

EFFECT OF COMPLEMENTARY FEEDING OF LIPID-BASED NUTRIENT
SUPPLEMENTS ON APPETITE IN 6- TO 18-MONTH-OLD RURAL MALAWIAN
CHILDREN

A Randomised Controlled Trial

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ABSTRACT

Background: Undernutrition in children is still widely prevalent in Malawi and elsewhere in sub-Saharan Africa and South Asia. In most developing countries, high prevalence of undernutrition is attributed to inadequate dietary intakes among other causes. Reduced appetite in children has been shown to markedly contribute to low dietary intakes. Some studies have documented improvements in child appetite following supplementation with various micronutrients, some of which, are contained in Lipid-based Nutrient Supplements (LNS). As such LNS hold a promise to improve appetite in children.

Objective: The objective was to test the hypothesis that infants and young children receiving complementary foods supplemented with LNS from 6 to 18 months of age would have lower proportion of days with anorexia reports than infants and young children receiving no supplements.

Data and methods: The present study is a sub-set of the International Lipid-based Nutrient Supplements (iLiNS) DYAD-M trial in which 869 pregnant women were randomly assigned to receive either LNS, Multiple Micronutrients (MMN) or Iron Folic Acid (IFA) in rural Malawi. Children born to these women formed the sample size for the present study. Children born to women in LNS group, received two sachets of LNS-20gM (20g of LNS) daily from the age of 6 to 18 months while those born from women in either IFA or MMN received no supplements. Independent sample t-test was used in order to compare the proportion of days during which anorexia was reported between the intervention and control groups.

Results: Maternal and infant baseline characteristics were comparable between the intervention and the control groups. The mean (SD) proportion of days during which anorexia was reported throughout the entire follow-up period were 3.21 (14.65) % and 3.66 (15.9) % in the intervention and control groups respectively (difference -0.45%, 95% CI -0.45 to -0.08, P=0.02)

The difference (95% CI) in mean proportions of days with anorexia reports between LNS and control groups for age intervals of Week₂₇₋₃₉, Week₄₀₋₅₂, Week₅₃₋₆₅ and Week₆₆₋₇₈ were -0.14% (95% CI -0.86 to 0.58), -0.47% (95% CI -1.26 to 0.32), 0.01% (95% CI -0.72 to 0.72) and -1.15% (95% CI -1.84 to -0.47) respectively. However, this result was statistically significant only in the oldest age interval (P=<0.001). Furthermore, adjustment of the analyses for various selected baseline variables such as maternal age, education, primiparity, maternal Body Mass Index (BMI), and household asset index; did not markedly alter the results.

Conclusion: Provision of SQ-LNS to IYC in resource-insecure settings during early life yields modest improvements in appetite as evidenced by a decrease in the prevalence of anorexia. This study also suggests that the effect of SQ-LNS in improving child appetite gets significant as the children grow older.

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ABBREVIATIONS

BMI	Body Mass Index
CI	Confidence Interval
COMREC	College of Medicine Research Ethics Committee
CONSORT	Consolidated Standards of Reporting Trials
GBDS	Global Burden of Disease Study
GDP	Gross Domestic Product
ICH-GCP	International Conference of Harmonization-Good Clinical Practice
IFA	Iron Folic Acid
iLiNS	International Lipid-based Nutrient Supplements
IYC	Infant/s and Young Child/ren
LMICs	Low- and Middle-Income Countries
LNS	Lipid-based Nutrient Supplements
MAM	Moderate Acute Malnutrition
MMN	Multiple Micronutrients
MUAC	Mid-Upper Arm Circumference
RCT	Randomised Controlled Trial
RUTF	Ready-to-Use-Therapeutic Food
SAE	Serious Adverse Event
SAM	Severe Acute Malnutrition
SD	Standard Deviation
SQ-LNS	Small Quantity-Lipid-based Nutrient Supplements
UNICEF	United Nations International Children's Emergency Fund
USD	United States Dollar
WAZ	Weight-for-Age Z-score
WHO	World Health Organization's

1. INTRODUCTION

Optimal nutrition is of paramount importance in early childhood as it ensures healthy growth, proper development and function of organs, a fit immune system and; neurological and cognitive development (UNICEF, 2012). Child undernutrition is, therefore, among the most important causes of increased morbidity and mortality, compromised cognitive ability and educational attainment, and child growth failure (Black et al., 2008; Black et al., 2013). The various forms of undernutrition among Infants and Young Children (IYC), including stunting, wasting and micronutrient deficiencies, constitute a significant global health concern ubiquitously prevalent in Malawi and many other developing countries such as those located in Sub-Saharan Africa and South Asia (Black et al., 2008; Black et al., 2013).

Undernutrition mostly occurs during the complementary feeding period following that infants between 6 and 23 months of age, a typical complementary feeding period, require nutrient dense food (Dewey and Brown et al., 2003). In most developing countries, especially those characterized by resource insecurity, the high prevalence of undernutrition in IYC is often ascribed to suboptimal dietary intakes, infections and the mother-child interaction (Waterlow, 1994). In part, sub-optimal dietary intakes result from failure of available foods to meet the particularly heightened nutritional needs required for growth and development of IYC (Vitta & Dewey, 2012), lack of food, lack of adequate health care and malabsorption of food nutrients due to illness. Anorexia, due to several factors, may also be on an important contributing causal pathway to sub-optimal dietary intakes (Golden et al., 2000). Correction of anorexia in IYC may, thus, improve intake of complementary foods and ultimately contribute to the prevention of undernutrition and its consequences.

Nutritional status of IYC has been targeted with several interventions (Bhutta et al., 2013), many of which aim at improving complementary feeding during the first 1000 days of a child's life (Victoria et al., 2010). One such strategy is home-based use of Lipid-based Nutrient Supplements (LNS) which have been designed to prevent undernutrition (Adu-Afarwuah et al., 2007; Phuka et al., 2008). Interest in the use of lipid-based foods to prevent child undernutrition has grown following the success of Ready-to-Use Therapeutic Foods (RUTF) in the treatment of severely malnourished children (WHO, 2007). Some studies have documented improvements in child appetite following supplementation with various micronutrients (Dossa et al., 2001; Dossa et al., 2002; Shakur et al., 2009; Latham et al., 1994; Mda et al., 2009;

Stoltzfus et al., 2004), some of which, are contained in LNS. As such LNS hold a promise to improve appetite in children. The aim of the present study was to assess whether supplementing IYC's diets with Small Quantity Lipid-based Nutrient Supplements (SQ-LNS) would improve their appetite for non-supplement foods.

2. LITERATURE REVIEW

2.1. Approach to literature review

The present study is a community based investigation of the effects of complementary feeding of SQ-LNS on appetite in 6- to 18-month-old IYC living in resource-insecure settings of Malawi. In an effort to provide background information for the presented research, the literature review describes human appetite and its assessment, defines undernutrition, describes causes of undernutrition, outlines prevalence and trends of undernutrition, and offers an overview of lipid-based nutrient supplements (LNS) as an option for prevention of moderate undernutrition. The literature review also describes the interaction between undernutrition and appetite.

A search of electronic publications using relevant key words was performed to identify relevant literature for the review. The literature was searched primarily from Medline using Ovid interface and secondarily through Medline interface. Additional searches were done through Google Scholar. The main search terms included, but were not limited to, appetite, anorexia, energy intake, undernutrition and sub-Saharan Africa. References from retrieved publications were also further reviewed for other relevant literature. Additional searches were also conducted for data and reports from international organizations such as WHO, the World Bank, and UNICEF.

2.2. Appetite in young children

2.2.1. Definition and factors

Appetite has been defined as *the internal driving force for the search, choice and ingestion of food* (de Graaf et al., 2004). Others have defined appetite as *a mild hunger usually directed at a choice of food items and often with expectations of reward* (Castonguay and Stern, 1990). Appetite exists in all higher life forms, and serves to regulate adequate energy intake to be used for basal metabolic needs and physical activity (Ellsworth et al., 2009). Appetite can also be

thought of as external expression of hunger. Hunger differs from appetite in that hunger is often regarded as a physiological concept, whereas appetite is usually culturally defined (Castonguay and Stern, 1990). Blundell (2000), suggests that appetite is a result of an interaction among psychological events and behaviour, peripheral physiology, and the central nervous system (CNS). These three systems operate in the equation of energy balance and dysfunction at any level can alter energy intake manifested as changes in appetite. A significant loss of appetite is referred to as anorexia.

2.2.2. Causes of poor appetite in children

Poor appetite in children is caused by various factors. Micronutrient deficiency has been identified as one of the most important causes of anorexia in children (Umeta et al., 2000). For instance, zinc deficiency has been associated with disturbances in taste perception and appetite (Ueda et al., 2006). Zinc supplementation given to stunted Ethiopian children significantly improved appetite compared to a placebo and it was concluded that combating zinc deficiency can increase growth of children through increased appetite (Umeta et al., 2000). Iron deficiency is also known to be associated with poor appetite. It has been reported that iron supplementation improved appetite and growth in anaemic Kenyan children (Lawless et al., 1994).

