

JADEN BENDABENDA

# The Associations of Malaria and Linear Growth Faltering in Infants and Young Children in Malawi



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ACADEMIC DISSERTATION

To be presented, with the permission of  
the Faculty of Medicine and Health Technology  
of Tampere University,  
for public discussion in the Jarmo Visakorpi auditorium  
of the Arvo Building, Arvo Ylpön katu 34, Tampere,  
on 24 January 2020, at 12 o'clock.

ACADEMIC DISSERTATION

Tampere University, Faculty of Medicine and Health Technology  
Finland

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Cover design: Roihu Inc.

ISBN 978-952-03-1415-6 (print)

ISBN 978-952-03-1416-3 (pdf)

ISSN 2489-9860 (print)

ISSN 2490-0028 (pdf)

<http://urn.fi/URN:ISBN:978-952-03-1416-3>

PunaMusta Oy – Yliopistopaino  
Tampere 2020

# ABSTRACT

The geographic overlap between stunting (small length for age) and malaria suggests there is a relationship between the two conditions, but current evidence is inconclusive. Interventions aimed to prevent stunting include improving the quality of complementary foods through home fortification with products such as lipid-based nutrient supplements (LNS). However, there are concerns that LNS, which contains iron, can increase the risk of malaria in children. Hence the need to establish whether a causal relationship exists between stunting and malaria; and whether LNS provision to prevent stunting can increase the risk of malaria.

The present study was therefore designed to determine: whether linear growth faltering can predict the risk of malaria; whether malaria causes linear growth faltering and poor child development; and whether LNS provision does not increase the risk of malaria. These aims were addressed using data from children enrolled in the International Lipid-based Nutrient Supplements Project (iLiNS) trials (iLiNS-DOSE and iLiNS-DYAD-M), conducted in rural Malawi between 2009 and 2015.

A total of 2,725 six-month old children were enrolled and followed during weekly morbidity home visits, where a diagnosis of presumed malaria was made. Additional malaria data were collected at clinic visits. Anthropometry data were collected at 6 months' and 18 months' clinic visits where length-for-age z scores [LAZ] were obtained. Stunting was defined as LAZ < -2. Developmental outcomes (fine motor scores, gross motor scores, language scores and Profile of Social and Emotional Development [PSED] scores) were assessed at 18 months' clinic visit.

At 18 months, 2,561 (94%) children had complete morbidity data and reported 28,032 morbidity episodes, 9.7% of which were episodes of presumed malaria. The mean (SD) incidence of all illnesses combined was 16.2 (13.1) episodes per child year, of which 1.1 (1.6) were episodes of presumed malaria. On average, the children made 5.5 visits to the hospital due to illness, of which 1.7 were due to malaria. The prevalence of malaria confirmed by microscopy increased from 4.7%

to 9.6% between 6 months and 18 months. There was no association of LAZ at 6 months with subsequent 12-month incidence of presumed malaria (incidence rate ratio [IRR] = 1.03; 95% CI = 0.98 to 1.09;  $p=0.394$ ) or prevalence of malaria parasitaemia at 18 months (prevalence ratio = 1.11; 95% CI = 0.93 to 1.33;  $p=0.259$ ).

Between 6 months and 18 months, the mean (SD) change in LAZ was -0.44 (0.77) and the prevalence of stunting increased from 26.7% to 41.2%. At 18 months, the mean (SD) for fine motor, gross motor, language and PSED scores were 20.9 (2.2), 17.3 (2.6), 26.2 (5.0) and 16.2 (5.4) respectively. There was no statistically significant association of presumed malaria incidence with change in LAZ ( $\beta = -0.02$ , 95% CI = -0.04 to 0.01,  $p = 0.069$ ). Presumed malaria incidence was significantly associated with increased proportion of children with stunting at 18 months (risk ratio = 1.04, 95% CI = 1.01 to 1.07,  $p = 0.023$ ). The association of presumed malaria incidence with child development at 18 months was significant for PSED scores ( $\beta = -0.21$ ; 95% CI = 0.39 to -0.03;  $p = 0.041$ ), but not for the other domains of child development.

