 months in Malawi. To measure the amount of breast milk intake, the dose-to-mother deuterium dilution technique was used, the method is currently considered the gold standard in assessment of breast milk intake in community settings. Energy and nutrient intakes were assessed using the interactive-24 hour dietary recall method. Motor and language development among infants at 18 months of age were assessed using the Kilifi Developmental Inventory (KDI) and adapted version of the MacArthur-Bates Communicative Development Inventory respectively. Regression analysis was used to determine predictors of breast milk intake and to assess the association between amount of breast milk and growth and development. The present study was conducted in Mangochi district, south of Malawi, Southern Africa.

Intake of breast milk among 9–10 months old infants (n=359) was not significantly different between those supplemented with SQ-LNS at a dose ranging from 10 to 40g/d and infants in the control group who did not receive SQ-LNS. The overall mean (SD) and the mean (SD) daily breast milk intake of infants in the control arm was 752.0 (244) and 730.6 (226) g respectively. The differences (95% CIs) in mean intake of infants provided with 10, 20, or 40 g SQ-LNS/d, compared with controls, were +62 (-218, +143), +30 (-240, +99), and +2 (-268, +72) g/d, respectively.
Children in the SQ-LNS intervention groups had significantly higher dietary energy, protein and fat intakes than those in the control group. However, no significant differences were observed in median intakes of energy from non-SQ-LNS complementary foods implying that SQ-LNSs increased intakes of energy and macronutrients without displacing locally available complementary foods.

To study the predictors of breast milk intake, path analysis was performed prior to conducting regression analysis, in order to establish potential causal relationships between variables. The regression analysis demonstrated that infant breast milk intake was significantly predicted by infant weight, and the following maternal factors, height, age and education. An increase in body weight of an infant by 1kg was associated with an increase in breast milk intake of 68g per day. On the other hand, an additional year spent in school by the mother was associated with a reduction in breast milk intake by about 11g per day. An increase in maternal age by a year was associated with a reduction in amount of breast milk intake by 6.5 grams, while an additional centimetre of maternal height was associated with an increase in breast milk intake by almost 7 grams.

The association between breast milk amount and growth and development was assessed using both univariate and multiple regression analyses. In multiple regression, breast milk intake (g/d) at 9–10 months was not associated with either growth between 12 and 18 months or development at 18 months, but was positively associated with attained length for age-z score at 12 months.

In conclusion supplementation of complementary foods with SQ-LNS did not result in reduction in breast milk intake at 9–10 months of age and displacement of complementary foods among rural Malawian infants. Amount of breast milk intake during later infancy was not associated with growth and development in early childhood. Infant weight, maternal height, age and education were the main predictors of breast milk intake among Malawian infants. Therefore, promotion, support and protection of optimal breastfeeding practices should continue to be part of the public health nutrition messages, while improvement of complementary foods with products like SQ-LNS should also be advocated for.
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LIST OF ORIGINAL PAPERS

The original articles of the thesis are:


III Chiza Kumwenda, Jaimie Hemsworth, John Phuka, Mary Arimond, Ulla Ashorn, Kenneth Maleta, Per Ashorn, Marjorie J. Haskell, Kathryn G. Dewey Factors associated with breast milk intake among 9-10 month-old Malawian infants. *Matern Child Nutr.* Published online 11 August 2015. doi:10.1111/mcn.12199
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>$^{2}$H$_2$O</td>
<td>Deuterium oxide</td>
</tr>
<tr>
<td>EED</td>
<td>Environmental Enteric Dysfunction</td>
</tr>
<tr>
<td>HAZ</td>
<td>Height-for-age z-score</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>IDA</td>
<td>Iron deficiency anemia</td>
</tr>
<tr>
<td>i-24-HR</td>
<td>Interactive 24-hour dietary recall</td>
</tr>
<tr>
<td>LAZ</td>
<td>Length-for-age z-score</td>
</tr>
<tr>
<td>MMN</td>
<td>Multiple micronutrients</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid-upper arm circumference</td>
</tr>
<tr>
<td>NSO</td>
<td>National Statistics Office</td>
</tr>
<tr>
<td>PAHO</td>
<td>Pan-American Health Organization</td>
</tr>
<tr>
<td>RUTF</td>
<td>Ready to use therapeutic food</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SQ-LNS</td>
<td>Small quantity lipid-based nutrient supplements</td>
</tr>
<tr>
<td>UN</td>
<td>United Nations</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations Children's Fund</td>
</tr>
<tr>
<td>VAD</td>
<td>Vitamin A Deficiency</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHZ</td>
<td>Weight-for-height z-score</td>
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</tbody>
</table>
1 INTRODUCTION

Almost all infants and young children have the potential to attain healthy growth and development [1]. One of the major determinants of poor infant and young child growth and development is undernutrition, which encompasses acute and chronic malnutrition and micronutrient deficiencies. In the short and immediate terms, undernutrition is also one of the major risk factors for morbidity and mortality during infancy and childhood [2]. Stunting (<-2 SD for length/height-age z-score) during infancy and early childhood is associated with adult short stature, less schooling and less economic productivity and in case of girls there is an increased risk of giving birth to stunted infants [3]. Furthermore, undernourished infants and young children who gain excessive weight have an elevated risk of chronic non communicable diseases [4, 5].

The etiology, determinants and timing of stunting have all been well documented [6, 7]. Albeit the progress made in reducing stunting [8] and knowing the causes and what works and at what cost to prevent stunting [9, 10], the prevalence of stunting remain high in most developing nations [11], including Malawi [12]. Consequently, global efforts to reduce levels of undernutrition have been heightened after noting that most low income countries failed to reduce levels of undernutrition to the agreed goals by 2015. The recent United Nations (UN) Sustainable development goals aim to end all forms of hunger and malnutrition by 2030.

One of the most effective public health approaches in combating infant and child under-nutrition is believed to be practicing optimal infant and young child feeding. WHO recommends exclusive breastfeeding of infants for the first 6 months from birth, thereafter appropriate and nutritious complementary foods are supposed to be introduced to meet the infants’ increasing nutritional needs [13]. Complementary foods in most low income countries are nutritionally poor [14, 15], and have been shown to be associated with undernutrition [16].

A number of strategies have been proposed to improve complementary foods to prevent malnutrition among infants and young children. Some of the strategies include provision of diversified foods [17], using fortified complementary products such as
micronutrient powders [18], micronutrient tablets, fortified infant cereals, fortified biscuits, and lipid-based nutrient supplements [10, 19]. Small quantity lipid-based nutrient supplements (SQ-LNS), in addition to providing multiple micronutrients, also provides essential fatty acids, protein and energy [20]. SQ-LNS have several advantages: they do not require change in dietary practices, and are less expensive than centrally processed, fortified complementary foods. SQ-LNS are also well accepted by infants [21, 22]. SQ-LNS have long shelf life even in tropical temperatures [23] and can potentially be produced locally [24].

The use of SQ-LNS in complementary feeding has received great interest over the past years, largely as a result of their efficacy and effectiveness in treatment of severe acute malnutrition when used as a ready to use therapeutic food (RUFT) [25–27]. Several investigators have been researching on the efficacy of SQ-LNS to prevent undernutrition [www.ilins.org; [28]].

SQ-LNS are both nutrient and energy dense products, however, their impact on breast milk intake among infants and young children has not been well documented. Infants self-regulate their total energy intake [29, 30], thus provision of SQ-LNS to breastfed infants has the potential to reduce breast milk intake and displace local complementary foods. Apart from energy density of complementary foods, infant breast milk intake may be influenced by other non-dietary determinants which operate differently in different environments. Unequivocal evidence has demonstrated that breastfeeding supports growth and development during the first 6 months of an infant’s life [31, 32]. Breast milk may contribute up to half or more of the energy and some nutrient needs of infants after 6 [33]. However, it is also not well known whether more breast milk compared to the complementary foods is important for growth and development after 6 months of exclusive breastfeeding.

The present study was mainly designed to assess the association between breast milk intake and growth and development among infants from rural Malawi. The study also assessed the impact of SQ-LNS on intakes of breast milk and complementary foods among infants. Finally the study assessed determinants of breast milk intake.
2 LITERATURE REVIEW

The literature review presents the background, the search strategy for publications related to the background of the study and finally the justification for the research. The review focuses on the following main topics; epidemiology and importance of malnutrition among infants and young children; causes of malnutrition; current approaches for combating malnutrition; the role of breast milk on growth and development beyond 6 months of age and the determinants of breast milk intake.

The search for the literature was done mainly through PubMed, using Medical Subject Heading Terms (MeSH) and key words and combination of words including: breastfeeding, breast milk, volume, determinants, development, growth, Africa south of the Sahara, complementary feeding, dietary assessment, 24 hour recall, energy intake, infants and young children. In addition, the reference lists in the eligible publications identified by the search were manually checked to identify further eligible studies that might have been missed in the electronic search. Relevant publications from UN agencies were also electronically searched. The review considered literature from studies, reviews and meta-analyses mainly dating back to the 70s. The literature search was conducted from December 2014, with the latest review conducted in the month of August, 2016. The review primarily focused on infants and young children up to the age of two years.

2.1 Epidemiology and importance of malnutrition among children

Malnutrition, especially stunting still remains a global health problem. By the year 2014, 159 million children under-five years of age were stunted worldwide [11], most of these children come from low income countries [34]. At global level the proportion of stunted children has been steadily declining, from 40% in 1990 to about 24% in 2014, on the other hand, in Africa prevalence of stunted children has not shown any significant decline, from 42 in 1990 to 32% in 2014 [11]. Within Africa, there are also wide variations in stunting levels, with North African countries having by far much
lower stunting levels than sub-Saharan Africa, at 17.2% and 35.7% respectively [11]. In Asia significant progress has been achieved almost halving stunting from 47.6% to 25.1% within the same time frame [11].

The global burden of micronutrient deficiencies is quite staggering, the problem dubbed hidden hunger affects millions of people especially in low income countries [35]. The most common micronutrient deficiency is iron, globally an estimated 2 billion people are iron deficient [36]. Iron deficiency is the major cause of anaemia among women of reproductive age group and young children [37] and is one of the major risk factors for morbidity and mortality among women. Among children, iron deficiency is also associated with poor physical and cognitive development [38]. Other micronutrient deficiencies of global health importance include vitamin A, iodine and zinc deficiencies. Vitamin A deficiency (VAD) is associated with increased incidence and severity of infections, the condition is one of the primary causes of morbidity and mortality among young children [39], especially in Africa and Southeast Asia [40]. VAD is also the main cause of preventable blindness in children, the WHO estimates that between 250 000 to 500 000 VAD children become blind every year and half of them die within 12 months of losing their sight (WHO, http://www.who.int/nutrition/topics/vad/en/).

Like is the case at global level, the major form of infant and young child undernutrition in Malawi is stunting. In comparison to the global and regional stunting rates at 24% and 32% respectively [11], stunting levels in Malawi are high. About 37% of all under-five children in Malawi are stunted [41]. Wasting (<-2 SD for weight-for-length z-score) and underweight (<-2 SD weight-for-age z-score) are at 3% and 12% respectively [41], the situation has not significantly improved since the early 90s. It is apparent that malnutrition levels in Malawi have been a public health problem for a very long time.

Micronutrient deficiencies are also quite common in Malawi. The deficiencies of public health importance include vitamin A, iron and zinc deficiency. Most recent estimates [42] indicate that in Malawi vitamin A and iron deficiencies among infants are at 22.9% and 50.9% respectively. Zinc deficiency is also likely to be high. The levels of anaemia among 6–59 months old children are also quite high, with 63% of these children being anaemic [43].