Apart from micronutrient deficiency, anorexia may result from inadequate regulation of hormones such as insulin and leptin (Arsenault et al., 2007). Insulin stimulates production and secretion of hormone leptin which takes place in the adipose tissue and orchestrates body fat and energy intake. Circulating leptin signals to the brain that body fat and energy intake are adequate. This results in suppression of appetite (Arsenault et al., 2007). It is not established whether regulation of these hormones is influenced by micronutrient levels although some scientists have reported that zinc may be involved in the regulation of serum leptin concentration (Chen et al., 2000).

2.2.3. Measurement of appetite in young children

According to De Graaf et al. (2004), human appetite can be assessed in two possible ways. Firstly, appetite can be measured with the help of subjective self-ratings. This is provided for following that humans have a capacity to introspect and can rate the strength of their conscious drive or motivation to eat. Subjective ratings, when correctly used, have been shown to be

reproducible, sensitive to exposures of food components and predictive of food intake (De Graaf 2004; Brown, 1995). It should, however be born in mind that assessment of human appetite may not always be accessible to introspection considering that people do not always eat when they are hungry, and they do not always refrain from eating when satiated.

Secondly, human appetite can be measured by assessing ad libitum intake of a test food of choice (De Graaf et al., 2004). Thus, the amount of food eaten within a certain context can be considered as a measure of appetite. This method of appetite testing has been evaluated in young Beninese children and validated as an appropriate option for appetite assessment (Dossa et al., 2002a). It is important to note that the extent to which actual food intake reflects appetite is still debatable. This is the case because there are many factors that may intervene between appetite and actual food intake. These include; cognitive factors, such as dietary restraint, but also external factors, such as availability, hedonic properties of food; and social circumstances. However, when measured under standardized conditions, actual food intake serves as a post hoc indicator of appetite (Dossa et al., 2002a). One important consideration in this respect is that the actual food intake should be observed (i.e., directly measured) and not derived from dietary records in which subjects record their own food intake. It is difficult to obtain a precise and valid estimate of energy intake on an individual level from dietary records alone (Dossa et al., 2002a)

Human appetite, especially in IYC, is also measured by maternal or care giver reports on their subjective assessment and ratings of the infant's appetite (Brown et al., 1995). This is because IYC do not have the capacity to effectively assess and express their appetite status. Considering the existing paucity of published quantitative information on children's appetite in scientific literature, care giver reports on child appetite have largely been considered anecdotal and not a robust ground for objective scientific conclusions. However, Brown et al. (1995), through a longitudinal study of infant's dietary intake in low-income, peri-urban community of Lima in Peru, explored to a greater detail, the epidemiology of maternal reports of poor infant appetite. In this study, in order to assess the validity of the mothers' reports on their infants' appetites, the infants' energy in-take on days with and without anorexia were compared. For those days, dietary in-takes were also measured by an observer in the home. When age, body weight, and the presence of specific symptoms of illness were adjusted for, it was found that intra-individual total energy intakes were nearly 15% less on days when the caregivers reported that their infants' appetites were diminished ($P < 0.001$). As such this study demonstrated the

correlation between maternal reports on child appetite status with actual food intakes (Brown et al., 1995).

Several clinical trials, conducted in Benin, Bangladesh, Kenya, South Africa, Tanzania and Iran, have measured child appetite (Dossa et al., 2001; Dossa et al., 2002b; Shakur et al., 2009; Latham et al., 1994; Mda et al., 2009; Stoltzfus et al., 2004) (Table 1). In most of the reviewed studies, both subjective and objective methods of assessing child appetite were used simultaneously with objective methods involving quantification of a test food of cultural relevance eaten ad libitum and subjective methods involving maternal report of their child's appetite. Two studies, conducted in Bangladesh and Tanzania employed the sole use of maternal child appetite reports as a child appetite assessment method. The reviewed study used multiple micronutrients, multi-micronutrients or specific minerals. No study with LNS as the intervention was identified.

Table 1: Summary of controlled studies measuring appetite in children

Study	Participants	Age	n	Intervention	Appetite assessment methods	Results
Dossa et al., 2002 (a blinded, placebo-controlled randomized trial), South Benin	A representative sample of stunted children in south of Benin	17-32 months	111	Multivitamin-multimineral supplements, VITALIA-tablets, containing 11 vitamins and 8 minerals or placebo for 6 weeks	– Measurement of a test food called “aklui” (a fermented maize porridge) eaten ad libitum. – Subjective maternal reports	Appetite status, measured by both methods, increased over time in both study groups but there were no inter-group differences before as well as after supplementation
Shakur et al., 2009.(a double blind randomized controlled interventional study), Bangladesh	Children suffering from non-specific etiology of poor feeding associated with failure to thrive	36-72 months	40	Oral zinc 20 mg/day mixed with multivitamin or oral multivitamin only for 21 days.	– Subjective maternal reports	Improvements in appetite were found in 60% of the children in the Zn+MV group. Of these, 50% achieved significant weight gain. Only 15% of the children in MV group had increased appetite and of these only 10% showed significant weight gain.
Dossa et al., 2001 (placebo-controlled single blinded randomized trial), Ze, Benin	A representative sample of young stunted and anemic children	18 – 30 months	154	multivitamin-multimineral plus iron, multivitamin-multimineral plus placebo, placebo plus placebo and placebo plus iron for 6 weeks	– Measurement of a test food called “riz-au-gras” (rice cooked in a tomato sauce) eaten ad libitum – Subjective maternal reports	There were no differences among groups in changes in appetite and growth performance
Latham et al., 1994 (A randomized, double-blind, placebo-controlled trial), Kenya	primary school children	6 – 11 years	87	Sustained-release ferrous sulfate (150 mg) or placebo tablets for 14 weeks	– Quantitative measurement of the ad libitum consumption of a midmorning snack	Children in the iron-treated group reported improvements in appetite, whereas those in the placebo group,

Study	Participants	Age	n	Intervention	Appetite assessment methods	Results
					called “uji” (a thin maize porridge). – Subjective self-assessment.	there was no significant difference in perceived appetite.
Mda et al., 2009 (A randomised, double-blind, placebo controlled study), South Africa	HIV-infected young children	6 – 24 months	140	multi-micronutrient supplement or placebo for 6 months	– Quantification of a honey and wheat cereal called “Nestlé’ Nestum No 2” eaten ad libitum.	Children who received the supplements achieved significant improvements in their appetites over the six-month period.
Hassanzadeh-Rostam et al., 2014 (a single-blinded, placebo-controlled study), Shiraz, Iran	A representative sample of children with low appetite and weight for age ratio below the 25th percentile	3 – 7 years	63	7.5 mL/d multivitamin-mineral syrup or a placebo for 2 months	– mothers’ reports of low appetite of their children – daily energy intake of 80% or less than estimated energy requirements, as determined by 24-hour food recalls	Appetite increased in both groups and no significant difference between the groups was identified
Stoltzfus et al., 2004 (a randomised , placebo controlled, double blind trial), Kengeja village, Pemba Island, Zanzibar, The United Republic of Tanzania	A representative sample of children with hemoglobin > 70g/L at baseline	6 – 71 months	459	A liquid preparation containing 20g/L iron as ferrous sulphate or an identical placebo for 12 months	– Subjective maternal reports	The intervention reduced poor appetite in the children, by ~40%, according to mothers’ report

2.3. Epidemiology of undernutrition

2.3.1. Definition of undernutrition

Various organisations have defined undernutrition in diverse ways. For example, UNICEF (2009) defines undernutrition as *the outcome of inadequate food intake and recurrent infectious diseases*. FAO/WHO/UNU (1985) defines undernutrition as *a dietary energy intake below the minimum requirement level to maintain the balance between actual energy intake and acceptable levels of energy expenditure*. The World Food Programme (WFP) (2005) defines undernutrition as *a state in which the physical function of an individual is impaired to the point where he or she can no longer maintain adequate bodily performance process such as growth, pregnancy, lactation, physical work and resisting and recovering from disease*. Undernutrition includes being underweight for one's age, too short for one's age (stunted), dangerously thin for one's height (wasted) and deficient in vitamins and minerals (micronutrient undernutrition) (UNICEF, 2009). Malnutrition, as a broad term, refers to all deviations from adequate nutrition including all forms of undernutrition and overnutrition both of which are public health problems with important consequences for survival, incidence of acute and chronic diseases, healthy development, and the economic productivity of individuals and communities (Black et al., 2013). The terms "malnutrition" and "undernutrition" have widely been considered synonymous although the distinction between them needs to be delineated at all times.