Regarding the safety of LNS, there were no significant differences in the incidence of presumed malaria, clinical malaria nor confirmed malaria across the different doses of LNS. Compared to the control group, the 95% CIs of the IRRs for presumed malaria, clinical malaria, and confirmed malaria were entirely below 1.20 (suggestive of non-inferiority) in all the intervention groups, except for the 40 g/d LNS where the incidence of confirmed malaria was 21% higher in this group than the control.

In conclusion, in a rural Malawian population, where both stunting and malaria are common, but with active surveillance and early treatment of infections, linear growth faltering at 6 months does not predict malaria incidence from 6 months to 18 months. In this population, malaria is not associated with change in LAZ but may increase the risk of stunting and reduce socio-emotional development. Finally, long term provision of iron-containing LNS at certain doses does not increase the risk of malaria in this population where iron deficiency and mosquito bed net utilization is high. These findings could be useful when designing growth promotion and malaria control interventions for infants and young children in malaria-endemic areas.

# TIIVISTELMÄ

Kasvun hidastumisen (ikäisekseen lyhyt) ja malarian maantieteellinen päällekkäisyys viittaa siihen, että ne olisivat yhteydessä toisiinsa, mutta tästä ei toistaiseksi ole selvää näyttöä. Kasvun hidastumista pyritään estämään mm. parantamalla kotona annettavaa täydennysravintoa. Tähän käytetään esimerkiksi rasvapohjaisia ravintolisiä (lipid-based nutrient supplements, LNS). On kuitenkin oltu huolissaan siitä, että rautaa sisältävät LNS-valmisteet voivat lisätä lasten malariariskiä. Siksi pitää selvittää, onko kasvun hidastumisen ja malarian välillä syy-yhteys ja voiko LNS-valmisteiden käyttö estämään kasvun hidastumista lisätä malarian riskiä.

Tämän tutkimuksen tavoitteena oli siksi selvittää, voiko pituuskasvun häiriö ennustaa malariariskiä, aiheuttaako malaria pituuskasvun häiriöitä, hidastaako se lapsen kehitystä, ja lisääkö LNS-valmisteiden käyttö malariariskiä. Näitä selvitettiin käyttäen kansainväliseen iLiNS-projektin (International Lipid-based Nutrient Supplements Project) tutkimuksiin (iLiNS-DOSE ja iLiNS-DYAD-M) Malawin maaseudulla vuosina 2009–2015 osallistuneiden lasten tietoja.

Tutkimuksiin otettiin kaikkiaan 2 725 kuuden kuukauden ikäistä lasta, joiden sairastuvuutta seurattiin viikoittain kotikäynneillä, joilla syntyi malariaepäily. Malariasta kerättiin lisätietoa klinikkakäynneillä. Antropometrisia tietoja kerättiin 6 kuukauden ja 18 kuukauden klinikkakäynneillä, joilla määritettiin LAZ-pistemäärä (length-for-age z), joka kuvaa pituutta ikään nähden. Kasvun hidastumisesta katsottiin olevan kyse, jos LAZ-pistemäärä oli  $< -2$ . Kehitystä (hieno- ja karkeamotoriikkaa, kielen kehitystä sekä sosiaalisen ja emotionaalisen kehityksen profiilia [Profile of Social and Emotional Development, PSED]) kuvaavat pistemäärät arvioitiin 18 kuukauden klinikkakäynnillä.

Täydelliset sairastuvuustiedot saatiin 18 kuukauden iässä 2 561 lapselta (94 %), ja heillä ilmoitettiin olleen 28 032 sairausjaksoa, joista 9,7 % oli oletettavasti

malariaa. Kaikkien sairauksien esiintyvyyden keskiarvo (SD) oli 16,2 (13,1) jaksoa lapsivuotta kohti, ja näistä 1,1 (1,6) oli oletettavasti malariaa. Lapset kävivät sairauden vuoksi keskimäärin 5,5 kertaa sairaalassa, ja näistä käynneistä 1,7 johtui malariasta. Mikroskoopilla varmistetun malarian esiintyvyys kasvoi 4,7 prosentista 6 kuukauden iässä 9,6 prosenttiin 18 kuukauden iässä. LAZ-pistemäärä 6 kuukauden iässä ei ollut yhteydessä oletetun malarian ilmaantuvuuteen 12 kuukauden iässä (ilmaantumistiheyksien suhde [IRR] = 1,03, 95 %:n luottamusväli = 0,98–1,09, p = 0,394) eikä malarialoisen esiintyvyyteen veressä 18 kuukauden iässä (vallitsevuussuhde [PR] = 1,11, 95 %:n luottamusväli = 0,93–1,33, p = 0,259).