In low income countries, malnutrition is evident quite early in life, even at birth [44] with the incidence of stunting peaking between 3 and 12 months [6]. The period between conception and 24 months is one of the critical “windows of opportunity” during which time growth faltering may be prevented [45]. The period is called as such considering that it is very difficult or impossible to reverse stunting after this period [46]. Thus global efforts, such as the Scaling Up Nutrition movement, (http://thousanddays.org/) have been heightened to target this window period.
2.2 Causes of malnutrition

Malnutrition results from an interplay of several factors operating on different levels. UNICEF developed a conceptual framework (Figure 1) to depict levels under which factors directly or indirectly operate with an ultimate manifestation of malnutrition [47]. The framework has been adapted by others to include maternal malnutrition as well as incorporate both short and long term consequences of malnutrition [2, 48]. Within the context of stunting, Stewart et al. [48] describe three levels, namely context, causes and consequences. Malnutrition is detrimental to survival, growth, and development of children, as such the Stewart et al. 2013 conceptual framework really does complete the whole spectrum of malnutrition from context to results.

The UNICEF [49] immediate causes of malnutrition are inadequate dietary intake and disease. Suboptimal dietary practices and disease may independently or through interaction cause malnutrition, if not controlled a vicious cycle may be formed [50]. Recently, the role of subclinical infections, especially inflammation of the small intestine by enteric pathogens is being recognized as one of the significant causes of undernutrition [51, 52]. The condition is referred to as environmental enteric dysfunction (EED) [53]. The gut of children may be infected by pathogenic microbes associated with increased intestinal permeability, impaired gut immune function and malabsorption.

Factors operating in the immediate environment of the infant have been categorized as underlying causes. These factors largely operate within household settings, they include household food insecurity, inadequate care and unhealthy household environment and lack of health services. Finally factors operating at population level have been termed basic factors, which are related to malnutrition through their influence on underlying factors. These factors include political, cultural, economic and social systems.
2.3 Approaches for promoting healthy growth and development

Many evidence based interventions have been designed to prevent malnutrition, they are now categorized as nutrition sensitive and specific based on the levels under which factors operate [10]. Evidence has so far accumulated on “what works” in order to prevent malnutrition [2, 10, 56]. The 2013 Lancet Nutrition series documented nutrition specific interventions targeting adolescents, women of reproductive age,
neonates, infants and children (Figure 2). Nutrition specific interventions are defined as interventions or programmes that address the immediate determinants of fetal and child nutrition and development [57]. More evidence is still coming from some potential interventions, for instance the use of SQ-LNS to promote growth and development (www.ilins.org).

Figure 2: Age specific nutrition specific interventions
(Adapted from: Bhutta et al. [10])

2.3.1 Optimal breastfeeding

The WHO defines optimal breastfeeding as initiation of breastfeeding within 1 hour of birth, exclusive breastfeeding of infants until 6 months of age, and continued breastfeeding until 2 years of age or older [13]. Optimal breastfeeding is associated with a reduction in morbidity and mortality among infants and young children [58]. The Lancet series on Maternal and Child nutrition of 2013, indicated that sub-optimal breastfeeding was responsible for 804,000 deaths among children under 2 years and about 12% of total deaths of under-five children in 2011 [59]. The most recent Lancet series on breastfeeding estimates that scaling up of breastfeeding to a near universal level could prevent 823,000 annual deaths in children younger than 5 years [60]. Overall optimal breastfeeding plays an important role in infant survival [9], which is
mainly attributed to exclusive breastfeeding [61]. From birth to 6 months, breast milk is sufficient for healthy growth and development among infants. Generally breastfeeding has been shown to be linked to better cognitive development among children even beyond childhood [60, 62]. In Malawi, appropriate breastfeeding practices are still below optimal levels, even though compared to regional and global estimates the country is doing much better (Table 1). The higher estimates of the appropriate breastfeeding practices in Malawi do not tarry with the prevalence of infant and young child malnutrition.

Table 1: Breastfeeding practices in Malawi, sub-Saharan Africa and globally

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<tr>
<td>Early initiation</td>
<td>94.5</td>
<td>60.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Exclusive breastfeeding</td>
<td>61.0</td>
<td>54.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Continued breastfeeding at 1 year</td>
<td>91.6</td>
<td>-</td>
<td>74.0</td>
</tr>
<tr>
<td>Continued breastfeeding at 2 years</td>
<td>71.5</td>
<td>60.0</td>
<td>49.0</td>
</tr>
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</table>

Estimates are from the recent Malawi Demographic and Health Survey [41]a, the Lancet breastfeeding series[60]b and Unicef [63]c.

Inadequate breastfeeding practices is one of the proximal factors causing malnutrition among infants and young children [48]. It would follow then that optimal breastfeeding would prevent stunting. However, so far the evidence on impact of breastfeeding on prevention of stunting is not convincing [10, 61, 64]. Even interventions promoting breastfeeding have not been associated with growth among children [65]. Presently, it is not clear why this is the case. It could be because methods for measuring breastfeeding practices fail to capture true practices or other variables that are not optimal for promoting healthy growth, like unfavourable infant gut environment [64]. This is why research in EED will prove critical in elaborating the apparent lack of effect of optimal breastfeeding on stunting. The quality of breast milk may also play an important role on growth and development [66]. Breast milk quality may be influenced by maternal diet [67] and nutritional status [68]. However, others have suggested that poor breast milk quality does not play a major causal role on infant and young child malnutrition, since the breast milk content of nutrients mainly related with growth is not affected by maternal status [69].

The prevalence of breastfeeding after 6 months of age (defined as continued breastfeeding by WHO) is common in low-income and lower-middle-income countries [60]. Evidence from meta-analyses of studies assessing the impact of any breastfeeding on mortality demonstrated a reduction in the risk of mortality by 50% among children
2.3.2 Complementary feeding and dietary diversification

Complementary feeding is the introduction of safe and nutritious foods in addition to breastfeeding around 6 months of age and usually runs from 6 to 24 months of age [76]. The quality and quantity of complementary foods are critical in preventing malnutrition. Dewey and Adu-Afarwuah, [77], proposed that in order for complementary feeding interventions to effectively prevent stunting, they should address proximal factors affecting linear growth including; quality and quantity of foods, breast milk and morbidity.

Evidence on the impact of interventions on complementary feeding has been generated mainly using two approaches, nutrition education on promotion of appropriate complementary feeding and provision of complementary foods [2]. In both food secure [78] and insecure [79] settings promotion of optimal complementary feeding without giving complementary foods produced promising results on prevention of stunting as well as absolute height and weight gains. Bhutta et al. [10] demonstrated that provision of complementary foods in food insecure populations was significantly associated with gains in Height-for-age z-score (HAZ) of 0.39 standard mean difference. The impact on stunting was insignificant, however, it has been suggested that provision of complementary foods in food insecure populations does have the potential to reduce stunting [80]. The development of complementary foods is still receiving scientific attention, considering that majority of families in low income countries cannot afford frequent consumption of animal-source foods [16, 81, 82].

Since no single food contains all essential nutrients [83], a diverse diet is essential in improving nutrition for infants and young children. Dietary diversity has actually been shown to be positively associated with nutritional status in children. Arimond and Ruel [17] using data from demographic surveys demonstrated that increased dietary diversity
is positively associated with HAZ-scores. A similar finding was reported by Marriot et al. [84] using the WHO infant and young child feeding indicators, minimum acceptable diet and dietary diversity. The most recent national data have shown that only 8% of children age 6–23 months meet the criteria for a minimum acceptable diet [41].

In low income countries complementary foods are monotonous and usually low in nutrient and energy density [15], such diets are associated with malnutrition in infants [85]. In rural Malawi complementary food is mainly made from dilute corn porridge, which has low nutrient and energy density [81]. Consequently, such complementary foods need to be enriched in order to meet the nutrient and energy needs of the infants and young children. Researchers have recently suggested that use of LNS would be one of the ways to home fortify complementary foods in order to cover the gap in both nutrient and energy needs for infant growth and development [20, 86]. This suggestion has mainly been motivated by evidence from studies testing the growth promotion potential of LNS [87, 88] after noting that the products are quite successful in treating severe acute malnutrition in children [89–91].

2.3.3 Potential role of Lipid-based Nutrient Supplements

Use of micronutrient-fortified energy dense spreads (lipid-based), especially the Ready to Use Therapeutic Foods (RUTF) have been widely used to accelerate rapid growth among severely acute malnourished children both in community and hospital settings [20, 91–93]. Several lipid-based food products have so far been developed, they largely differ in energy and micronutrient concentration [94]. The RUTF version is given in large amounts to ultimately provide large amount of energy (200–300g/d), in most cases temporarily replacing most or all other complementary foods [94]. Other lower dose (45–60g/d) lipid-based food products have also been developed to prevent acute malnutrition [95, 96]. Evidence from studies assessing the impact of LNS on nutrient and energy intakes is shown in Table 2.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Participants (n)</th>
<th>Design</th>
<th>Assessment of energy and nutrient intake</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Thakwalakwa et al. 2015, [97]</td>
<td>Moderately underweight Malawian children aged 8–18-month– (188).</td>
<td>A randomized, controlled, assessor-blinded clinical trial. Children were randomized to either receive 71g of com-soya blend (CSB) or 43g of lipid-based nutrient supplement (LNS) per day or no intervention food (control)</td>
<td>Interactive 24-hour dietary recall method</td>
<td>Total mean energy intake was significantly lower in the control and CSB groups, 548 kcal and 551 kcal respectively compared to 692 kcal ($P = 0.011$). The mean intake of energy and protein were 144 and 46 ($P &lt; 0.001$) larger, respectively, in the LNS group than among the controls.</td>
</tr>
<tr>
<td>Flax et al. 2015, [98]</td>
<td>Honduran children aged 6–18 months old (298)</td>
<td>Cluster randomized trial, children were randomized into LNS or control group. Dietary data were collected at baseline, 3, 6, 9 and 12 months.</td>
<td>24 hour dietary recall</td>
<td>Energy intake in the control group was 461.4 compared to 594.5 Kcal in the LNS group ($P &lt; 0.001$); Protein 12.8 in the control vs 15.6 g in LNS group ($P &lt; 0.001$) at 3 months of the intervention. Similar trend was observed at each study visit.</td>
</tr>
<tr>
<td>Flax et al. 2008, [99]</td>
<td>Underweight Malawian children aged 6– to 17-month-old, (16)</td>
<td>Longitudinal observational study. Children were selected from six villages based on pre-defined criteria.</td>
<td>12 hour observations by the research team</td>
<td>Energy intake was not calculated, but the number of regular food episodes did not change before and during supplementation. Intake of Nutributter significantly increased daily energy intake from complementary foods by 85 kcal.</td>
</tr>
<tr>
<td>Adu Aforwauh et al. 2007, [87]</td>
<td>Healthy Ghanaian infants aged 6–12 months (313)</td>
<td>Randomized controlled trial, infants participated in the trial for 6 months. They were randomized to receive micronutrient sprinkles (SP), crushable Nutritabs (NT) or Nutributter (NB) or to the control group</td>
<td>24-hour dietary recall</td>
<td>Excluding the contribution of energy from Nutributter, resulted in a non-significant difference in energy intake from complementary foods between groups.</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Intervention</td>
<td>Method</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>-------------------------------------------------------------------------------</td>
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<tr>
<td>Christian et al. 2015, [100]</td>
<td>Bangladeshi children aged 6–18 months old (5536)</td>
<td>A cluster-randomized controlled trial, aimed at testing the effect of two local, ready-to-use foods (chickpea and rice-lentil based) and a fortified blended food compared with medium LNS doses</td>
<td>24 hour dietary recall</td>
<td>LNS contributed about 20–30% of older (12–18 months) children’s daily energy requirement.</td>
</tr>
<tr>
<td>Maleta et al. 2004, [101]</td>
<td>Underweight and stunted Malawian children aged 42 to 60 months of age (61).</td>
<td>Randomized parallel group intervention study, in which children were randomized to either LNS or corn-soy blend groups</td>
<td>Interactive 24 hour dietary recall</td>
<td>More children in the LNS group achieved the recommended daily intakes for energy, calcium, zinc and vitamin A compared to the corn-soy blend ($P &lt; 0.05$). Compared to corn-soy blend, LNS did not lead to significant reduction in consumption of local food, thus higher intake of energy, fat, iron, and zinc in latter group.</td>
</tr>
<tr>
<td>Siega-Riz et al. 2014, [102]</td>
<td>Honduran children aged 6–18 months old, (300).</td>
<td>A 12-month cluster randomized controlled trial, LNS intervention ($n = 160$) and control group ($n = 140$)</td>
<td>24-hour dietary recall method</td>
<td>At 6-month follow-up, less children in the LNS group were Vitamin B12 deficient (43.6%) compared with the control (67.7%; $P = 0.03$). The LNS group had a higher mean concentration for folate at 6 months ($P = 0.06$), and improvements continued through 12 months for folate ($P = 0.002$).</td>
</tr>
<tr>
<td>Ickes et al. 2015, [103]</td>
<td>Moderately acute malnourished Ugandan children, aged 6–59 months, (128)</td>
<td>The study assessed diet adequacy and the quality of complementary foods of 128 Ugandan children, aged 6–59 months. The children participated in a 10-week programme for children with moderate acute malnutrition</td>
<td>24 hour dietary recall</td>
<td>LNS contributed 28%, 55%, of total calories and protein to children’s diets among children 6–8 months. LNS also provided more than half of the total requirements for Vitamin C, Zinc and Calcium.</td>
</tr>
</tbody>
</table>
Evidence from studies using smaller dose (20–50g/d) lipid-based Nutrient Supplements (LNS) with higher concentration of micronutrients has given promising results on prevention of growth faltering and promoting growth [87] as well as preventing severe stunting [104]. The evidence base is still being expanded, more trials are being implemented to test the growth promoting potential of SQ-LNS [105].