2.3.2. Population groups most at risk of developing undernutrition

Undernutrition commonly affects all groups of people in a community, but infants and young children are the most at risk of developing undernutrition and bearing its consequences (Blössner et al., 2005). This is because IYC have particularly increasing needs for growth and development, enhanced infectious burden, and small gastric capacities. Low intakes of good quality complementary foods are on the causal pathway (Dewey, 1999). Undernutrition, growth faltering in particular, is common during the complementary feeding period, indicative of suboptimal complementary feeding practices (Dewey, 1999). Pregnant women are also considered a high risk

group as undernourished women would give birth to low birth weight children creating an intergenerational cycle of undernutrition (Dewey, 1999)

2.3.3. Window of opportunity for prevention of undernutrition

The most critical window of opportunity to meet a child's nutritional requirements, is in the first 1000 days of life, including pregnancy period and ending with the child's second birthday (Victoria et al., 2010). During this period, children have increased nutritional needs to support rapid growth and development, are more susceptible to infections, have heightened sensitivity to biological programming and are totally dependent on others for nutrition, care and social interaction. (UNICEF, 2013). If children do not get the right food in the first two years of life, the damage done to their physical growth is irreversible (UNICEF, 2013) and health and nutritional interventions implemented after this period (to children who have already experienced growth failure) may not be effective (Checkley et al., 2003). Acting during this critical window of opportunity is imperative considering that the goal in fighting undernutrition is not only to treat it when it occurs, but also to prevent it from happening in the first place.

However, Prentice et al. (2013), without seeking to undermine the importance of the 1000 day optimal window of opportunity for prevention of undernutrition, or to discourage interventions during this critical period, suggest that improvements in child growth after early faltering might have significant benefits on schooling and cognitive achievement. As such, the focus on the first 1000 days should not inhibit merit for consideration to implement growth promoting nutritional interventions in preprimary and primary school-age children (Prentice et al., 2013).

2.3.4. Assessment of undernutrition

Undernutrition in children can manifest itself in several ways, and it is defined by measures of child physical growth and composition which are used to assess a child's nutritional status. These include height-for-age index measures, weight-for-height index measures and weight-for-height index measures. Each of the child nutritional status indices provides different information about growth and body composition, used in child nutritional status assessment (WHO Multicentre Growth Reference Study Group, 2006).

The height-for-age index measure indicates linear growth retardation and cumulative growth deficits. Children whose height-for-age Z-score is below minus two standard deviations (-2 SD) are considered short for their age, or stunted, and are chronically malnourished. (WHO Multicentre Growth Reference Study Group, 2006). Children who are below minus three standard deviations (-3 SD) are considered severely stunted. (WHO Multicentre Growth Reference Study Group, 2006). Stunting denotes absence of adequate nutrition over an extended period and is also affected by recurrent and chronic illness. As such, height-for age index, epitomizes the long-term effects of malnutrition in a population and is not sensitive to, short-term changes in dietary intake.

The weight-for-height index measures body mass in relation to body height or length and describes current nutritional status resulting from Children whose Z-scores are below minus two standard deviations (-2 SD) are considered thin, or wasted, and are acutely malnourished. Wasting represents the failure to receive adequate nutrition in the short term. It may result from inadequate food intake or a recent episode of illness causing loss of weight and the onset of malnutrition. Children whose weight-for-height is below minus three standard deviations (-3 SD) are considered severely wasted. (WHO Multicentre Growth Reference Study Group, 2006).

Weight-for-age is a composite index of height-for-age and weight-for-height. It takes into account both acute and chronic malnutrition. Children whose weight-for-age is below minus two standard deviations (-2 SD) from the median of the reference population are classified as underweight. Children whose weight-for-age is below minus three standard deviations (-3 SD) from the median of the reference population are considered severely underweight (WHO Multicentre Growth Reference Study Group, 2006).

2.3.5. Causes of undernutrition

Undernutrition is an upshot of a complex interaction of several factors and it is usually not possible to point at a single factor in order to explain the cause of high prevalence of child undernutrition. The UNICEF conceptual framework of causes and consequences of undernutrition outlines the

different levels of causality including immediate, underlying and basic causes (Figure 1) (UNICEF, 1990).

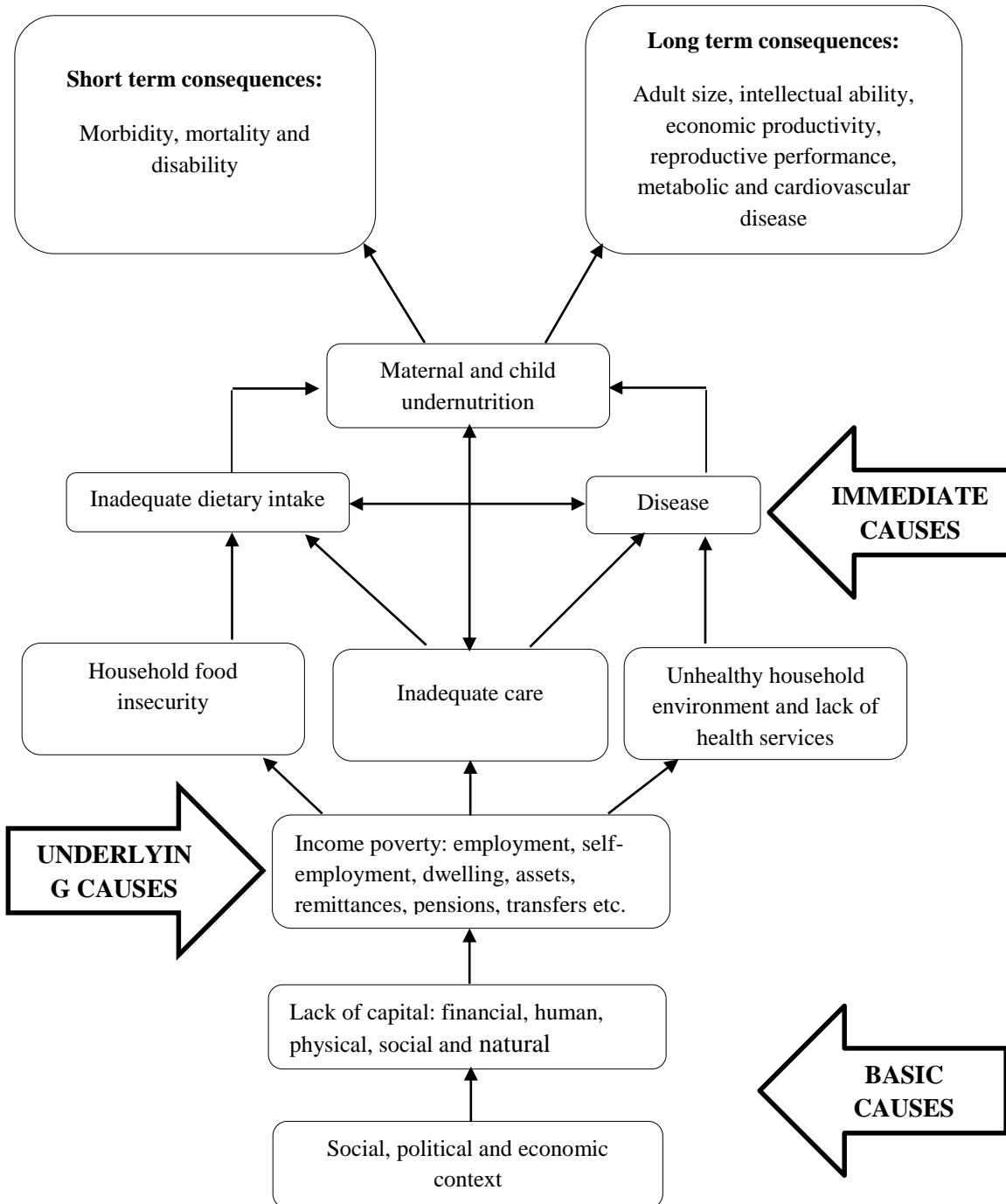
The immediate causes of child undernutrition are suboptimal dietary intakes and severe and repeated infectious diseases, particularly in underprivileged populations (UNICEF, 1998; Blössner et al., 2005). Poor diets include those that may be low in quantity, with limited nutrient density or variety, or those that are eaten rather infrequently (Burgess, 2008). Examples of infectious diseases in this regard are HIV/AIDS, diarrhea, respiratory tract or ear infections, measles, hookworms and other helminthes (Burgess, 2008). It is commonly known that infectious diseases lead to inadequate dietary intake and that inadequate dietary intake makes a person more susceptible to infectious diseases (Katona et al., 2008). As such, these two factors work in a synergistic relationship creating a vicious circle. Whether one factor or the other starts the vicious circle is not definitively established (Scrimshaw et al., 1968).

The underlying causes of undernutrition are noticeable at community and household level. These include factors such as income poverty, employment, food security, care, available health services and hygiene and sanitation (Burgess, 2008). Inadequate diet and disease are closely related to the general standard of living, the environmental conditions, and whether a population is able to meet its basic needs such as food, housing and health care (Blössner et al., 2005). Nutritional status is clearly compromised by diseases with an environmental component, such as those carried by insects or protozoan vectors, or those caused by an environment deficient in micronutrients. But the effects of adverse environmental conditions on nutritional status are even more pervasive. Environmental contamination including destruction of ecosystem, loss of biodiversity, climate change, and effects of globalization, has contributed to an increasing number of health hazards affecting nutritional status (Johns and Eyzaguirre, 2000). In addition, overpopulation disrupts the ecological equilibrium in which the population may exceed the carrying capacity of the environment. This is counterproductive to food production and leads to inadequate food intake and/or the consumption of non-nutritious food, and thus to undernutrition (Burgess, 2008).