Kuuden ja 18 kuukauden iän välillä keskimääräinen (SD) LAZ-pistemäärän muutos oli -0,44 (0,77) ja kasvun hidastumisen esiintyvyys lisääntyi 26,7 prosentista 41,2 prosenttiin. 18 kuukauden iässä pistemäärien keskiarvot (SD) olivat seuraavat: hienomotoriikka 20,9 (2,2), karkeamotoriikka 17,3 (2,6), kielen kehitys 26,2 (5,0) ja PSED 16,2 (5,4). Oletetun malarian ilmaantuvuuden ja LAZ-pistemäärän muutoksen välillä ei ollut tilastollisesti merkitsevää yhteyttä ( $\beta = -0,02$ , 95 %:n luottamusväli = -0,04–0,01, p = 0,069). Oletetun malarian ilmaantuvuus oli merkitsevästi yhteydessä niiden lasten lisääntyneeseen osuuteen, joiden kasvu oli hidastunut 18 kuukauden iässä (riskisuhde = 1,04, 95 prosentin luottamusväli = 1,01–1,07, p = 0,023). Yhteys oletetun malarian ilmaantuvuuden ja lapsen kehityksen välillä 18 kuukauden iässä oli merkitsevä PSED-pistemäärien osalta ( $\beta = -0,21$ , 95 prosentin luottamusväli = 0,39 – -0,03; p = 0,041) mutta ei muilla lapsen kehityksen osa-alueilla.

LNS-valmisteiden turvallisuudesta todettiin, että oletetun, kliinisen tai varmistetun malarian ilmaantuvuudessa ei ollut eroa erisuuruisia LNS-annoksia käyttäneillä. Verrokkiryhmään verrattuna niin oletetun, kliinisen kuin varmistetunkin malarian IRR-arvojen 95 prosentin luottamusvälit olivat alle 1,20 (mikä tarkoittaa vertailukelpoisuutta [non-inferiority]) kaikissa interventoryhmissä, paitsi 40 g/vrk LNS-valmistetta saaneessa ryhmässä, jossa varmistetun malarian ilmaantuvuus oli 21 % suurempi kuin verrokkiryhmässä.



Yhteenvetona voidaan sanoa, että malawilaisessa maalaisväestössä sekä kasvun hidastuminen että malaria ovat yleisiä, mutta jos lapsia seurataan aktiivisesti ja infektiot hoidetaan hyvissä ajoin, pituuskasvun häiriö 6 kuukauden iässä ei ennusta malarian ilmaantuvuutta 6–18 kuukauden iässä. Tässä populaatiossa malaria ei liity LAZ-arvon muutokseen mutta voi lisätä kasvun hidastumisen riskiä ja heikentää sosioemotionaalista kehitystä. Lopuksi rautaa sisältävien LNS-valmisteiden pitkäaikainen käyttö tietyn suuruisina annoksina ei lisää malarian riskiä tässä populaatiossa, jossa raudanpuute ja moskiittoverkon käyttö sängyn ympärillä ovat yleisiä. Näistä löydöksistä saattaa olla hyötyä suunniteltaessa vauvojen ja pikkulasten kasvun edistämiseen ja malarian hallintaan tähtääviä toimenpiteitä malarian endeemisillä alueilla.