2.3.4 Vitamin A supplementation

Vitamin A is required for normal functioning of the visual system, maintenance of cell function for growth, epithelial integrity, red blood cell production, immunity and reproduction [106]. No wonder VAD is associated with several adverse effects among infants and young children. VAD is linked to blindness; impaired cognitive function; morbidity (incidence and/or severity) due to diarrhoea, measles, acute respiratory infections, malaria and other infectious diseases [107].

Epidemiological evidence suggests that VAD is associated with stunting [108, 109], and higher vitamin A intake in VAD endemic areas has been shown to reduce the risk of stunting [110]. However evidence on the potential for Vitamin A to improve child growth is lacking [111]. The link between vitamin A and linear growth or prevention of stunting is still not clear [112]. In studies showing a positive association between vitamin A supplementation and linear growth, the effect has been observed among infants with low serum retinol concentrations [113]. Vitamin A appears to have an additive role on prevention of stunting with other nutrients like zinc. Thus the role of vitamin A on linear growth should be considered from a wholistic approach of a group of coexisting factors that can modify growth [85].

2.3.5 Zinc supplementation

Zinc is essential for cell growth, differentiation and metabolism [114, 115]. Zinc also plays an important role on the integrity of the immune system [116], its deficiency is evidenced by poor immune system [117] resulting in decreased resistance to infections [118]. Zinc deficiency does negatively impact on infant and young child growth, and increases the risk and severity of infections [119]. The main causes of zinc deficiency in low income countries are inadequate dietary intake or poor absorption of zinc [119] and suboptimal breastfeeding [120]. Zinc is one of the ”problem nutrients”, considering that there is the greatest discrepancy between their content in complementary foods and the estimated amount required by the infant [16]. For such nutrients, supplementation
Preventive zinc supplementation in populations at risk of zinc deficiency reduces the risk of morbidity from childhood diarrhoea and acute lower respiratory infections [123] and might increase linear growth and weight gain in infants and young children [10, 124]. The pathways through which zinc contributes to reduction in risk of morbidity has been suggested to be through enhancement of the host immune system [118, 125, 126] as well as through direct effect on microbial growth [127]. So far the evidence linking zinc deficiency to impaired human growth is strong, however, the results of studies on efficacious and effectiveness of zinc supplementation on growth are still inconclusive [112].

2.3.6 Iron supplementation

Iron is essential for all tissues in a young child’s developing body [128]. Iron deficiency is one of the major causes of anemia and poor growth in infants and young children [37]. Iron supplementation and fortification improves iron nutriture in infants and young children [129], subsequently preventing anemia and iron deficiency [18]. Like is the case with zinc, the role of iron on growth is still debatable, furthermore, evidence on the impact of iron supplementation on motor and cognitive development is mixed [130].

It has also been demonstrated that routine iron supplementation among breastfed infants benefits those with low hemoglobin (Hb) and potentially harm those with normal Hb. Dewey et al. [131], had found that compared to the placebo group, iron replete children supplemented with iron had significantly lower gains in length and weight among Swedish and Honduran infants. In iron replete young infants the negative impact of iron could be due to a limited ability to regulate iron absorption, considering their inability to downregulate iron absorption in replete state [132].

Furthermore the lack of an association in other trials may be explained by short duration of the studies since length or height takes relatively longer time to change than weight [85]. Furthermore, the mode of providing iron has an impact on iron nutriture in infants [133], thus differences observed could partly be explained by differences in the modes of administration of iron [134].

Since iron is essential for both the host and some pathogens [128], the risk of morbidity is higher when iron is given to children in malaria endemic areas [135]. Thus it has been suggested that iron supplementation be based on the prevalence of anemia [128] and in malaria endemic areas on the basis that malaria prevention and treatment is available [136].
2.3.7 Provision of multiple micronutrients supplementation

In low income countries, staple diets are predominantly cereal based [15], such diets have low concentrations of many micronutrients which are in poor bioavailable forms. Due to low intakes of animal based food in such settings, the prevalence of multiple micronutrient (MMN) deficiencies is likely to be high among vulnerable groups like infants, pregnant women and lactating mothers. To address the problem, one of the main approaches suggested is to provide MMN [10]. Others have even pointed out that in order to improve healthy growth and development among infants and children, the use of MMN is the desired strategy rather than single nutrients [137].

Use of MMN has been shown to improve body stores of different nutrients. For instance Salam et al. [138] reported that use of MMN improved iron Hb concentration and prevented 57% of Iron deficiency anemia (IDA) and VAD by 21%. However, the effect of MMN on linear growth is insignificant [10]. There are also safety concerns on the use of MMN, in some contexts the risk of morbidity increases when supplementing infants with MMN [139].

2.3.8 Disease and parasitic infection prevention and management

There is a vicious cycle between malnutrition and infections [140]. The most notable infections in low income settings linked to malnutrition are diarrhoeal and other enteric infections. Even though, the link between diarrhoeal diseases and child undernutrition has been questioned [141] and continue to be questioned. Humphrey, [51] contends that diarrhoea does not explain most of the stunting related to enteric infections, but rather it is tropical enteropathy that explains much of stunting in low resource settings. Nevertheless, overall, diarrhoeal diseases contribute to malnutrition [142].

The contribution of nematodes to malnutrition during infancy and childhood has also received considerable attention [143]. Parasitic infections operate through reduced dietary intake mainly due to loss of appetite; digestion and absorption can also be impaired ultimately contributing to stunting in low income countries [144]. One of the strategies to prevent enteric infections is through promotion of optimal breastfeeding. Human breast milk contains numerous anti-infective and immune-regulatory components that helps protect infants from certain infections [145, 146]. Furthermore, breastfeeding does not expose infants and children to environmental pathogens, as is usually the case with contaminated water and infant foods given to infants in low income countries [147].
2.3.9 Water and sanitation

Poor water and sanitation conditions have been linked to malnutrition among infants and young children. The impact of health and nutrition interventions aimed at improving or preventing malnutrition have so far been quite small, others have hypothesized that this is likely to be due to issues related to sanitation and hygiene [148, 149]. Several studies have so far been designed to explore the relationship between water, sanitation and hygiene (WASH) and child malnutrition, health and development [(www.washbenefits.net/; [150)]. The evidence from these trials will help to elucidate the link and pathways through which water and sanitation interventions would contribute to improving nutritional outcomes among infants and children.

2.4 Impact of SQ-LNS and other high energy dense foods on breast milk intake

Considering the benefits of breast milk to infants and young children globally [151], public health advocates are still raising concerns on the potential of LNS to displace breast milk. Presently the evidence on whether or not SQ-LNS displaces breast milk is inconclusive. Table 3 shows studies that assessed the impact of SQ-LNS on breast milk intake, evidence from other studies aimed at assessing the impact of energy density and meal frequency are also include. The evidence from Table 3 confirms that infants' breast milk intake is indeed sensitive to energy density of complementary foods. Small-quantity LNS is an energy dense product and hence its impact on breast milk intake needs to be investigated further in well-designed randomized controlled trials.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Participants (n)</th>
<th>Design</th>
<th>Measurement of breast milk</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galpin et al. 2007, [152]</td>
<td>Malawian infants aged 6 months (44)</td>
<td>Randomized controlled trial of 3 complementary feeding regimens (LNS 25g, 50g and Corn-soy blend); Breast milk intake was assessed before and after a month of the intervention</td>
<td>Dose-to-the-mother deuterium dilution method</td>
<td>Breast milk intake (g/kg/d) was reduced by about 7–13% across the three groups; there were no intergroup differences.</td>
</tr>
<tr>
<td>Owino et al. 2011 [153]</td>
<td>Democratic of the Congo infants aged 8.5–9.5 months, (58).</td>
<td>Randomized controlled trial, Ready to use complementary food (RUCF) and standard corn-soy blend (UNIMIX); Breast milk intake was assessed after about 3 months of consuming intervention foods</td>
<td>Dose-to-the-mother deuterium dilution method</td>
<td>Breast milk intake was not significantly different between the RUCF and the UNIMIX groups respectively 705/d/ and 678 g/d ($P = 0.69$)</td>
</tr>
<tr>
<td>Owino et al. 2007 [154]</td>
<td>Zambian infants aged 9-months, (43).</td>
<td>Randomized controlled trial; children were either assigned to 3 groups; a) fortified blend of maize, beans, bambara nuts, and groundnuts (CBM), b) CBM with α-amylase; c) control group: Breast milk intake was assessed after about 3 months of consuming intervention foods</td>
<td>Dose-to-the-mother deuterium dilution method</td>
<td>No significant differences in mean daily breast milk intake among the groups: CMB= 614; CMBA=635; control=653. ($P = 0.87$)</td>
</tr>
<tr>
<td>Islam et al. 2008, [155]</td>
<td>Bangladeshi infants aged 6–11 months old (18)</td>
<td>Randomized controlled trial in medical facility. Assessed the effect of energy density of complementary foods on breast milk intake, using 3 formulations</td>
<td>Test weighing</td>
<td>Mean breast milk intake decreased by 11% when energy density was increased from 0.5 to 1.5 kcal/kg ($P &lt; 0.001$)</td>
</tr>
<tr>
<td>Bajaj et al. 2005, [156]</td>
<td>Indian infants aged 6–10 months (20)</td>
<td>Randomized controlled trial in medical facility. Assessed the effect of increasing energy density of complementary foods by adding cooking oil on breast milk intake</td>
<td>Test weighing</td>
<td>Infant mean daily breast milk intake was reduced by 121 g when oil was added to complementary food compared to no-oil foods ($P = 0.008$)</td>
</tr>
<tr>
<td>Singh et al. 2005 [157]</td>
<td>Indian infants aged 6–10 months (20)</td>
<td>Randomized controlled trial in a medical facility assessing the effect of complementary food feeding frequency on breast milk intake</td>
<td>Test weighing</td>
<td>Increasing feeding frequency from 3 to 4 per day reduced breast milk intake by 61.2 g. ($P = 0.05$)</td>
</tr>
</tbody>
</table>
2.5 Factors influencing breast milk intake