The basic causes for undernutrition are found at national and international level. The social, economic and political context creates the structural causes for undernutrition and these causes are

deeply interrelated, and affect the others mutually. These are often characterized by a weak state, that lack incentives for leaders to perform pro-poor development (Collier 2009), making good governance an issue to consider. Weak states also suffer from weak institutional arrangements, and therefore often do not have the capacity to actually govern the state (Buzan 1991). This makes it hard to implement political initiatives and deliver services to the citizens.

Figure 1. Conceptual framework of causes and consequences of undernutrition. Adopted from Black et al., 2008.



2.3.6. Consequences of child undernutrition

Childhood undernutrition, as a disorder, is associated with both short- and long-term negative consequences for the affected individuals and populations (Black et al., 2013). These include reduced final adult height, momentous child mortality and morbidity, delay or deficits in development including lower cognitive function and poor school performance (Pelletier et al., 1994; Grantham-McGregor et al., 2007). UNICEF estimated that almost half of the 6.3 million global deaths of children under-5 years in 2013 were attributable to undernutrition (You et al., 2014). The Global Burden of Disease Study (GBDS), estimated that 1.4 million deaths in 2010 were attributable to child and maternal undernutrition, a significant decrease from 3.5 million in 1990 (Lim et al., 2010). Undernutrition also exposes IYC to various other infections. The existence of bidirectional causal pathways between undernutrition and infections have been well documented (Dewey et al., 2011) forming a vicious circle whereby poor nutrition upsurges the risk of contracting infections and infections impair nutritional status, (Krawinkel et al., 2012).

In addition, undernutrition (stunting, in particular) has been associated with adult health and disease (Piwoz et al., 2012). Impaired early childhood development in turn has been associated with limited final academic achievement, adult productivity, and earning potential (Victoria et al., 2008). As such, not only is undernutrition a major public health problem of global importance, but it is also an entrenched developmental and economic concern (Lutter et al., 2011; Victoria et al., 2008).

2.3.7. Prevalence of undernutrition

Undernutrition remains a major public health problem of global importance with Sub-Saharan Africa and south Asia having the highest prevalence (Grantham-McGregor et al., 2007). An estimated 171 million and 165 million (26%) of children younger than 5 years, approximately 90% of whom live in Asia and Africa (de Onis et al., 2012), were stunted in 2010 and 2011 respectively. Nearly one third of children below the age of five years in Low-income and Middle-Income Countries (LMICs), about 35 % of whom lived in Africa, were estimated to be stunted in 2011

(Black et al., 2013; de Onis et al., 2013). Globally, an estimated 52 million children under-five years of age, or 8%, were wasted (i.e., weight-for-height below $-2SD$) in 2011 with 70% of these living in Asia (WHO, 2009). With regards to, micronutrient deficiencies in children under five, in 2009 47% of children of the world were anaemic (293 million children) (McLean, 2009) and 33% were vitamin A deficient (190 million children) (WHO, 2009).

The global prevalence of stunting has taken a decreasing trend recently. For instance, there has been a reduction in it from 39.7% in 1990 to 26.7% in 2010. However, this trend has not been consistent in all regions of the world and stunting remains a major global health priority because of the large number of children affected by it and the associated severe short and long term health consequences (de Onis et al., 2013). Prevalence of underweight has also been decreasing steadily with a global reduction of 36% between 1990 and 2011. In 2011, the proportions of underweight children were highest in south-central Asia and western Africa with prevalence of 30% and 22% respectively (Black et al., 2013). A similar downward trend has been noted in the global prevalence of wasting with 11% decrease between 1990 and 2011 (Black et al., 2013).

2.4. Interrelationship between appetite and undernutrition in young children

There exists a bidirectional causal relationship between poor appetite and undernutrition. As already alluded to, undernutrition, micronutrient deficiency in particular, has been shown to cause poor appetite and growth failure in IYC (Lawless et al., 1994; Umeta et al., 2000). In turn, poor appetite results in inadequate dietary intakes which contribute to high prevalence of undernutrition in IYC (Waterlow, 1994). Even in developing countries, where undernutrition is often associated with household socioeconomic status, a child's own appetite plays a critical role in the development of undernutrition (Brown et al., 1995; Garcia et al., 1990). As such, improvement of appetite in stunted children, if possible, may contribute to the improvement of their linear growth and prevent a host of consequences associated with infant and young child undernutrition. Thus, nutritional interventions directly targeting correction of undernutrition may also improve child appetite.

2.5. Lipid-based nutrient supplements

LNS are a variety of fortified, lipid-based products based on similar ingredients, but varying in energy dose and concentration of micronutrients (Arimond et al., 2013). LNS are typically made from vegetable oil, peanut paste, milk powder, and sugar, with added vitamins and minerals, thus providing many of the fatty acids and micronutrients that are necessary for brain development (Arimond et al., 2015). LNS products have low water content hence limit bacterial growth and can mask the metallic taste of added micronutrients. LNS products do not require special storage facilities such as refrigeration and they have a considerably long shelf life (Briend, 2001). They also have a point-of-use added advantage because they can be consumed without the need to be cooked (Chaparro et al., 2010). LNS are, therefore, ideal for use in developing countries, especially in poor areas (Briend, 2001).

Ready to use therapeutic foods (RUTFs) have been shown to effectively treat severe acute malnutrition (SAM) in children both at community level and hospital rehabilitation centers (Ciliberto et al., 2005). Overtime, this has encouraged scientists to develop various lipid-based products with an aim to treat moderate acute malnutrition (MAM) (which includes wasting, or weight-for-height below -2 SD of the standard) and prevention of wasting or stunting. LNS designed for prevention of wasting or stunting are meant to be given in much smaller amounts (20–50 g/d). This is indicative of the high concentration of micronutrients, essential fatty acids and a small amount of protein, making them of reasonably low cost and, capable to not displace other foods that constitute a child's non-supplement diet. As such LNS are particularly suitable for home-fortification (Dewey et al., 2012).

LNS are excellently accepted across the world. Short term trials conducted in Burkina Faso, Ghana and Malawi have shown LNS to be highly accepted by mothers and infants (Adu-Afarwuah et al., 2011; Hess et al., 2011; Phuka et al., 2011) albeit a study conducted in South Asia, reported poor acceptability of LNS among malnourished children (Ali et al., 2013). The benefits of LNS in low income country settings have also been well described. Some scientists through several postnatal prevention trials conducted in Malawi, Ghana, and Haiti; providing a 20- to 50-g daily dose of

LNS to 6- to 18-mo-old children have concluded that providing LNS for 6–12 months may modestly promote infant or young child growth, either in the entire target population or a selected subgroup (Adu-Afarwuah et al., 2007; Phuka et al., 2008; Mangani et al., 2013; Iannotti et al., 2014).

2.6. Justification for the present study

As elaborated by the literature review, several studies have documented the impact of various nutrient supplements such as multi-micronutrient and Multivitamin-multimineral supplements multiple multivitamins and micronutrients on appetite in children (Table 1) (Dossa et al., 2001; Dossa et al., 2002a; Shakur et al., 2009; Latham et al., 1994; Mda et al., 2009; Stoltzfus et al., 2004). Mostly, these studies have found that multivitamins or micronutrients such as iron, zinc improve appetite in children. Considering that these micronutrients are also contained in LNS, these supplements (LNS) hold a promise to improve appetite in children. As such it is important to investigate the effect of LNS formulation on appetite in children despite the understanding that LNS are primarily formulated to treat or prevent the development of child undernutrition by improving energy density of infant diets and supplying selected micronutrients associated with growth promotion (Arimond, 2013).

In addition, as evidence for effectiveness and efficacy of LNS interventions for the prevention of undernutrition is rapidly growing (Arimond, 2013), there is noticeable paucity of evidence on possible effect of LNS on child appetite. No controlled studies so far have assessed the possible effect of LNS intervention on appetite in infants and young children. Therefore, the present study was designed to fill in some of the gaps in research regarding the effects of LNS intervention on children's appetite in a low income country setting. Furthermore, the findings of an investigation such as this would inform future programming of LNS.

3. AIMS OF THE STUDY

3.1. Main Aim of the study

This study aimed to determine whether provision of Small Quantity-Lipid-based Nutrient Supplements (SQ-LNS) to rural Malawian children aged 6–18 months, improves their appetite.

3.2. Specific aims of the study

- To compare the overall mean proportion of days during which anorexia was reported between children enrolled in the LNS group and those in the control group for the entire study follow-up period.
- To analyse if age modifies the effect of LNS on the proportion of days during which anorexia was reported.

3.3. Study hypothesis

Infants and young children receiving complementary foods supplemented with LNS from 6 to 18 months of age in the study area in rural Malawi would have lower proportion of days with anorexia reports than those who received no supplements.