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# ABBREVIATIONS

ACS	Active case survey
ARI	Acute respiratory infections
CI	Confidence interval
DALYs	Disability-adjusted life years
DSMB	Data and safety monitoring board
EPI	Expanded Program on Immunization
HSA	Health surveillance assistant
HAZ	Height-for-age z-score
Hb	Haemoglobin
HFIAS	Household Food Insecurity Access Scale
HRP2	Histidine-rich protein 2
IFA	Iron and folic acid
iLiNS	International Lipid-based Nutrient Supplements Project
IMCI	Integrated management of childhood illness
IRR	Incidence rate ratio
ITN	Insecticide-treated mosquito bed net
KDI	Kilifi Developmental Inventory
LAZ	Length-for-age z-score
LMICs	Low- and middle-income countries
LNS	Lipid-based nutrient supplements

LOWESS	Locally weighted regression smoothed curves
MDHS	Malawi demographic and health survey
MeSH	Medical Subject Headings
MMN	Multiple micronutrients
PCD	Passive case detection
PCR	Polymerase chain reaction
PR	Prevalence ratio
PSED	Profile of social and emotional development
RDT	Rapid diagnostic test
RR	Risk ratio
SAE	Serious adverse event
SD	Standard deviation
SDGs	Sustainable Development Goals
SSA	Sub-Saharan Africa
UNICEF	United Nations Children's Fund
WAZ	Weight-for-age z-score
WBC	White blood cells
WHO	World Health Organization
WLZ	Weight-for-length z-score
ZPP	Zinc protoporphyrin

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three original research articles, referred to within the text by the Roman numerals:

- I. **Bendabenda J**, Patson N, Hallamaa L, Ashorn U, Dewey KG, Ashorn P, et al. Does anthropometric status at 6 months predict the over-dispersion of malaria infections in children aged 6-18 months? A prospective cohort study. *Malar J*. 2019 Apr;18(1):143. <https://doi.org/10.1186/s12936-019-2778-y>
- II. **Bendabenda Jaden**, Noel Patson, Lotta Hallamaa, John Mbotwa, Charles Mangani, John Phuka, Elizabeth L Prado, et al. 2018. “The Association of Malaria Morbidity with Linear Growth, Hemoglobin, Iron Status, and Development in Young Malawian Children: A Prospective Cohort Study.” *BMC Pediatrics* 18 (1): 396. <https://doi.org/10.1186/s12887-018-1378-2>.
- III. **Bendabenda J**, Alho A, Ashorn U, Cheung YB, Dewey KG, Vosti SA, Phuka J, Maleta K, Ashorn P. The effect of providing Lipid-based Nutrient Supplements on morbidity in rural Malawian infants and young children: a randomized controlled trial. *Public Health Nutr*. 2016 Jul;19(10):1893-903. doi: 10.1017/S1368980016000331. Epub 2016 Mar 9. PMID: 2695661



# 1 INTRODUCTION

Good nutrition is essential for children's growth, hence undernutrition has detrimental effects on the growth and development of children (1). To underline the global importance, indicators of undernutrition, specifically stunting (small length for age) and wasting (low weight in relation to height) (2,3) are among the selected indicators for the 17 Sustainable Development Goals (SDGs) endorsed by the United Nations General Assembly in 2015 aimed at mobilizing resources to end global hunger and poverty, as well as improve education and health, among others (4). The global focus on undernutrition has helped to reduce stunting in children under 5 years of age from 32.6% in 2000 to 22.2% in 2017 (1).

Globally, 151 million children under 5 years of age were considered stunted (length-for-age z-scores [LAZ] <-2) in 2017. More than one third of these children lived in sub-Saharan Africa (SSA), where malaria burden is also very high: 90% of the 219 million cases and 435,000 deaths due to malaria occur in this region(1,5,6).

The interaction between undernutrition and infections in children in low- and middle-income countries (LMICs) can create a vicious cycle of repeated infections and worsening nutritional status (1). The geographic overlap of undernutrition and infections suggests that linear growth faltering may play an important role in malaria epidemiology (7–9). However, four reviews on the relationship between undernutrition and malaria were inconclusive; explained by the differences in the populations studied and the malaria parasite species, among others (10–13).

Nutrition interventions aimed to improve the quality of complementary foods only have modest impact on growth (14), possibly due to a high burden of infections in children (15,16). Studies in which morbidity treatment was integrated with a complementary feeding intervention demonstrated improved linear growth (17) and developmental outcomes in children (18,19), suggesting the importance of reducing the burden of infections along with improved diet to promote child growth and

development. There is a plethora of studies showing a significant inverse association of diarrhoea with growth (20–22), but few on malaria, which has limited conclusions on the effect of malaria on stunting (23).