Several factors may interact or act independently to influence infants and young children’s breast milk intake. Factors may be related to infant, caregiver (usually maternal) and the environment. Energy density and feeding frequency of complementary foods are inversely associated with breast milk intake among infants [155]. It is against this knowledge that there are concerns that use of SQ-LNS may negatively impact on breast milk intake. Even non-caloric food and drinks reduce breast milk intake among infants and young children [158]. Controlling for potential confounding, male infants consume more breast milk compared to female infants [152]. From birth the former tend to have more lean tissue than the latter [159] and theoretically would require more energy per kg body weight and hence consume relatively more breast milk to meet higher energy needs. Evidence from some studies from across the world including Malawi is shown in Table 4.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Participants (n)</th>
<th>Design</th>
<th>Statistical test used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galpin et al. 2007, [152]</td>
<td>Malawian infants aged 6 months (44).</td>
<td>Randomized controlled trial of 3 complementary feeding regimens (LNS 25g, 50g and Corn-soy blend): Breast milk intake was assessed before and after a month of the intervention</td>
<td>Multiple linear regression (standardized β coefficients</td>
<td>Male sex (β=-0.34, P = 0.01) Maternal body fat (β=-0.37, P = 0.007) Infant weight (β=0.53, P &lt; 0.001)</td>
</tr>
<tr>
<td>Haisma et al. 2003 [160]</td>
<td>Brazilian infants aged 4 months old (70)</td>
<td>Cross-sectional, community-based study, in urban Pelotas, Southern Brazil.</td>
<td>Pearson correlation coefficient followed by multiple linear regression analysis</td>
<td>Infants’ weight-for-height and weight-for-age percentiles were positively correlated with breast milk intake (r=0.28, P = 0.018) and r=0.38, P = 0.001 respectively.</td>
</tr>
<tr>
<td>Islam et al. 2005, [155]</td>
<td>Healthy Bangladeshi children aged 9–18 months (10)</td>
<td>Children were randomly assigned to sequences of High Density-Low Density-High Density or Low Density-High Density-Low Density diets along with ad libitum breastfeeding. The study was partly aimed at evaluating the effects of varied energy density of CFs on breast-milk intakes</td>
<td>T-test and the Turkey’s test to compare the daily mean intakes of breast milk</td>
<td>Consumption of high density diet resulted into a significant reduction in breast milk intake compared to periods when low density diets were consumed; 192±115 and 234±121 g/d, respectively (P = 0.03)</td>
</tr>
<tr>
<td>Sachdev et al. 1991, [158]</td>
<td>Health male infants age 1 to 4 mo (45)</td>
<td>Infants were randomly assigned to an exclusively breastfed group or breast milk plus supplemental water group</td>
<td>Pearson correlation and analysis of covariance</td>
<td>Both breast milk intake (274 vs 210 ml) and total fluid intake (274 vs 233 ml) were significantly higher in the exclusive breastfeeding than the supplemented group respectively. This was after controlling age, height, length and room temperature.</td>
</tr>
<tr>
<td>Perez-Escamilla et al. 1995, [161]</td>
<td>Honduran mother-child pairs from low-income settings (141).</td>
<td>Randomized trial, women were randomly assigned to exclusively breast-feed for the first 6 mo or to complement breast milk with pre-prepared solid foods beginning at 4 mo.</td>
<td>Multivariate analysis</td>
<td>Infant breast milk energy intake was positively associated with birth weight at 4, 5 and 6 months; β=78.7; β= 91.3 and β=56.2 respectively. Milk energy density was inversely associated with breast milk intake</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Methods</td>
<td>Findings</td>
<td></td>
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<tr>
<td>Dewey et al. 1991 [162]</td>
<td>American infants age 3 months (73)</td>
<td>Longitudinal study, comparing growth patterns, nutrient intake, morbidity, and activity levels of breast-fed and formula-fed infants.</td>
<td>Infant birth weight ($\beta=33$) weight at 3 months ($\beta=0.56$), and total time nursing ($\beta=0.24$) were positively associated with breast milk intake.</td>
<td></td>
</tr>
<tr>
<td>Nazlee et al. 2011 [163]</td>
<td>Pakistani mother-baby pairs (33)</td>
<td>Participants were taking part in a study aimed at assessing the effect of Maternal Depression on intake of breast milk in infants</td>
<td>Among rural Pakistani infants, leaner babies with the highest breast milk intake had leaner mothers</td>
<td></td>
</tr>
<tr>
<td>Ettyang et al. 2005, [164]</td>
<td>Kenyan infants, aged 2–4 months (10)</td>
<td>The aim of the study was to evaluate the association between intake of breast milk of pastoral infants who were exclusively breast-fed and the body composition of their mother.</td>
<td>Infant milk intake was positively correlated to maternal pregnancy triceps ($r = 0.679$, $P &lt; 0.05$) and pregnancy MUAC ($r = 0.725$, $P &lt; 0.05$).</td>
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</tbody>
</table>
Infant birth weight is also positively related to breast milk intake [152, 161, 162], heavier infants consume more breast milk compared to leaner infants which is also related to satisfying energy needs. Complementary foods, independent of energy density reduce [152, 162, 165] breast milk intake [29, 152]. Maternal body composition is also related to breast milk intake, maternal body fat has been shown to be inversely correlated with breast milk intake [163].

Some factors influence breastfeeding practices which may impact breast milk intake. For instance, maternal age, older mothers are more likely to breastfeed than younger mothers [160]. In some cultures infant sex influences caregivers attention and care of infants [166], in some settings male infants in certain cultures are likely to be breastfed more than female infants [167]. Maternal socio-economic factors also influence optimal breastfeeding practices and may impact breast milk intake. In high income countries, educated mothers are more likely to breastfed than uneducated counterparts [168], this relationship is not uniform across different social economic settings. In low income countries, women from high social economic strata are likely to have lower breastfeeding rates than those from deprived societies. This is likely due to the availability of resources to afford complementary foods or other forms of milk [169]. Thus there is a need to explore context specific determinants of breast milk intake so that appropriate strategies and interventions can be designed to foster optimal breastfeeding.

2.6 Association between breast milk and infant growth and development

Breast milk alone provides the energy and nutrients that infants need for healthy growth and development for the first 6 months of life [170], thus exclusive breastfeeding is recommended during this period [171]. During the second half of infancy, breast milk may provide up to half or more of the infants’ nutritional needs [33]. In the medical literature, the impact or contribution of breast milk to growth and development has mainly been assessed broadly using breastfeeding practices rather than the actual quantitative breast milk. Meta-analyses and systematic reviews on the effect continued breastfeeding up to two years of age or beyond on child growth and development have demonstrated that evidence is scarce and contradictory [172]. The lack of conclusive evidence may be related to the measures of breastfeeding practices which depend on less valid methods for capturing true practices [64]. Even interventions aimed at promoting optimal breastfeeding practices have also failed to demonstrate significant impact on child growth [65].
Simondon et al. [173] demonstrated that Senegalese children who were breastfed for longed period (up-to and beyond 2 years) of time had greater length increments compared to those with shorter breastfeeding duration and those who were weaned. A similar finding was reported by Onyango et al. [174] among Kenyan children, where they showed that prolonged breast-feeding was associated with faster growth in both length and weight over a 6-mo period in a cohort of 9–18-mo-old children. Kenyan children in the longest-duration group gained 3.4 cm and 370 g more than those in the shortest duration group. In addition, the strongest association between breast-feeding and linear growth was observed in households that had no latrine. Similarly among Peruvian children breastfeeding frequency was associated with linear growth between 12 and 15 months [175]. The paucity of evidence, warrants the need to conduct further research in order to delineate the role played by breast milk rather than breastfeeding practices on growth and development.

2.7 Justification for the present study

The literature review has demonstrated that undernutrition still remains a public health problem in low income countries including Malawi. While evidence has accumulated over the years on strategies to prevent malnutrition among infants and young children, it is clear from the review that more needs to be done to increase the evidence base. The review has shown that the use of SQ-LNS for prevention of stunting is receiving considerable scientific attention. The review has further shown that evidence on the impact of SQ-LNS on breast milk intake is still limited.

Apart from the impact of SQ-LNS, there are other potential predictors of breast milk intake. The review has demonstrated that breast milk intake may be influenced by several determinants which are location specific. Determinants of breast milk intake during complementary feeding period are still not well explored. Therefore, there is a need to identify specific predictors of breast milk intake in specific settings.

At the same time, the review has also shown that breast milk remains very critical for survival of infants and young children in low income countries. However, it has also been shown that the role of breast milk intake on growth and development from 6 months of age, during the complementary feeding period, is not fully understood. Specifically, whether more breast milk intake as compared to intake of complementary foods is associated with healthy growth and development remains to be elucidated. The present study was therefore designed to fill the gaps as shown from the review, with an ultimate aim of contributing to the design of targeted public health interventions like the use of SQ-LNS in the context of breastfeeding.
3  AIMS OF THE STUDY

The main aim of this study was to assess the impact of amount of breast milk on healthy growth and development among infants and young children from rural Malawian setting. The children were a sub-sample of a larger iLiNS-DOSE trial participants aged 9–18 months. The specific objectives were as follows:

1. To compare breast milk intakes among 9-10 month old infants consuming complementary foods supplemented with 0, 10, 20 or 40 g of SQ-LNS per day (I)

2. To evaluate the difference in energy and macronutrient intakes from complementary foods between a control (no supplement) group and 3 groups that received 10, 20, or 40 g SQ-LNS per day (II)

3. To identify factors which predict breast milk intake among 9 month-old rural Malawian infants (III)

4. To examine the association between breast milk intake at 9–10 months of age and growth and development in infancy and early childhood (IV)
4 METHODS

4.1 Approach to the study and the authors contribution

The main aim for the present study was achieved using data from four different studies based on the same participants (Figure 3). Since participants of these studies were a sub-sample of a larger iLiNS DOSE randomized efficacy trial (Figure 6: Flow of participants in the study), the author collaborated with various individuals in study implementation. The enrolment of the participants from the main iLiNS-DOSE trial is provided in chapter 6 of this Thesis. Specifically, the author designed studies III and IV, developed data collection tools for study I and participated in the development of data collection tools for study II. The author prepared statistical analysis plans for studies I, III and IV, the plan for the former is published on www.ilins.org website. The author trained and supervised the research assistants for studies I and II. Furthermore the author analysed the saliva samples and analysed the data for studies I, III and IV.

Study I assessed breast milk intake among 359 infants at 9–10 months of age, when infants had been in the main trial for about three months. The study was designed to test the hypothesis that provision of SQ-LNS at a daily dose of 10, 20, or 40 g/d would not reduce breast milk intake. The main outcome was difference in mean breast milk intake between the intervention and control groups.

Study II was designed to evaluate the difference in energy and macronutrient intakes from complementary foods between a control and the intervention groups.

Study III analysed factors predicting breast milk intake among infants. All the infants from study I were included in study III. The main outcome for study III was associations between breast milk intake and selected covariates.

Finally study IV assessed the association between breast milk intake at 9–10 months and growth and development at 18 months of age.
Figure 3: Study designs for the thesis

**Aims**

- To compare breast milk intakes between SQ-LNS and control groups
- To evaluate the difference in energy and macronutrient intakes from complementary foods between the SQ-LNS and control groups
- To identify factors which predict breast milk intake
- To examine the association between breast milk intake and growth and development

**Study**

- **Study I (n=359)**: Breast milk intake measurement study
- **Study II (n=568)**: Complementary food assessment study
- **Study III (n=358)**: Factors associated with breast milk intake
- **Study IV (n=358)**: Association between breast milk intake and growth and development

**Outcomes**

- Breast milk intake
- Energy and macronutrient intake
- Predictors of breast milk intake
- Association between breast milk intake and growth and development
4.2 Study setting and participants

4.2.1 Study area

The study was carried out in southern Malawi in areas surrounding Mangochi District Hospital and Namwera Health Centre (Figure 4). Malawi is a landlocked country in Southeast Africa and has an estimated population of 16 million people, about 85% of Malawians live in rural areas [176] and earn their livelihood mainly through rain-fed subsistence Agriculture. Malawi has three seasons, namely; rainy season (December to April), cool dry (May to August) and dry hot (September to October).