4. MATERIALS AND METHODS

4.1. Study Site

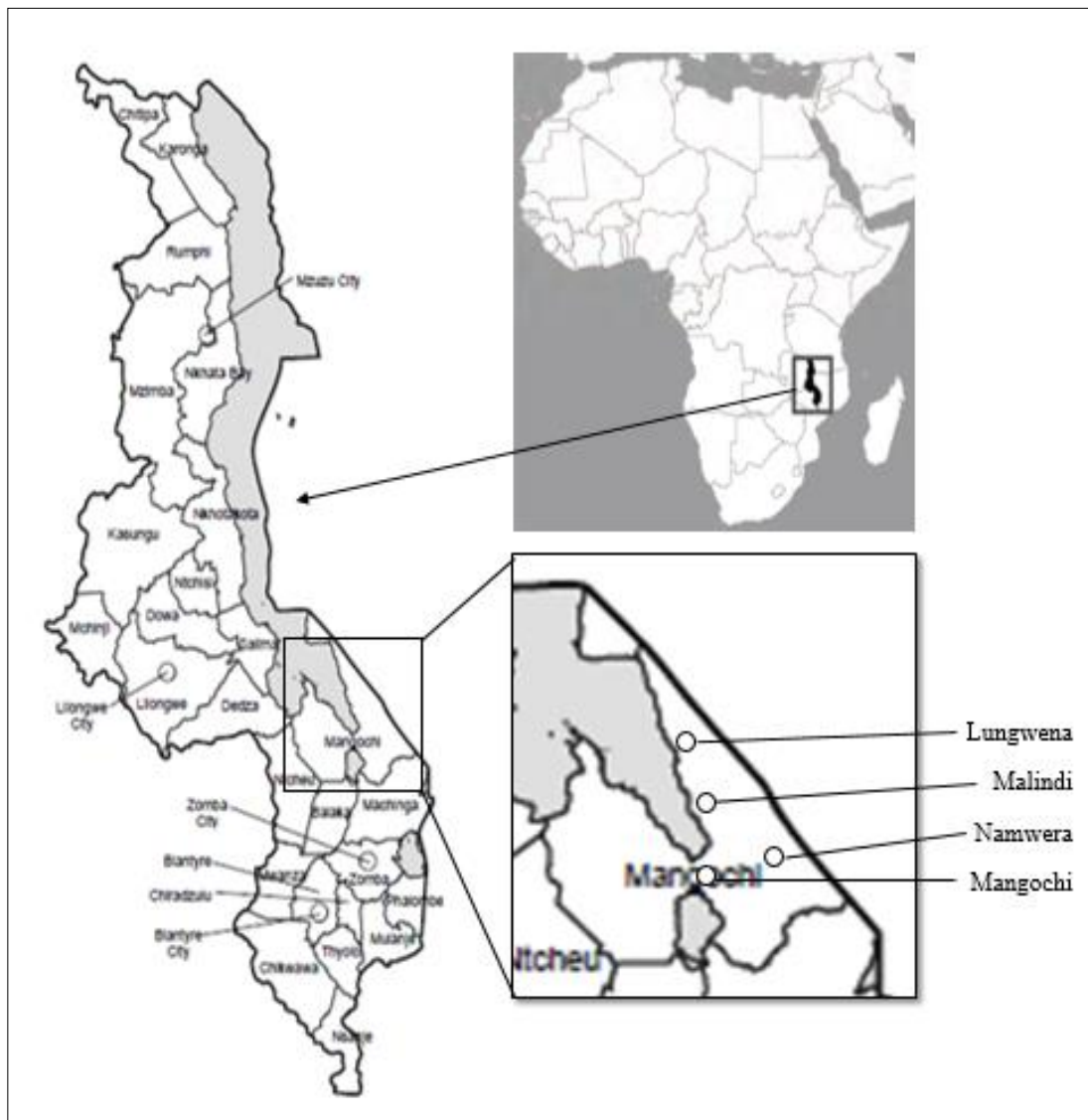
The present study was conducted in Mangochi district, Southern Malawi. Malawi is a small land-locked country located in the sub-Saharan Africa, a region with one of the highest prevalences of various forms of undernutrition. It is bordered by Tanzania to the North and North-East, Mozambique to the East, South and South-West and Zambia to the West. The total surface area for the country is estimated to be 118,484km² of which 94,276km² is land and the remaining area is mostly composed of Lake Malawi (National Statistical Office (NSO) [Malawi] and ICF, 2011) (Figure 2). In 2012, the population of Malawi was estimated at 15.9 million with the under-five age cohort accounting for about 17% (WHO, 2014).

The Gross Domestic Product (GDP) for Malawi is estimated to be 5.1 billion US dollars (World Bank, 2012) and Malawi is one of the most impoverished countries of the world. The economy of Malawi is predominantly based on agriculture, which accounts for 30% of the GDP (National Statistical Office (NSO) [Malawi] and ICF, 2011). The gross-national income per capita for Malawi was about 730 international dollars in the year 2012 and only 16% of the population lived in urban areas (WHO, 2014) Crude birth and death rates are 39.5/1000 population and 10.4/1000 population respectively (National Statistical Office (NSO) [Malawi] and ICF, 2011). Life expectancy at birth was indicated to be 47 years, which is the lowest amongst all countries of the world (WHO, 2010). Malawi is also among countries with high HIV prevalence. Currently, 11% of adult population are HIV infected.

Amongst under-5 children in Malawi, 37% are stunted (short for their age); 3% are wasted (thin for their height); and 12% are underweight (thin for their age). (National Statistical Office (NSO) [Malawi] and ICF, 2017). In Malawi, there has been a decline in the mortality of children under 5 from 234 per 1000 live births in 1992 to 63 deaths per 1000 live births in 2015-16 with rural areas registering more deaths than urban areas (77 deaths per live 1,000 births versus 61 deaths per 1,000 live births (National Statistical Office (NSO) [Malawi] and ICF, 2017).

Specifically, enrolment of study participants was carried out in geographic proximities of one public district hospital (Mangochi), one semi-private hospital (Malindi) and two public health centres (Namwera and Lungwena), all in Mangochi district, Southern Malawi (Figure 2). The Mangochi district hospital outpatient department served an approximated semi-urban population of 100,000 while the other hospital and health centres each offered health care services to a rural population of 30,000. In all the four sites, the population mainly subsisted on farming and/or fishing. In part, Mangochi district was chosen because the research team has had on-going child and maternal health studies in the district for about 15 years.

Figure 2: Map of Malawi indicating study sites. Modified from National Statistical office of Malawi (2011).



4.2. Study Design

The present study is a subset of a larger intervention trial, iLiNS-DYAD-M, a randomized, controlled, outcome assessor-blinded clinical trial (trial registration: www.clinicaltrials.gov, trial identification NCT01239693). The trial was conducted by the International Lipid-based Nutrient Supplements (iLiNS) project, a research group whose general objective is to investigate the use of LNS as an option for prevention of undernutrition. The trial had three intervention arms – LNS, MMN and IFA – and two types of participant follow-up schemes namely, “simplified follow-up” and “complete follow-up”. The complete participant follow-up scheme implied providing intervention and following-up the women during pregnancy until 6 months post-partum and then their children receiving the intervention and followed-up until 18 months after delivery. The simplified participant follow-up scheme only implied provision of intervention and following-up the women during pregnancy.

Healthy pregnant women were enrolled into the trial and, assigned to receive either LNS, MMN or IFA. In this MSc research project, singleton children born to women in the complete intervention and follow-up formed the study sample and those born to women assigned to pregnancy intervention only were excluded. Furthermore, children born to women allocated to the complete follow up scheme and assigned the LNS group formed the intervention group while those born to women randomised into either IFA or MMN study arms formed the control group.

4.3. Study participants

The targeted population included pregnant women who made antenatal care visits at any of the four study clinics from February 2011 to August 2012. Those who met all inclusion and none of the exclusion criteria, were enrolled into iLiNS-DYAD-M trial. The following were inclusion criteria: ultrasound confirmed pregnancy of not more than 20 completed gestation weeks, permanent residence in the study catchment area, availability during the period of the study, and signed or thumb-printed informed consent.

Exclusion criteria included age younger than 15 years, need for frequent medical attention due to a chronic health condition, diagnosed asthma treated with regular medication, severe illness warranting hospital referral, history of allergy toward peanuts, history of anaphylaxis or serious allergic reaction to any substance, requiring emergency medical care, pregnancy complications evident at enrolment visit (moderate to severe oedema, blood haemoglobin concentration <50 g/L, systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg), earlier participation in the iLiNS-DYAD-M trial (during a previous pregnancy), or concurrent participation in any other clinical trial.

The study sample for the current analysis included all 6- to 18- months-old singleton children born to women enrolled into the complete follow-up scheme of the main study. All women enrolled in Namwera were allocated into the simplified follow-up scheme by design. As such, children born to women enrolled in this site were all excluded.

4.4. Study Interventions

Women in the IFA group, the first control, received one micronutrient capsule/day containing 60 mg iron and 400 mg folic acid from enrolment to delivery, and 2 doses of intermittent preventive malaria treatment with sulfadoxine-pyrimethamine (3 tablets of 500 mg sulfadoxine and 25 mg pyrimethamine orally), one at enrolment and the other between weeks 28 and 34 of gestation. This is a standard antenatal care package as stipulated by the Malawian health policy. From delivery to 6 months postpartum, women in the IFA group received a placebo tablet containing 200 mg Ca.

Women in the MMN group, the second control, received one micronutrient capsule/day from enrolment to 6 months postpartum that contained IFA and 16 additional micronutrients, as shown in Table 2. The women also received the intermittent preventive malaria treatment during pregnancy.