Mangochi district is mainly occupied by Yao speaking Malawians, majority of them are Muslims. The main occupation in the study area was subsistence farming seconded by small scale businesses mainly selling of farm produce [177]. The main staple food in the area is maize and fish also forms a significant source of food. The main health services in the study areas are from free public facilities, Mangochi District Hospital and Namwera Health centre, with the former being the referral centre for the latter. There are also private health centres serving both study areas.
4.2.2 Participants

The study participants were part of a larger randomized controlled trial, the iLiNS-DOSE trial. Healthy infants <6 months of age were identified through community census, and all those who met the inclusion/exclusion criteria were invited to participate in the trial. Infants were eligible for enrolment into the main iLiNS-DOSE trial if they were 5.50–6.49 months of age, resided in the two study catchment areas, if they would be available during the 12-months study period and were not concurrently participating in any other clinical trial.

The data for the present studies I, II, III and IV are from sub-studies nested in the main iLiNS-DOSE trial. The studies were designed to assess both breast milk and complementary food intakes in infants participating in the LNS trial. Thus some participants from the main iLiNS-DOSE trial were randomised to participate in both study I (Breast milk intake assessment, n=400) and study II (Dietary assessment, n=688). Mother-infant pairs were eligible for the studies if the infant was enrolled in the main iLiNS-DOSE trial, infant age was between 9.0 and 10.0 months, the mother was breastfeeding the infant, the mother and infant would be available for the full study period and the mother consented for both herself and her infant to participate. Studies III and IV used the same participants from studies I and II.

4.3 Lipid-based Nutrient Supplements for iLiNS-DOSE Trial

The SQ-LNS that was used in the iLiNS-DOSE trial was produced and packaged by Nutriset S.A.S. (each package weighed 140 g). Raw ingredients included vegetable oil, peanut paste, dried skimmed milk, maltodextrin, sugar, and a mineral and vitamin mix (Nutritional composition of the SQ-LNS are shown in Supplemental Table 1 for article I). SQ-LNS was delivered to caregivers at 2-week intervals by the study team. The infants’ caregivers were advised to mix one spoonful (5 g) of SQ-LNS with 2 tablespoons of porridge prepared in the home and feed the entire mixture to the infant. After 2 weeks, the study team collected empty SQ-LNS cups and leftover SQ-LNS and then provided a new supply to the caregiver, for the next coming two weeks.

4.4 Data collection

The follow up scheme for the participants in the present analysis (studies I and II) was different from that of the main iLiNS-DOSE trial. In the main iLiNS-DOSE trial all participants were followed up by two-weekly home visits and six-monthly clinic visits.
4.4.1 Demographic and socio-economic data

Demographic and socioeconomic data were assessed by administering questionnaires to the mother of the infant. The data were collected as part of the iLiNS-DOSE trial. Morbidity and socioeconomics data collectors collected data from study participants at the study clinic as well as at homes of participants. All participants provided the data at baseline and in subsequent study visits. Socioeconomic status was assessed using household asset score as a proxy. Household asset index was based on baseline ownership of a set of assets (radio, television, refrigerator, cell phone, boats and stove), lighting source, drinking water supply season, sanitation facilities, and flooring materials. The household ownership of the aforementioned set of assets was combined into an index (with a mean of zero and standard deviation of one) using principal components analysis in STATA 12. Higher asset index scores indicated relatively socioeconomically better.

Household food insecurity was measured using the Household Food Insecurity Access (HFIA) Scale. The HFIA scale is based on a set of questions that captures perceptions and reported experiences of three domains of food insecurity: anxiety and uncertainty about the household food supply; insufficient quality; and insufficient food intake and its physical consequences [178]. The HFIA score was generated using nine questions related to household food insecurity. Each household received a score from
0–27 based on a simple sum of the frequency of occurrence of each food insecurity condition, where ‘never’ = 0, ‘sometimes’ = 2 points, and ‘often’ = 3 points. The higher the score, the higher the degree of household food insecurity that the household experienced in the previous four weeks.

4.4.2 Anthropometric measurements

Anthropometric measurements were taken at enrolment into the iLiNS-DOSE main trial as well as during the breast milk intake assessment study; the measurements were done in triplicate. Maternal anthropometric variables included weight and height. Infants’ anthropometric variables collected were weight, length, head circumference and Mid-upper arm circumference (MUAC). Mothers were weighed in light clothing to the nearest 0.01 kg using an electronic scale (SECA 846; Chasmors Ltd, London England), and their height was measured to the nearest 0.1 cm using a stadiometer (Harpenden, Holtain Ltd, Crosswell, UK). Infants were weighed nude to the nearest 0.01 kg using an electronic scale (SECA 735; Chasmors Ltd, London England). The study weight measuring scales were calibrated every morning. MUAC and head circumference were measured using nonstretchable plastic tape measures [177].

4.4.3 Breast milk and non-breast milk fluid intakes

Infant breast milk intake (g/d), was determined by using a 2-compartment steady state model of water turnover in the mother-infant dyad, using the dose-to-the-mother $^{2}$H$_{2}$O dilution method developed by Coward et al. [179]. The method has been validated [180] and is considered the gold standard for assessing breast milk intake in community settings. In the two compartment model, the mother’s body water is the first compartment and the baby’s body water is the second compartment. These two compartments are connected by the flow of milk from the mother to the baby [181]. In a steady state model, the total water input is equal to the total water output, for the mother this true. However, the total body water for the baby changes linearly during the course of the study due to growth. Intake of human milk and water from sources other than human milk can be calculated by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby, using solver function in excel [160, 181]. Deuterium enrichment in the saliva can be measured by using isotope ratio mass spectrometry or Fourier transform infrared spectroscopy. In the present study deuterium enrichment was measured using the FTIR (FTIR 8400 Series; Shimadzu Corporation) at College of Medicine, Mangochi laboratory.
On study day 0 (baseline), morbidity assessment for the mother-infant pairs during the previous 7 days was done using a questionnaire. If either the mother or her infant experienced fever or symptoms of diarrhoea or vomiting within the past 7 days, the pair was requested to report back to the clinic after a week. For healthy mother-infant dyads, maternal and infant weights were measured, then saliva samples were collected from both the mother and infant. Subsequent saliva samples were collected from the mother-infant dyad in their home on study days 1, 2, 3, 4 and 13. On study day 14, mother-infant pairs came to the study clinic for final saliva sample collection and weight measurement.

Saliva from the mother-infant dyad was collected as detailed in the International Atomic Energy Agency protocol [181]. To collect saliva from mothers, a small piece of cotton wool was carefully placed in their mouth for about 2 min until it was fully soaked with saliva. Mothers then transferred the soaked cotton wool directly to the empty barrel of a 20-mL disposable syringe. Saliva was squeezed out of the soaked cotton wool into a vial labeled with participant details including the study number, date, and time of sample collection. The procedure was repeated by using a new piece of cotton wool, until a total of 4 mL saliva was collected. Infant saliva was collected by tightly wrapping a piece of cotton wool around the tip of a wooden stick and placing the stick in the infant’s mouth, between the lower gum and cheek for about 1–2 min or until the cotton wool was soaked with saliva. Cotton wool was separated from the stick and put into a 20-mL disposable syringe for saliva collection, just as described for mothers. In infants the procedure was repeated until a total of 2 mL saliva was collected. An oral dose of 30g $\text{H}_2\text{O}$ (99.8% purity, Cambridge Isotopes Labs, Andover, MA, USA) was then administered to mothers soon after collecting baseline saliva.

4.4.4 Energy and nutrient intake assessment

Dietary intakes were assessed using a repeat 4-pass interactive 24-hour recall (i24-HR), the method was validated before being employed. The method has also been validated for use in Malawi [182]. The i24HR was designed to assess all non-breast milk foods and beverages consumed by the infant on the previous day. The information was collected from the main-caregiver who fed or observed the infant consuming the recalled food or drink (usually the mother).

Caregivers were instructed to use standard cups and bowls when feeding the index child on the day preceding the recall. The cup, bowls as well as a pictorial chart containing pictures of different food groups common in the study area were provided two days
prior to the dietary assessment day. The data collection team member explained the use of the given tools to be used the following day.

On the interviewing day, the respondents were asked if the infant was ill on the day of intake, if the intake was usual, increased or decreased compared to usual. The data collection followed four passes. The first pass, involved asking the caregiver to list all the food and drinks the infant consumed during the previous day. In the second pass, the details of the listed foods were asked, including the time and place of consumption and the person who fed the infant. In the third pass, the caregiver was asked to estimate the portion size served to the infant and the amount left-over, using appropriate food models as well as some real foods. After completing the third pass, the data collector compared the marked pictorial chart with the i-24-HR to assess if they agree, and discrepancies of the two were discussed and corrected.

The final pass involved summarizing of the food and beverages recorded, the respondent was then asked if what was recorded was the accurate representation of what the infant had eaten. Non-breast milk energy and nutrients consumed by infants were calculated as averages from the two recall days using a food composition table specifically developed from regional [183, 184] as well as international sources [185]. Additional details for the calculation of the energy and nutrient content of the foods are in paper II of this thesis.

4.4.5 Developmental data

Developmental assessment was conducted when the children had reached a mean age of 18 months. Assessments were conducted at the iLiN-DOSE clinic by well-trained data collectors [186]. Motor development was assessed using the Kilifi Developmental Inventory (KDI), which is based on the Griffiths Mental Development and the Merrill-Palmer Scales [187]. Children were evaluated on 35 gross motor skills, such as walking and climbing, and 34 fine motor skills, such as threading beads on a string. The score was the total number of skills the child successfully completed in each of the sub-scales (gross and fine motor) and the total motor score (sum of all 69 skills).

All testing materials were purchased or made locally, according to the specifications in the KDI manual [187]. The KDI was piloted prior to its use in order to ascertain its validity in the local context. The data collectors assessed and rated the child’s mood, interaction with the assessors and activity level. The child’s score was the total number of skills he or she was able to perform. The child’s mood during the KDI assessment was rated as positive (smiling/laughing or occasional smiles) or not positive (crying/inconsolable, occasional crying, changeable/mood swings, or no visible emotions). The
child’s interaction with the assessor during the KDI was rated as positive (friendly) or not positive (avoidant and withdrawn, clings to family member, hesitant/when approached will accept reluctantly, difficult to engage in tasks, or inappropriate approaches to assessor). The child’s activity level during the KDI was rated as positive (active and maintains interest) or not positive (unarousable, sleepy, can hardly be awakened, sleepy but easily awake, does not spontaneously engage in activity, and awake but loses interest).

Children’s language development was assessed using an adapted version of the MacArthur-Bates Communicative Development Inventory [188]. The score was the total number of meaningful words the child could say out of a hundred word vocabulary checklist reported through an interview with a caregiver. Description of the methods in detail has been provided elsewhere [189].

We assessed language development using a 100-word vocabulary checklist based on the MacArthur-Bates Communicative Development Inventory (CDI) [190], developed in part following previous adaptations of this tool in Bangladesh [191] and Kenya [192]. The 100-word vocabulary list was developed after conducting interviews with two sets of mothers of children age 14 to 33 months. The first group of mothers was asked what words their children said, and probing specific categories from the MacArthur-Bates CDI, such as animals, food and drink, and clothing. The results of these interviews lead to development of a list of 352 words. Another set of women (n=41) was asked whether their children said each of these 352 words. Finally, 100 words with a positive correlation with total vocabulary score and a positive correlation with child age was developed. The child’s score was the total number of words the child said, out of the one hundred word list, as reported by the caregiver, which was the mother for 98% of children.

4.5 Statistical analysis

4.5.1 Sample size calculation

The sample sizes for studies I, III and IV were calculated based on the need to detect a group difference in milk intake of ≥86 g/d with a power of 80%, 95% confidence and the effect size of 0.5 in the breast milk intake assessment study I. A sample size of 89 mother-infant pairs was needed per group, assuming an attrition rate of about 12%, the sample size was increased to 100 per group (Total of 400). A difference in breast milk intake of 86 g/d translates to about 10% of total daily energy needs of infants at 9–11 months of age. Eighty six grams of breast milk intake represents about 55–60 kcal,
which is 8–9% of the total energy needs of 686 kcal/d at that age [16]. The reduction in breast milk intake of this magnitude was assumed to be unlikely to result in negative health consequences to the infant and was therefore chosen as the non-inferiority margin. The sample size for study II was calculated to detect a 20% increase in energy intake from complementary foods between the control and intervention groups with 80% power, 95% CI and an attrition rate of 15%. A sample size of 172 was required for each group, the control, 10, 20 and 40g/d-LNS groups.

4.5.2 Data analysis

Statistical analyses for studies I, II and IV were performed using Stata 12 (StataCorp, College Station, TX), Stata Version 13 was used for study III. For all studies differences between groups were compared by using ANOVA and t-test for continuous variables and a chi-square test for proportions. Missing data due to loss to follow-up and misplaced participant data files were considered missing at random [193] and so no data imputation was performed. For study I, the non-inferiority of differences in breast milk intake of each of the LNS groups compared with the control group was assessed by using 2-sided 95% [194]. Non-inferiority was deemed established if the lower bound of the 2-sided 95% CIs of the LNS group was above the set non-inferiority margin, a difference in breast milk of 86 g between the LNS and control groups. The primary analysis was conducted on the basis of intention to treat. A secondary, per-protocol analysis was conducted by using data on estimated LNS intake.

For study III, the selection of explanatory variables to be used in the models was mainly based on evidence from the literature on their potential to correlate and associate with breast milk intake. Pearson correlation analysis was performed to determine the correlation between continuous predictor variables and the outcome variables. Variables significantly correlated or associated with breast milk intake in the bivariate analysis were included in the multiple regression analyses. Energy from non-breast milk sources, maternal education and age were included in the regression models based on their biological plausible relationship with breast milk intake. Path analysis was conducted to gain an understanding of the pathways through which the independent variables were related to each other and ultimately to breast milk intake. The analysis was mainly done to establish whether or not certain independent variables mediated the association of some of the independent variables with breast milk intake. Path analysis is a statistical technique used to examine hypothesized causal relationships between two or more variables [195]. Linear regression was used to assess the associations between explanatory variables and breast milk intake.
For study II, the analysis of study group differences on energy and macronutrient intakes were first done with the use of an unadjusted ANOVA. When the overall difference was significant at the 5% level, further pairwise significance testing was performed to determine whether LNS dose amount groups differed from control and from each other. Confounding variables were examined with a univariate regression model with unadjusted total energy (kcal/d) as the outcome and the individual variables as the exposure. Variables with $P < 0.2$ in the univariate model were then used with LNS dose amount (control as reference group) in the multivariate analysis to ascertain predictors of energy intake from complementary foods. Principal component analysis was used to generate the household socioeconomic status by combining the variables maternal occupation, household crowding, housing material, roof material, sanitation facilities, and cooking fuel. The quintiles of the first principal component were used to categorize households into 1 of 5 socioeconomic status levels.

For study IV, bivariate analysis was conducted using simple linear regression to assess the independent association between breast milk intake or % total energy intake from breast milk and each growth and developmental outcome [attained Length-for-age z-score (LAZ) at 12 months, change in LAZ between 12 and 18 months, motor and language scores]. Variables associated with the outcomes in the bivariate analysis were included in multiple regression models. Some covariates were identified a priori to be included in the adjusted models based on the known biologically plausible relationship with growth and development: they included maternal education and height, household asset score, family care index and household food insecurity. The primary analysis was conducted on the basis of intention to treat.
5 ETHICAL APPROVAL

All the studies were cleared by the College of Medicine Research and Ethics Committee of University of Malawi and the Regional Ethics Committee of Pirkanmaa Hospital District, Finland. The iLiNS-DOSE trial is registered at National Institutes of Health [108] clinical trial registry (clinicaltrials.gov) with registration number NCT00945698. For study I and II additional clearance was also given by the University of California, Davis and the London School of Hygiene and Tropical Medicine respectively.
6 RESULTS

The results are presented to follow a sequence so that breast milk intake results will appear first. The rest will be presented following the order; dietary assessment, determinants of breast milk intake and finally the association between breast milk intake and growth and development.

6.1 Enrolment, follow up and background characteristics

The present study enrolled participants from the iLiNS-DOSE main trial. A total of 595 mother-infant pairs were approached for enrollment into this sub-study. The flow of participants is shown in Figure 6, and their baseline characteristics are shown in Table 5. Some participants in the dietary assessment were not randomized into the breast milk intake sub-study, hence the difference between 651 and 595 as shown in Figure 6.
Figure 6: Flow of participants in the study
Table 5: Comparison of baseline (at 5.5mo–6 mo) characteristics of those participating and those not participating in breast milk intake (study I)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Studies I, III, IV (n=359)</th>
<th>iLiNS-DOSE Participants not included in study I (n=1337)</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of males (%)</td>
<td>47%</td>
<td>51%</td>
<td>0.74</td>
</tr>
<tr>
<td>Length for age z-score at 5.5–6 months of age</td>
<td>-1.43 (1.03)</td>
<td>-1.39 (1.06)</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight for length z-score at 5.5–6 months of age</td>
<td>0.27 (1.10)</td>
<td>0.25 (1.12)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height2 (cm)</td>
<td>155.1 (5.4)</td>
<td>155.0 (5.7)</td>
<td>0.27</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.7 (8.1)</td>
<td>53.4 (8.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Age (y)3</td>
<td>26 (17–43)</td>
<td>25 (21–30)</td>
<td>0.77</td>
</tr>
<tr>
<td>Education (y)3</td>
<td>4 (0–12)</td>
<td>5 (2–7)</td>
<td>0.32</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 (3.0)</td>
<td>21.9 (2.9)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

1Figure is obtained after subtracting 595 from the total iLiNS-DOSE sample size of 1932; 2Height was measured at enrolment into the main trial; 3Maternal age and education are medians (interquartile range)

6.2 Impact of SQ-LNS on breast milk intake (I)

The impact of SQ-LNS on breast milk intake was assessed among 359 infants at the age of 9–10 months. The mean (SD) daily breast milk intake for the entire sample and the un-supplemented infants was 752 (244) g and 730 (226) g respectively. These levels of breast milk intake are quite high compared to the global estimate of 616 (172) g at the same age group. The difference (95% CI) in mean intake of infants provided with 10, 20 or 40 g of SQ-LNS/d, compared to controls, was +62 (-18 to +143), +30 (-40 to +99), and +2 (-68 to +72) g/d, respectively (see Table 2, article I). Therefore, mean breast milk intake in all of the groups that received SQ-LNS doses was non-inferior to the un-supplemented complementary diets (control group) (non-inferiority margin was set as a difference in breast milk intake between the intervention and control group of 86 g/d) (Figure 7). Additionally, non-breast milk oral water intake did not differ by group (P=0.39) and was inversely (r=-0.22, P<0.01) associated with breast milk intake. Non-breast milk oral water intake represents intake of complementary foods and drinks.
6.3 Energy and macronutrient intakes from complementary foods of 9–10 month old Malawian infants (II)

About 87% (568 out of 651) of the infants randomised into the dietary assessment study had complete dietary data. An overview of the reasons for not including some participants have been shown in Figure 1 of article III. The mean intakes for energy and macronutrients are presented in Table 6. Mean energy intakes among infants in the SQ-LNS intervention groups ranged from 388 to 406 kcal/day and were higher than in the control group, 345 kcal/d, \( P < 0.05 \). Protein and fat intakes were also significantly higher in the SQ-LNS intervention groups than in the control group, the former ranged from 9.0 to 9.4 in the intervention compared to 8.3 g/d in the control group. While fat was 7.0 in the control group, in the intervention group fat intake ranged from 10.1 to 13.0 g/d. There were no significant differences in median intakes of energy from non-SQ-LNS complementary foods between the intervention and control groups \( P = 0.11 \).
Table 6: Energy and macronutrient intakes among the study participants

<table>
<thead>
<tr>
<th></th>
<th>Control (n= 123)</th>
<th>10 g LNS/d (n=130)</th>
<th>20 g LNS/d (n=158)</th>
<th>40 g LNS/d (n= 157)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/d</td>
<td>345 (247, 463)a</td>
<td>396 (309, 532)b</td>
<td>406 (300, 535)b</td>
<td>388 (304, 548)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>8.2 (5.7, 11.4)a</td>
<td>9.3 (7.4, 12.0)b</td>
<td>9.4 (6.5, 12.3)b</td>
<td>9.0 (6.2, 11.7)b,a,b</td>
<td>0.040</td>
</tr>
<tr>
<td>% energy</td>
<td>9.6 (8.4, 10.7)b</td>
<td>9.8 (8.7, 10.7)b</td>
<td>8.9 (8.0, 10.0)a</td>
<td>8.6 (7.4, 9.8)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>7.0 (3.9, 10.8)a</td>
<td>9.3 (6.9, 15.4)b</td>
<td>11.9 (7.6, 17.2)b</td>
<td>13.0 (8.7, 19.1)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% energy</td>
<td>18.0 (13.6, 23.6)a</td>
<td>22.6 (18.6, 27.3)b</td>
<td>26.8 (19.7, 32.4)c</td>
<td>29.7 (24.1, 35.3)c</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The predictors of energy intake from complementary foods were SQ-LNS intake, breastfeeding status, maternal education, agricultural season (rainy season), and reported lower-than-usual food intake on the day of the dietary assessment (see Table 3 of article II). Breastfeeding and reported lower intakes were negatively associated with total energy intake from complementary foods, which concurs with what was observed in the breast milk assessment study (i.e an inverse association between breast milk intake and non-breast milk oral intake).

6.4 Predictors of breast milk intake (III)

Breastfeeding frequency was quite high among infants in Mangochi and Namwera, over 90% of the infants were breastfed for more than 6 times a day. Breast milk intake was also higher than the regional or global estimates. In the bivariate analysis, breast milk intake was significantly associated with infant weight and maternal height at 0.05 level (see Table 3 of article III). Infant weight was the strongest independent variable associated with breast milk intake and the following maternal variables height, age and education. An increase in infant weight by a kilogram was associated with an increase in breast milk intake of 68 g/d. Maternal education and age were inversely related to breast milk intake (Table 7). Energy from complementary foods was also inversely associated with breast milk intake, though the association did not reach statistical significance ($P = 0.063$). From the path analysis, infant weight had the strongest relationship with breast milk intake ($\beta=0.28$), followed by maternal height ($\beta=0.21$) (Figure 4 of article III). Maternal BMI and height were both positively related to infant weight, but the relationship of maternal height to breast milk intake was only partially explained by its association with infant weight.
Table 7: Regression model for the association between breast milk intake (g day\(^{-1}\)) and independent variables

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient</th>
<th>Confidence Interval</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant weight (kg)</td>
<td>67.6</td>
<td>39.2 to 96.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>-6.5</td>
<td>-10.8 to -2.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Maternal education</td>
<td>-10.9</td>
<td>-19.0 to -2.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>6.8</td>
<td>1.7 to 12.0</td>
<td>0.009</td>
</tr>
<tr>
<td>Energy from non-breast milk sources (kcal day(^{-1}))</td>
<td>-0.2</td>
<td>-0.3 to 0</td>
<td>0.063</td>
</tr>
</tbody>
</table>

6.5 Association between breast milk and growth and development (IV)

The mean (SD) daily breast milk intake for the present study was 752 (244) g which contributed more than half (504 kcal) of the daily total energy intake of Malawian infants at 9–10 months. The mean (SD) change in LAZ-score for children in the present study between 12 and 18 months was -0.17 (0.6). The mean (SD) for language scores was 32 (24) (out of 100 meaningful words from the vocabulary checklist) while median fine, gross and overall motor scores (from the Kilifi developmental inventory) were 21, 18, 38 respectively.