Women in the LNS intervention group received sachets of SQ-LNS from enrolment to 6 months postpartum. The daily dose (20g) of maternal SQ-LNS contained the same micronutrients as those available in the MMN capsule, 4 additional minerals, protein, and fat; and it delivered 118 kcal of

energy (Table 2). From 6 to 18 months, infants and young children born to mothers enrolled in the LNS group received a version of LNS teller made for children.

In the course of implementing the trial, there was a temporary discontinuation of distribution of LNS to study participants from August 1 to September 11, 2012. This was due to a new quality assurance procedure in the supplement production which recommended the testing of LNS for the presence of *Cronobacter sakazakii*. Subsequently, the study implementation team suspended the distribution of the otherwise untested supplements pending test results. After the study implementation team was communicated to that the supplements did not contain *Cronobacter sakazakii*, they resumed provision of LNS to study participants. As a follow on to the suspension, 160 pregnant and 127 postpartum women in the LNS group did not receive LNS for periods ranging 1 to 20 days, and 121 children did not receive LNS for periods ranging from 1 to 41 days.

TABLE 2: Nutrient and energy contents of the dietary supplements used in the study¹

	IFA ²	MMN	Maternal LNS	Child LNS
Ration	1 tablet	1 tablet	20-g sachet	20-g sachet
Total energy, kcal	0	0	118	118
Protein, g	0	0	2.6	2.6
Fat, g	0	0	10	9.6
Linoleic acid, g	0	0	4.59	4.46
α -Linolenic acid, g	0	0	0.59	0.58
Vitamin A, mg RE	0	800	800	400
Vitamin C, mg	0	100	100	30
Vitamin B-1, mg	0	2.8	2.8	0.3
Vitamin B-2, mg	0	2.8	2.8	0.4
Niacin, mg	0	36	36	4
Folic acid, mg	400	400	400	80
Pantothenic acid, mg	0	7	7	1.8
Vitamin B-6, mg	0	3.8	3.8	0.3
Vitamin B-12, mg	0	5.2	5.2	0.5
Vitamin D, mg	0	10	10	5
Vitamin E, mg	0	20	20	6
Vitamin K, mg	0	45	45	30
Iron, mg	60	20	20	6
Zinc, mg	0	30	30	8
Copper, mg	0	4	4	0.34
Calcium, mg	0	0	280	280
Phosphorus, mg	0	0	190	190
Potassium, mg	0	0	200	200
Magnesium, mg	0	0	65	40
Selenium, mg	0	130	130	20
Iodine, mg	0	250	250	90
Manganese, mg	0	2.6	2.6	1.2

¹ IFA, iron and folic acid; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient; RE, retinol equivalent.

² The group that received IFA from enrolment to delivery received 200 mg Ca/d from delivery to 6 months postpartum.

4.5. Sample size calculation and justification

The target sample size for child outcomes (“Complete follow-up”) was 288 / group and 864 in total. The sample size calculation was based on being able to detect differences between three groups equivalent to an effect size of 0.3 (difference between groups, divided by the pooled SD) for each continuous outcome assuming power of 80% and $\alpha=0.05$. This required 216 participants per group, for a total of 648 subjects. Allowing for up to 25% loss to follow-up by the time the children turned 18 months of age, we needed to recruit 864 subjects.

4.6. Data collection

During the entire follow-up period, data on child appetite were collected once every week during home visits made by research assistants. A structured questionnaire, which was designed for collection of data on participant morbidity, illness signs, trial supplement use and the use of health services; was used. The research assistants who conducted the assessments were trained to use the questionnaire.

All collected data was reviewed by field supervisors daily for accuracy and completeness and then transferred to data entry clerks for double data entry in the field office. The database program incorporated range and consistency checks. Data was transferred weekly to the central data bank at the study site offices and was backed up by internet transfer to UC Davis and the University of Tampere. Programs were developed to detect any outlier data. All data collected by each research assistant was also checked for mean values and number preference, and any retraining was scheduled if necessary. The field workers met with the field supervisors weekly at a central office to discuss any problems or challenges.

At baseline, we recorded a series of relevant characteristics for both mothers and children participating in the trial. These included maternal age, maternal education (number of completed school years was used as a proxy), household socio-economic status index, maternal BMI, child

weight at the age of 5 months, child sex and site. The aim for recording and analyzing the selected baseline characteristics was to show that these potential confounding variables are equally distributed between the two groups. Additionally, it is usual practice when reporting an RCT to demonstrate the integrity of the randomisation process by showing that there is no significant difference between baseline variables following Trial profile in Consolidated Standards of Reporting Trials (CONSORT) guidelines (Begg et al., 1996).

4.7. Methods for protecting against other sources of bias

In this study, outcome-assessor blinding was used in order to prevent bias. Randomization into the trial, group allocation, and distribution of study food supplements was done by a research assistant who did not participate in evaluation of outcomes. The interventions were double-masked between the IFA and the MMN groups, for example the supplementary tablet looked identical and neither the participants nor the research team had knowledge about the nutrient contents of the supplement tablets. For the LNS group, field workers who delivered the supplements were the only ones who knew the actual participants who received LNS.

4.8. Definition of study outcomes

4.8.1. Child appetite

Appetite was assessed by maternal reports, a method shown to be valid in Lima, Peru (Brown et al., 1995). Mothers were asked in the local language, “For each of the days in the past week, how has your child’s appetite been?” The answer was rated on a 3-point scale with options “normal”, “reduced” and “none”. Thus the current study does not make an effort to validate maternal reports on their infants’ appetite state; rather, it relies on the maternal reports as an acceptable measure of infant and young child appetite.

4.9. Statistical methods

4.9.1. Preparation of child appetite data

During data collection, child appetite status was recorded as being “Normal”, “Reduced” or “None”. We determined weekly proportion of days during which anorexia was reported from daily appetite status reports expressed as a percentage of all days with valid data for the week in consideration. We considered anorexia to be present when the child’s appetite was reported to be either reduced or absent on a particular day. We used the following formula: weekly proportion of days during which anorexia was reported (%) = (number of days in a week (in a visit) when child appetite was reported to be either reduced or absent) / (sum of days with valid data on child appetite status during that week) × 100.

Following this, we calculated various child anorexia indices for all participants as mean proportion of days when anorexia was reported during the specified study follow-up weeks. The child anorexia indices are, in this report, named by the words “Week” followed by a range of study follow-up week numbers marked as subscripts to indicate the time period of interest and age of children in weeks.

For the main analysis, we calculated anorexia index for “Week₂₇₋₇₈” covering all time points throughout the entire follow up period from week 27 to week 78 of children’s age. Thereafter, we split the entire follow-up period into four narrower age intervals namely “Week₂₇₋₃₉”, “Week₄₀₋₅₂”, “Week₅₃₋₆₅” and “Week₆₆₋₇₈” and calculated anorexia indices for each of them. The study follow-up period was split to create an opportunity to assess if age would have an effect on anorexia indices. We used the following formula: The proportion of days during which anorexia was reported (%) = (sum of all weekly proportion of days with anorexia reports) / (sum of weeks with valid data on appetite within the age interval of interest) × 100.

4.9.2. Statistical analysis

All statistical analyses were performed with STATA software version 12.1 (StataCorp, College Station, TX, USA). Independent sample t-test was used in order to compare anorexia indices between the intervention and control groups for the entire study follow-up period (Week₂₇₋₇₈) and for four separate age windows expressed in weeks (Week₂₇₋₃₉, Week₄₀₋₅₂, Week₅₃₋₆₅ and Week₆₆₋₇₈). A one sided test of significance was used, with P value of <0.05 denoting statistically significant differences in anorexia indices between the intervention and control groups. Confidence Intervals (CI) at 95% were provided for the various differences in the proportion of days during which anorexia was reported.

A sensitivity analysis was carried out by constructing a regression model for each of the five anorexia indices in which the study results were adjusted for selected covariates all recorded at enrolment. These included maternal age, maternal education, primiparity, maternal Body Mass Index (BMI), and household socio-economic status index (arrived at by summarizing household assets).

Statistical analysis for the present study was based on the principle of modified intention-to-treat. The modification concerned two participants who were accidentally allocated to another group than that into which they were actually randomized. For each participant, the randomization code was pre-packed and sealed in an individual envelope that was opened and used for group allocation at enrolment. For these two individuals, the randomizer made a recording error, i.e. s/he noted down in a data collection form an incorrect group code or wrote the code with unclear handwriting. The incorrect code was later transcribed into the computer software that was used to plan participant visits and allocate interventions. These two participants were told to belong to the erroneously recorded intervention group and they received that intervention throughout the trial – hence they were also analyzed in that group (rather than the one written on the randomization slip). Another modification is that children with form 27 data collected less than 10 times during the follow up period will be excluded in the analysis. Additionally, all twin children will also be excluded.

As supplemental evidence aimed at improving precision of the intention-to-treat results, per-protocol analysis was also performed. This analysis was restricted to infants and young children who achieved a $\geq 70\%$ adherence rate in reported consumption of study supplement (LNS) and all children in the control group. The adherence rate was estimated by calculating the proportion of days when the study child was reported to have consumed the study supplement. This was calculated with the following formula: Intervention adherence rate (%) = (number of days when the participant was reported having consumed the supplement) / (the total number of days in the follow-up) $\times 100$.