Table 8: Associations between breast milk variables* and LAZ at 12 months and change in LAZ between 12 and 18 months†‡

<table>
<thead>
<tr>
<th>Variables</th>
<th>LAZ-scores at 12 months (n 163)</th>
<th>Change in LAZ-scores from 12 to 18 months (n 158)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95 % CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Breast milk intake (g/d)</td>
<td>0.15 (0.03 to 0.27)</td>
<td>0.012</td>
</tr>
<tr>
<td>Breast milk intake (g/d), adjusted model§</td>
<td>-0.01 (-0.10 to 0.08)</td>
<td>0.831</td>
</tr>
<tr>
<td>Breast milk intake (g/kg/d)</td>
<td>-0.14 (-0.25 to -0.02)</td>
<td>0.012</td>
</tr>
<tr>
<td>Breast milk intake (g/kg/d), adjusted model§</td>
<td>-0.003 (-0.10 to 0.09)</td>
<td>0.952</td>
</tr>
<tr>
<td>% total energy intake from breast milk intake</td>
<td>-0.05 (-0.18 to 0.09)</td>
<td>0.493</td>
</tr>
<tr>
<td>% total energy intake from breast milk intake, adjusted model§</td>
<td>-0.08 (-0.18 to 0.02)</td>
<td>0.185</td>
</tr>
</tbody>
</table>

*Variables measured when infants were 9–10 months old; †Analysis was done using linear regression; ‡Standardized regression coefficients; §Adjusted for maternal height, education, infants weight at 9–10 months, household assets, household food insecurity and family care index.
Breast milk intake at 9–10 months expressed as g/d or g/kg body weight/d was not associated with growth between 12 and 18 months (Table 8) development at 18 months (Table 9). Breast milk in g/d had a weak positive association with attained LAZ at age 12 months (standardized $\beta=0.15$, $P=0.012$), when breast milk intake was expressed as g/kg body weight per day the direction of the association was reversed (standardized $\beta=-0.14$, $P=0.021$). Percent of total energy intake from breast milk, was negatively associated with fine motor scores (standardized $\beta=-0.18$, $P=0.015$) after controlling for maternal height, education, infant’s weight at 9–10 months, household assets, household food insecurity and family care index. In the bivariate analysis, percent of total energy intake from breast milk was negatively associated with language development (standardized $\beta=-0.14$, $P=0.033$), however, the association was attenuated in the adjusted model.
Table 9: Association between breast milk variables* and developmental scores at 18 months†‡

<table>
<thead>
<tr>
<th>Variables</th>
<th>18-Month Developmental scores</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fine motor (n 188)</td>
<td>Gross motor (n 172)</td>
<td>Total motor (n 185)</td>
<td>Language (n 194)</td>
<td></td>
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<tr>
<td></td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Breast milk intake (g/d)</td>
<td>-0.02 (-0.13 to 0.10)</td>
<td>0.771</td>
<td>0.06 (-0.07 to 0.18)</td>
<td>0.356</td>
<td>0.06 (-0.05 to 0.18)</td>
<td>0.277</td>
<td>0.07 (-0.04 to 0.19)</td>
<td>0.204</td>
</tr>
<tr>
<td>Breast milk intake (g/d), adjusted model§</td>
<td>-0.05 (-0.16 to 0.06)</td>
<td>0.436</td>
<td>0.04 (-0.08 to 0.16)</td>
<td>0.558</td>
<td>0.03 (-0.08 to 0.13)</td>
<td>0.652</td>
<td>0.08 (-0.02 to 0.18)</td>
<td>0.190</td>
</tr>
<tr>
<td>Breast milk intake (g/kg/d)</td>
<td>-0.08 (-0.20 to 0.03)</td>
<td>0.168</td>
<td>-0.02 (-0.15 to 0.11)</td>
<td>0.594</td>
<td>-0.04 (-0.16 to 0.07)</td>
<td>0.473</td>
<td>0.05 (-0.07 to 0.16)</td>
<td>0.403</td>
</tr>
<tr>
<td>Breast milk intake (g/kg/d) adjusted model§</td>
<td>-0.04 (-0.14 to 0.07)</td>
<td>0.553</td>
<td>0.04 (-0.08 to 0.15)</td>
<td>0.553</td>
<td>0.04 (-0.07 to 0.14)</td>
<td>0.579</td>
<td>0.08 (-0.02 to 0.18)</td>
<td>0.194</td>
</tr>
<tr>
<td>% total energy intake from breast milk intake</td>
<td>-0.19 (-0.31 to -0.06)</td>
<td>0.003</td>
<td>-0.01 (-0.15 to 0.13)</td>
<td>0.865</td>
<td>-0.12 (-0.24 to 0.01)</td>
<td>0.068</td>
<td>-0.14 (-0.26 to -0.01)</td>
<td>0.033</td>
</tr>
<tr>
<td>% total energy intake from breast milk intake adjusted model§</td>
<td>-0.18 (-0.29 to -0.06)</td>
<td>0.015</td>
<td>-0.02 (-0.15 to 0.11)</td>
<td>0.755</td>
<td>-0.12 (-0.24 to 0.004)</td>
<td>0.087</td>
<td>-0.12 (-0.23 to 0.01)</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Variables measured when infants were 9–10 months old; †Analysis was done using linear regression; ‡Standardized regression
The overall aim for designing the present study was to explore the association between amount of breast milk and growth and development during the complementary feeding period among infants and young children. Thus we first aimed to assess breast milk intake among infants; then secondly assessed energy and macronutrient intakes from complementary foods, thirdly assessed predictors of breast milk intake, and finally the main aim of the study was achieved by assessing the association between amount of breast milk intake and growth and development. In the subsequent sections the results of each aim are discussed and possible reasons for the findings provided. The strength and limitations of the overall study will be presented at the end of the discussion.

7.1 Impact of SQ-LNS on breast milk intake

The present study was designed to assess the impact of supplementing complementary foods with SQ-LNS on breast milk intake among infants. Breast milk continues to be critical for child survival, health and nutrition status beyond the 6 months of age [13]. Thus too much displacement of breast milk by complementary foods, especially if they are also nutritionally poor is likely to be detrimental to infants. Compared to the infants in the control group, all the intervention groups had higher but not statistically (non-superior based on non-inferiority analysis) significant breast milk intakes. Therefore, supplementing complementary foods with SQ-LNS did not result in significant reduction of breast milk intake. The study had a number of strengths. The inclusion of a true control group that did not receive any study supplements allowed the assessment of the independent effect of SQ-LNS on breast milk intake. Breast milk intake was assessed using an objective method, the dose-to-mother deuterium dilution technique, which provides a quantitative estimate of breast milk intake. Furthermore, per-protocol analysis was planned apriori to confirm the intention-to-treat findings. The other strength was the randomized controlled design of the study, which further minimized biases.
The main study weaknesses were inability to observe SQ-LNS intake and high attrition rate during the trial implementation which necessitated a further selection of infants from the main study into the present sub-study. It is possible that caregivers may not have fed the SQ-LNS as planned, including sharing within the households and indeed with those in the control group. However, this source of bias was minimized by conducting fortnightly visits to the study participants to check on the usage of the SQ-LNS. Furthermore, caregivers reported high compliance on SQ-LNS use [196], similar findings from communities nearby the present study areas showed high compliance levels [197]. Additionally, the per protocol analysis in this study gave similar findings to the intention to treat analysis of breast milk intake, which further allowed an assessment of the impact of SQ-LNS on breast milk intake. The second enrolment had the potential to introduce selection bias, which was minimized by randomly selecting participants from all treatment groups. Bias was further reduced by blinding the study implementation team to group assignments.

The study finding is in line with other published data from low income countries which used SQ-LNS [152] and blended fortified foods [153, 154] but contrasts the evidence from some studies [157, 198]. Indian infants aged 6–10 months, significantly reduced their breast milk intake when their complementary food was supplemented with oil [156]. Overall infants from around Mangoche Boma and Namwera had higher breast milk intakes compared to the estimate from developing countries of 616 g/d. Studies in the sub-Saharan region have also reported higher breast milk intakes ranging from 678 g/d to 705 g/d [153] among older infants. So far, very few studies have been designed to assess the impact of SQ-LNS on breast milk intake, despite the heightened interest in the use of LNS products to prevent malnutrition. Consequently, our study results would need to be viewed as contributing to the evidence base rather than conclusive.

The lack of significant reduction in breast milk intake after supplementation with SQ-LNS may be explained by a number of possibilities. In our study area breastfeeding is almost universal and high breastfeeding frequencies have been reported from the same study area [99]. Among our study sample there was very little variability in breastfeeding frequency, as such we did not observe any significant association between breast milk intake and breastfeeding frequency. However, overall higher breastfeeding frequency is likely to be positively associated with breast milk intake. In other studies, small SQ-LNS has been shown to boost appetite among infants [199], whether this appetite is also for breast milk it is not known, but it is likely that infants’ breast milk intake would also improve with general improvement in appetite.

The other possibility would be related to infant growth. Breast milk intake was assessed after infants had been in the trial for 3 months. It would be that both SQ-LNS
and control groups experienced similar growth benefits independent of the SQ-LNS and hence both groups consumed extra calories from breast milk. Indeed, growth was not different between infants in the SQ-LNS arm and the control group [177]. Thus it is plausible to conclude that SQ-LNS given in the range of 10–40g does not have a significant negative impact on breast milk intake.

The other reason would be related to low total energy bioavailability from complementary foods. It has been hypothesized that even if children are consuming diets with adequate energy and nutrient densities, they would still not fully benefit due to EED or asymptomatic infections [177]. EED has been shown to be quite common in most low income countries, including Malawi [200]. Thus children would still be demanding more breast milk in order to meet their energy needs.

7.2 Energy and macronutrient intake

There have been concerns regarding the potential of SQ-LNS to displace local foods from infants’ diets. The present study was designed to assess whether or not supplementation of complementary foods with SQ-LNS would result in displacement of local complementary foods. There were no significant differences in total energy intakes by children from non-SQ-LNS complementary foods implying that although SQ-LNS did increase intakes of energy and macronutrients they did not displace locally available complementary foods. However, the 40g SQ-LNS dose reduced the mean energy intake contributed by legumes, nuts and seeds. Reduction in legume intake has also been noted elsewhere [154]. These findings are indicative of dose response association, meaning that as the dose of LNS increase, the probability of displacing certain food groups increases. These results are important in many respects, but more importantly they fit well in the global strategy for infant and young child feeding of the WHO, which promotes the use of suitable local foods [13].

The main potential for error in this study was reliance on caregivers’ (usually the mother) recalls, memory lapses during the dietary interviews is one of the sources of bias in estimating intakes [201]. To minimise bias due to memory and measurement, the data collection team was specially trained on the assessment method. The other strategies included the use of the food calendar as well as the use of replicas of the actual foods consumed to help the recall process. Additionally the 24 hour dietary recall was repeated on all the subjects, which improves the validity of the dietary intakes measurements [201]. Furthermore, since we did not observe actual intake of SQ-LNS compliance may not have been as anticipated, as has previously been reported in some parts of sub-Saharan Africa [103]. However, the study was designed as a randomized
trial and minimized the potential for systematic error of under or over reporting SQ-LNS intake. Thus provision of SQ-LNS up to 40g per day to complementary foods does not significantly displace intake of locally available infant and young child foods. This observation holds true among healthy infants, the results may not be generalized to sick infants and children.

The use of energy dense food supplements in interventions to enhance the energy density of complementary foods has the potential to lower the intake of local foods [155, 156, 202]. Evidence from certain studies have indeed shown a decrease in energy intake from complementary foods after supplementation [203]. On the other hand, some studies have shown that use of SQ-LNS in complementary foods among healthy infants [87, 98] and among moderately malnourished children [204] does not result in displacement of complementary foods. Christian et al. [100] also demonstrated that supplementing complementary foods of Bangladeshi infants with locally made supplementary foods and LNS did not have a significant impact on intake of local complementary foods or breastfeeding frequency. Among infants participating in LNS studies in Malawi [205] and Burkina Faso [206], provision of LNS to children increased the feeding frequency from complementary foods. In a study aimed at assessing the effect of energy and micronutrient supplements among Indonesian children, Beckett, Durnin [207] speculated that the absence of observable impact of the intervention was not due to displacement of the habitual diet.