4.11. Ethical Considerations

The iLiNS-DYAD-M trial was conducted in accordance to International Conference on Harmonisation - Good Clinical Practice (ICH-GCP) guidelines and it adhered to the principles of Helsinki declaration as well as clinical research regulatory guidelines in Malawi. Recruitment of study participants did not commence before the College of Medicine Research and Ethics Committee (COMREC), University of Malawi and the Ethics Committee of Pirkanmaa (Pirkanmaan Sairaanhoidopiirin eettinen toimikunta), Finland approved the trial protocol. Furthermore, signed or thumb-printed (in case of inability to write) informed consent form was a prerequisite to study enrolment and all participants were afforded a chance to withdraw their study participation at any point of the trial without being asked to provide a reason for the withdrawal by the study implementation team. All suspected Serious Adverse Events (SAEs) were documented throughout the entire study follow-up period and submitted to and assessed by an independent Data Safety and Monitoring Board (DSMB).

Before commencement of the trial, the study team members held numerous discussions with community leaders and organized village meetings to discuss research objectives and procedures. Pregnant women coming to antenatal clinics received further information about the trial before finalising their decision to participate.

5. RESULTS

5.1. Flow of study participants

Between February 2011 and August 2012, the iLiNS project trial implementation team members approached a total of 9310 women. From these, 1391 (14.9%) met all inclusion and none of the exclusion criteria and were enrolled and randomly assigned to one of the three intervention groups. A total of 869 (62.47%) of the enrolled women were assigned to the complete follow-up scheme of the study. Of these, 290 (33.37%), 291 (33.49%) and 288 (33.14%) pregnant women were assigned the IFA, MMN and LNS groups respectively. After maternal loss-to-follow-up and birth outcomes that were not live births, a total of 781 singleton children were identified; 263 (33.67%) in the IFA group, 264 (33.8%) in the MMN group and 254 (32.52%) in the LNS group. At the end of the one-year-long follow-up period, 227 (86.31%) and 231 (87.5%) completed the study in the IFA and MMN group respectively, and were summed up to form the control group in accordance with the design of the current study. 222 (87.4%) children completed the study in the LNS group. Success rate for participant follow-up was similar for both intervention and control groups. The flow of study participant is elaborately detailed in CONSORT recommended format (Figure 3).

5.2. Background characteristics of trial participants

At enrolment, the mean number of completed formal education years was 4 in both study groups. The intervention groups were also similar in terms of their average demographic and socioeconomic characteristics, obstetric history, and maternal nutritional status. In addition, at the start of the intervention period for children, both groups were similar in terms of child nutritional status with WAZ of -1 in both groups. Background maternal and child characteristics are shown by intervention group (Table 3).

Figure 3: Trial profile in Consolidated Standards of Reporting Trials (CONSORT)-recommended format

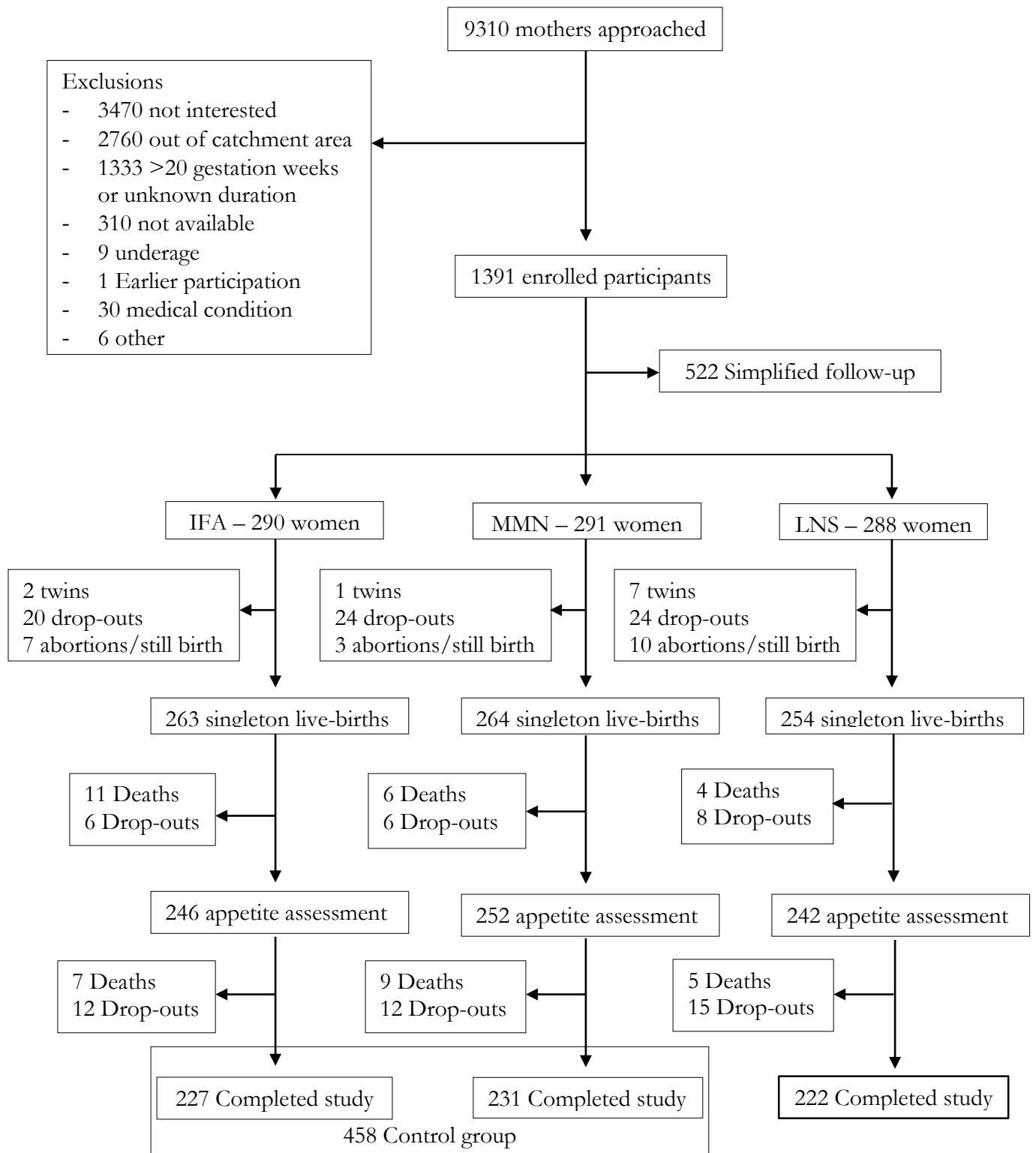


Table 3: Background characteristics by intervention group¹

	LNS	Control (IFA + MMN)
Maternal Characteristics		
Participants, n	288	581
Age, y	25 (6)	25 (6)
Education completed, y	4 (4)	4 (4)
Socio-economic index	0.1 (1)	0.0 (1)
BMI < 18.5, kg/m ²	7	5
Primiparous	24	24
Site		
Mangochi	109 (38)	216 (38)
Malindi	47 (16)	98 (16)
Lungwena	132 (46)	267 (46)
Child characteristics		
Participants, n	242	498
Male child	133 (49)	245 (47)
Weight at 6 months, kg (mean, SD, N)	7 (1)	7 (1)
Age at start of intervention, months	6 (0)	6 (0)
WAZ at start of intervention	-1 (1)	-1(1)

¹ Values are means (SD), n (%) or (%). LNS, lipid-based nutrient supplement; IFA, iron and folic acid; MMN, multiple micronutrient.

5.3. Proportion of days during which anorexia was reported

For the entire study follow-up period (Week₂₇₋₇₈), intention-to-treat analysis revealed that mean (SD) proportion of days during which anorexia was reported were 3.21 (14.65) % and 3.66 (15.90)% for intervention and control groups respectively (difference -0.45, 95% CI -0.45 to -0.08, P=0.02) (Table 4).

The difference (95% CI) in mean proportions of days with anorexia reports between LNS and control groups for age intervals of Week₂₇₋₃₉, Week₄₀₋₅₂, Week₅₃₋₆₅ and Week₆₆₋₇₈ were -0.14% (95% CI -0.86 to 0.58), -0.47% (95% CI -1.26 to 0.32), 0.01% (95% CI -0.72 to 0.72) and -1.15% (95% CI -1.84 to -0.47) respectively. However, this result was statistically significant only in the oldest age interval (P=<0.001). Furthermore, adjustment of the analyses for various selected baseline maternal variables did not markedly alter the results (Table 4).

A sensitivity analysis which was restricted to children who achieved >70% adherence to the intervention also yielded similar results for the entire follow-up period as well as the four separate age windows (Table 5).

Table 4 Prevalence of child anorexia by intervention group: Intention-to-treat analysis

Time points	Unadjusted results by intervention group				Covariate-adjusted results ⁴	
	LNS	Control (IFA + MMN)	Difference in means (95% CI)	P-value ⁵	Difference in means (95% CI)	P-value ⁵
Weeks 27-78 (SD), Percent	3.21 (14.65)	3.66 (15.90)	-0.45 (-0.45,-0.08)	0.02	-0.42 (-0.79,-0.56)	0.02
Weeks 27-39 (SD), Percent	3.23 (14.75)	3.37 (15.02)	-0.14 (-0.86,-0.58)	0.71	-0.12 (-0.84,0.61)	0.75
Weeks 40-52, (SD), Percent	3.62 (15.76)	4.09 (17.19)	-0.47 (-1.26,0.32)	0.25	-0.41 (-1.21,0.38)	0.31
Weeks 53-65, (SD), Percent	3.56 (15.49)	3.57 (15.43)	-0.01 (-0.72,0.72)	0.99	0.02 (-0.71,0.75)	0.96
Weeks 66-78, (SD), Percent	2.46 (12.37)	3.61 (15.83)	-1.15 (-1.84,-0.47)	<0.01	-1.14 (-1.83,-0.44)	<0.01

⁴ Adjusted for baseline maternal age, education, primiparity, BMI, and household socio-economic index.

⁵ Obtained from t-test.

Table 5 Prevalence of child anorexia by intervention group: per-protocol analysis

Anorexia Indices	Unadjusted results by study groups				Adjusted results ⁶	
	LNS	Control (IFA + MMN)	Difference in means (95% CI)	P-value ⁷	Difference in means (95% CI)	P-value ⁷
Weeks 27-78 (SD), Percent	3.15 (14.54)	3.66 (15.90)	-0.51 (-0.89,-0.12)	0.01	-0.47 (-0.86,-0.78)	0.02
Weeks 27-39 (SD), Percent	3.10 (14.72)	3.67 (15.02)	-0.27 (-1.03,-0.50)	0.49	-0.25 (-1.02,0.53)	0.53
Weeks 40-52 (SD), Percent	3.54 (15.48)	4.09 (17.19)	-0.55 (-1.38,0.29)	0.20	-0.49 (-1.33,0.35)	0.26
Weeks 53-65 (SD), Percent	3.60 (15.69)	3.57 (15.43)	-0.26 (-0.74,0.7)	0.95	0.08 (-0.70,0.85)	0.85
Weeks 66-78 (SD), Percent	2.38 (11.97)	3.61 (15.83)	-1.12 (-1.96,-0.49)	<0.01	-1.20 (-1.93,-0.45)	<0.01

⁶ Adjusted for baseline maternal age, education, primiparity, BMI, and household socio-economic index

⁷ Obtained from t-test

6. DISCUSSION

6.1. Comparison of results and study hypothesis

The present study tested a hypothesis that provision of SQ-LNS to infants and young children from 6 to 18 months rather than no supplements would decrease the mean proportion of days with anorexia reports in rural Malawi. From 6 to 18 months of age, infants and young children in the LNS group had a significantly, but modestly, lower mean proportion of days with anorexia reports than those in the control group. Similarly, modestly lower means in proportion of days with anorexia reports in infants and young children in the LNS group were observed in all four child age disaggregates of the follow-up period than among infants and young children in the control group albeit only significant during the final age bracket. Hence the study findings support the hypothesis that infants and young children receiving complementary foods supplemented with LNS from 6 to 18 months of age in the study area in rural Malawi would have lower proportion of days with anorexia reports than those who received no supplements.

The various interventions given to mothers during pregnancy, may have had an effect on the final child appetite outcome. Stunting often begins in utero (de Onis et al., 2013) and some of the stunting that occurs after birth is feared to be programmed during pregnancy period (Martorell & Zongrone 2012). As such, introduction of maternal interventions such as LNS or MMN would have different effects on development or programming of undernutrition. Considering that undernutrition is known to cause reduced appetites in children (Lawless et al., 1994; Umeta et al., 2000), this would have a ripple effect on appetite statuses of children born to mothers who received the interventions. However, the two study groups were comparable at the onset of the child intervention in terms of baseline characteristic for children. As such, it is safe to consider the findings of this study to have been influenced by child intervention only and not combined with residual effect of maternal interventions.

6.2. Comparison of study findings with other studies

Our results are consistent with the findings of an earlier study, also from Malawi, in children receiving either LNS or Corn Soy Blend (CSB) (Thakwalakwa et al., 2014). In this study, it was found that energy in-take from non-supplement foods was higher in the LNS group than CSB group. The authors suggested that the particularly high energy intakes in the LNS group resulted from low displacement of non-supplement foods by LNS. In the current study, it is very likely that improved appetite in children in the LNS group may have resulted, in part, from this mechanism.

Findings of the current study also corroborate several short-term acceptability trials from Malawi, Ghana and Niger which aimed to assess acceptability of LNS. These studies have shown that mothers perceive that LNS yields improved appetite in children (Adu-Afarwuah et al., 2011; Phuka et al., 2009; Tripp et al., 2011). The present study has shown a difference in the mean proportion of days with anorexia reports for the entire age interval with children in the LNS group having a lower proportion of days with anorexia reports. However, the results suggest that the effect of LNS in reducing anorexia in children is largest in the oldest children. It has to be considered that the significant difference observed in the final age window may have been a chance finding following that multiple comparisons were performed. Nevertheless, the finding of statistically significant lower prevalence of anorexia in the LNS group than in the control group in the final age interval is important, because it leads to the suggestion that LNS intervention, may yield more significant improvements in child appetite if the supplementation period is much longer than one year.

Controlled studies examining the effect of SQ-LNS on appetite in infants and young children are scarce. However, some studies are very closely related to the present study as they have reported the effect on appetite of various micronutrients, typically contained in LNS (Table 2) and have employed a child appetite assessment method similar to the one utilised in the current study. Dossa et al. (2001) through a blinded, placebo controlled randomized trial carried out in South Benin, reported that multivitamin-multimineral supplements given 17 – 32-month-old children increases appetite. Other researchers, through a double blind randomized controlled intervention study conducted in Bangladesh have documented improved appetite in 60% of children in that study following supplementation with oral zinc mixed with multivitamins albeit the sample size in this

study was particularly small (n=40) (Shakur et al., 2009). Mda et al. (2009) has documented significant improvements in child appetite over 6 month period in HIV infected young South African children. Stoltzfus et al. (2004) also reported 40% decrease in the prevalence of poor appetite after supplementing the diets of children with Iron as ferrous sulphate for 12 months to a representative sample of Zanzibari children with haemoglobin >70g/L at baseline.

6.3. Strengths and limitations of the study

The present study has strengths inherent in typical Randomised Controlled Trial (RCT) study design. These include random allocation of study participants to study intervention groups which resulted in similarity in both maternal baseline and infant background characteristics shown in Table 3, rigorous quality assurance during data collection which led to high data quality, and group allocation blinding of outcome assessors which eliminated bias at data analysis stage. In addition, implementation procedures of the current study were highly controlled, such that, LNS was delivered at study participant's homes at appropriate and predetermined intervals throughout the entire follow-up period. Moreover, all data were collected by adequately trained and regularly performance-tested data collectors and data collection supervisors (who conducted all quality control checks). Also worth mentioning is that the study sample size was calculated to provide power of 95% confidence interval.

Despite its strengths, the study had some limitations that should be borne in mind when construing its findings. For instance, internal validity of the present study may have been compromised due a temporary discontinuation of distribution of LNS to study participants which transpired to allow for an investigation on whether the supplements contained *Chronobacter sakazakii* or not. Secondly, our inability to directly observe and assess the consumption of LNS by study children at home may have potentially compromised internal validity of the study. Thirdly, there was also a relatively substantial amount of missing data. Another limitation was the subjectivity of the maternal or caregiver reports on child appetite. Although this method has been validated in Lima, Peru (Brown et al., 1995), objective assessment of quantities of food eaten by study children would

have yielded much more precise results. However, it is because the results of this study were robust to several sensitivity analyses that we believe the listed weaknesses did not bias our conclusion.

6.4. Future studies

The present study has shown that provision of SQ-LNS to IYC in resource-insecure settings yields modest improvements in their appetite as evidenced by a decrease in the prevalence of anorexia during the one-year-long intervention period. However, it is not known whether the modest improvements in child appetite gained during the one-year-long study intervention and follow-up period are long lasting or transient. As such, post-intervention follow-up on study subjects is encouraged in future studies on the effect of LNS on child appetite.

This study also suggests that the effect of SQ-LNS in improving child appetite gets significant as the children grow older. A question remains as to whether longer LNS supplementation durations than one year would continue to yield significant improvements in child appetite. Future studies may consider extending the intervention period.

The present study also leaves a gap in research as it does not demonstrate the actual pathways through which appetite is improved. For instance, it is not definitively established whether LNS first alleviates undernutrition and then improve child appetite or if they directly reduce appetite and then alleviate undernutrition as a follow on. Further studies are, thus, required to investigate and conduct pathway analyses in a possible causality relationship between appetite and undernutrition in children.

7. CONCLUSION

Provision of SQ-LNS to IYC in resource-insecure settings during early life yields modest improvements in appetite, as evidenced by a decrease in the prevalence of anorexia. This study also suggests that the effect of SQ-LNS in improving child appetite gets significant as the children grow older.

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Peter Harmony Phiri.

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