Our study is in agreement with the latter studies, showing that SQ-LNS in doses of up to 40 g/d increase intakes of energy and macronutrients among infants, without displacing locally available complementary foods. The difference between our study findings and those reporting an inverse association may be explained by a number of factors. Most of the evidence showing inverse associations comes from research laboratory based settings [202, 208], our study was conducted among free living individuals. In the latter settings, dietary intake may not reflect the usual eating patterns. Related to the same, is the study duration, short feeding times are unlikely to provide evidence on habitual response to high energy diets.

The other reason could be related to the nutritional and health status of the children. Evidence from children recovering from infections shows a reduction in energy intake from complementary foods when high energy density supplements have been used [202, 209]. At the same time, our study population may also have had subclinical infections, especially those of the gut such as EED. Environmental enteropathy dysfunction in rural Malawi is quite prevalent among infants and young children [200, 210]. This condition has been hypothesized to partly explain the lack of positive impact of nutrition interventions on health and nutrition status in children from low income countries [149].
7.3 Predictors of breast milk intake

Study III was designed to identify factors which predict breast milk intake among 9 month-old rural Malawian infants. In the present study breast milk intake was associated with infant weight and the following maternal factors; height, age and education. Maternal BMI, energy intake from non-breast milk sources, residence and meal frequency were not associated with breast milk intake. We also conducted path analysis in order to further understand the pathways through which the above variables were related to each other and ultimately to breast milk intake. The pathway analysis also demonstrated that the main determinant of breast milk intake is infant weight.

These results should be interpreted in light of the limitations of the study. The examination of a number of associations, may have resulted in giving some significant associations which may have be due to chance [211]. However, sensitivity analyses gave similar results which increased the validity of the findings. Other potential sources of error are use of maternal self-reports on age which may be unreliable, due to memory loss. Dietary intake was also not observed, hence under or over-reporting of intake may not be ruled out. Taken together, the present study demonstrated that infant weight is a significant predictor of breast milk intake when the latter is expressed both as grams per day and grams per day per kilogram body weight of the infant.

There are many possible explanations to our research findings. Infants self-regulate their energy intake [30], which would follow that heavier infants would need greater energy intake and therefore consume more breast milk to meet total daily energy requirements. However, since our study was cross sectional it is not possible to separate which factor influenced the other i.e whether more breast milk intake resulted in heavier infant weight or heavier infants demanded greater breast milk. However, others have demonstrated that breastfeeding is not significantly associated with growth children [65], implying that weight of the infant does have an influence on amount of breast milk consumed.

Maternal education has also been shown to be inversely associated with certain breastfeeding practices such as frequency and duration in some studies [212, 213], while other studies have shown positive associations between maternal education and breastfeeding practices [214–218]. The observed differences are likely to be partly due to differences in social-economic status between low and high income groups as well as low and high income countries across different environments.

Maternal height was positively associated with breast milk intake in our study. It is very likely that the observed association would have been mediated through infant growth. In the path analysis model, maternal height was directly associated with breast milk intake but the relationship was also partly mediated through infant weight.
Published data has also shown a positive association between maternal height and infant breast milk intake [219]. Indeed, it is well established that maternal height is positively associated with infant birth length and weight [220, 221], which may be linked to an enhanced breastfeeding success early in infancy and possible have persistent effect on breast milk intake throughout the breastfeeding period.

The inverse association between maternal age and breast milk intake in our study may partly be related to the fact that younger mothers benefited more from the encouragement to practice optimal breastfeeding practices in our study population than older women. Indeed the direction of the relationship between maternal variables and breastfeeding outcomes has been shown to be site specific. In high income countries, younger mothers are more likely to practice suboptimal breastfeeding practices [222], the relationship in low income countries may be the reverse. Older mothers may not have been taking the breastfeeding messages as seriously as young mothers, others have shown that younger mothers are more likely than older mothers to follow optimal breastfeeding practices if both have same exposure of encouragement [223].

7.4 Association between breast milk intake and growth and development

The objective of study IV was to examine the association between breast milk intake at 9–10 months of age and growth and development in infancy and early childhood. Breast milk intake at 9–10 months was not associated with either growth between 12 and 18 months or development at 18 months. Breast milk intake was however associated with LAZ-score at 12 months. The study had a number of weaknesses worth considering when interpreting the results. Birth size was not measured in our sample, and consequently was not controlled for, size at birth is also an important determinant of growth during infancy and childhood [224]. Furthermore, the assumption that breast milk intake levels at 9–10 months may have persisted into later infancy and during early childhood may not have been completely true. Some of the developmental outcomes that were used in the study relied on participants’ recall, however, they were not blind to intervention group which may have introduced bias.

Currently there is a paucity of studies on association between quantitative breast milk intake and growth and development. Therefore, our results are compared to studies on breastfeeding practices focusing on growth and development. There are a couple of pathways through which breast milk may exert its impact on growth and development. Breast milk may promote infant and young child growth through its nutritional as well as anti-infectious properties [225], as well as through maternal-infant bonding during the breastfeeding process.
Some studies on breastfeeding practices such as continued and duration of breastfeeding have demonstrated positive association with growth among children. Simondon, Simondon [173] demonstrated among West African children that prolonged (up to 24 months) breastfeeding was positively associated linear growth among Senegalese children. Similarly, among East African children, Onyango, Esrey [174] reported that continued breastfeeding was positively associated with height gain among children age 9–18 months. They observed a dose response in the effect on duration of continue breastfeeding on growth, children breastfed for the longest duration gained about 3·4cm and 370g more than those in the shortest duration group. The association was pronounced among children coming from households with poor sanitation conditions. Similarly Alvarado, Zunzunegui [226] demonstrated a significant association between breastfeeding and growth among 5–7 months old infants, the benefits was also greatest among infants from households with poor socio-economic indicators. Analysis of data from the pre-second World War 2 period in the United Kingdom demonstrated breastfeeding during infancy as opposed to bottle feeding was associated with greater height gain during childhood [227].

Evidence from other breastfeeding studies does not demonstrate significant associations between growth and breastfeeding practices [62, 228]. Systematic reviews and meta-analyses have also demonstrated that breastfeeding has non-significant but positive associations with growth. Recently Giugliani, Horta [65] in a systematic review showed that there is little to no difference in growth (weight, height or length) between breastfed and non-breastfed children. Results of the present study are in line with this evidence showing non-significant effect of breastfeeding on growth.

Several studies have examined the association between breastfeeding and developmental outcomes among infants and children. So far the evidence has also been mixed even after controlling for potential confounding such as maternal characteristics and socio-demographic factors [74], with some studies showing positive and others demonstrating no significant associations. Among younger infants, Dewey et al. [229] demonstrated that children who were exclusively breastfed for 6 months were more likely to be walking by 12 months compared to those who were exclusively breastfed for 4 months. Leventakou et al. [230] also demonstrated that longer duration of breastfeeding is positively associated with language and motor development among 18 month children. The benefits of breast milk on development have been reported to be sustained into early childhood. Thorsdottir et al. [231] demonstrated exclusive breastfeeding was associated with verbal and motor development at 6 years of age. Like is the case with growth, the benefits of breastfeeding on development are much pronounced in a subset of infants, premature infants, in this instance [232].
The lack of a clear association between breast milk and growth and development may be as a result of a number of factors. Firstly, breast milk intake levels may not have been enough to influence significant growth or developmental changes. However, since breast milk contributed more than half of the energy intake for the infants there could be other reasons for the non-significant effect. As indicated earlier, evidence is accumulating showing the lack of desired results from nutrition interventions to be due to subclinical infections among children. It could also be that the level of breast milk intake in later infancy was more important for other body functions for instance physical activity [207, 233] as well as fighting or prevention of infections. Further analysis also demonstrated that growth was not significantly correlated or associated with total energy total intake (energy from complementary foods plus breast milk intake). Additionally, correlational analysis showed that breast milk intake was negatively associated with energy from non-breast milk sources, but the correlation was not statistically significant. The study shows that Malawian infants have relatively higher breast milk intakes, which contributes more than half of the total energy needs at 9–10 months of age. However, higher intake levels of breast milk are not associated with growth and development in this population.
The main strength for the present study is related to design. The sub-studies are based on a longitudinal randomized controlled trial. This set up enabled exploration of the impact of breast milk on growth and development. The other strength is related to the measurement of breast milk intake, breast milk was measured among free living children using what is currently considered as the gold standard method. The study also used a fairly large sample size which enables generalizability of the findings to the Malawian population. The present study obtained data on both breast milk and dietary intake concurrently, which enabled the independent assessment of the contribution of breast milk to growth and development.

The study had a number of limitations worthy of noting, nutrient and energy density of breast milk was not measured. Instead published values were used, since there is much variability in energy content of the breast milk, it is likely that we may have either over or underestimated the contribution of breast milk. On the same note, the other source of error may emanate from use of food composition tables from other parts of the world, nutrient content of foods vary from one place to another [234]. The use of the 24-hour dietary recall method is also prone to recall bias as well as measurement errors which may not be ruled out. However, the assessment tool validated for use specifically for the present study, and also validated for use in rural Malawi (Thakwalakwa et al. 2012). The interactive as opposed to the ordinary 24 dietary recall minimises memory loss by using local food calendar and models. Therefore, the measurement error for the interactive 24 hour recall was likely to have been minimal. The other limitation is that the association between breast milk intake and growth and development was done after three to nine months, the intake at 9–10 months may not have been reflective of the intakes at 12 and 18 months.
9 SCIENTIFIC CONCLUSIONS

The aim of the present study was to assess the relationship between breast milk intake and growth and development. After the implementation, the following conclusions can be made:

1. Breast milk intake among infants is not significantly reduced when their complementary foods are supplemented with SQ-LNS. Breast milk intake is significantly higher than the reported global estimate of 616g per day.

2. Supplementation of complementary foods of infants with SQ-LNS increases intakes of energy and macronutrients without displacing locally available complementary foods.

3. The main determinants of breast milk intake among 9–10 month-old infants are infant weight and the following maternal factors; height, age and education.

4. Breast milk intake at 9–10 months is neither associated with growth between 12 and 18 months nor development at 18 months. Breast milk is however associated with LAZ-score at 12 months.
The present study has demonstrated that provision of SQ-LNS does not lead to a significant reduction in breast milk intake and that the use of SQ-LNS does not replace complementary foods. Thus SQ-LNS would indeed be a potential vehicle for home fortification of complementary foods as the product does not have significant negative effect on both breast milk and local diets of infants. However, more evidence from efficacy and effectiveness trials is needed to substantiate the use of SQ-LNS as one of the strategies for preventing malnutrition, considering the inherent limitations of the evidence currently being generated. The study showed significant amount of breast milk intakes when infants are 9-10 months old, however, these levels of breast milk intake have not been shown to be associated with healthy growth and development during early childhood. The lack of an apparent association between the amount of breast milk consumed and growth and development may point to the need to conduct a thorough assessment of factors which impact growth after 6 months of exclusive breastfeeding. The study also demonstrated that breast milk intake is mainly determined by infant weight.

The present study therefore necessitates the need for more studies to be conducted to address the gaps identified. Following is a proposal of studies to be conducted:

1. Conduct a study assessing the impact of breast milk on growth and development using breastfed and weaned/non breastfed children. All participants in the present study were breastfed, the use of non-breastfed participants would allow assessment of independent contribution of breast milk on healthy growth and development among young children.

2. Conduct a study to measure energy and nutrient content of breast milk during late infancy and relate them to health growth and development.

3. Conduct a study on the relationship between maternal factors and growth and development in rural settings

4. Conduct a study to assess the major determinants of growth and development after the first 6 months of age.
This study was carried out at the Department for International Health, University of Tampere School of Medicine and at the School of Public Health and Family Medicine, University of Malawi. The study received financial support from the Mathile Institute for the Advancement of Human Nutrition, the International Atomic Energy Agency, the University of Tampere, and the Bill & Melinda Gates Foundation issued to the University of California, Davis. I received help from many people during the study, too numerous to mention them individual but I would like to thank all of them. However, the following individuals deserve special mention:

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