One of the contributing factors to stunting in children in LMICs is believed to be consumption of low quality complementary foods (24). To improve the quality of complementary foods, home fortification with products such as small quantity lipid-based nutrient supplements (LNS) has been explored as one of the novel strategies (25). However, LNS fortified with iron may raise concerns of increased risk of malaria in children in malaria-endemic areas (26–28). Currently, iron supplementation is not recommended in children in malaria-endemic countries unless effective malaria control interventions are certain to be provided (28). This recommendation was based on evidence from studies that provided iron tablets or multiple micronutrients (29–31). Few studies have reported that food fortificants such as LNS may be safe (15,32–34), but this evidence is still deemed inconclusive (35,36). More evidence on the safety of LNS in malaria-endemic areas is still needed.

The present study was designed to determine whether linear growth faltering is associated with higher risk of malaria, whether malaria is associated with linear growth faltering, and whether LNS provision aimed to reduce linear growth faltering increases the risk of malaria.

## 2 REVIEW OF LITERATURE

### 2.1 Approach to the literature review

The purpose of this literature review is to provide the evidence on the associations between growth faltering and malaria, and the safety of LNS in malaria-endemic settings available at the time of designing this study. The review provides background of the two conditions such as: prevalence and trends of stunting and malaria that reveal the importance of the two conditions; methods of assessing the two conditions; possible role of malaria in growth faltering and the role of stunting in malaria epidemiology; as well as the safety of LNS for promotion of growth in malaria-endemic settings. Finally, the review provides a summary of the knowledge gaps on this topic, which forms the basis for the current study.

Published electronic references were obtained from PubMed, HINARI, and Google Scholar databases using Medical Subject Headings (MeSH). Some of the keywords used in the search were: malaria AND “linear growth faltering OR stunting” OR “nutritional status” AND children; “LNS” OR supplements AND “malaria” OR morbidity. Initial searches were conducted in 2014 and were constantly updated when the study aims were modified, with the latest review conducted in December 2018. Additional articles were obtained from the references of the extracted articles. Further information was collected from websites of international organizations (i.e. the WHO, UNICEF, World Bank, etc), governmental and non-governmental organizations.

## 2.2 Growth faltering

### 2.2.1 Definitions

Human growth and development is defined by the increase in somatic size, shape, and functional maturity associated with age (37,38). Growth faltering refers to the child's failure to achieve the growth and developmental milestones at the appropriate age. Linear growth faltering specifically refers to an abnormally slow rate of gain in a child's length or height determined by length-for-age z-score (LAZ) or height-for-age z-score (HAZ) (39). The degree of linear growth faltering in a population is expressed as the mean LAZ or the prevalence of stunting (i.e. percentage of children with LAZ < -2) (40).

### 2.2.2 Assessment and classification of linear growth in children

#### 2.2.2.1 Anthropometry

Anthropometry is the commonly used method of assessment of nutrition status of a child or a population (41). The commonly used measures of anthropometric status are weight and length (or height<sup>1</sup>) in combination with age and sex. These measurements are used to calculate indices and indicators<sup>2</sup> of nutritional status of a child or a population (42).

---

<sup>1</sup> Length or height is measured based on the child's age and whether the child is sitting or lying down during the assessment. If the child is lying down (recumbent), length is measured; if the child is standing, height is measured. Generally, length is measured in children less than 2 years of age, while if the child is aged 2 years or older and able to stand, height is measured. Standing height is believed to be 0.7 cm less than recumbent length (144). For this thesis, all children were aged less than 2 years, hence length was used throughout.

<sup>2</sup> Indices are defined as combinations of measurements, useful for interpretation of the measurements e.g. weight for length; indicators involve the use or application of indices e.g. proportion of children below a certain cut-off in the particular index which can be used to describe nutrition status of a community (42).



#### 2.2.2.2 Anthropometric indices: length-for-age

Anthropometric variables (length and weight) used in combination with sex and age provide useful information about a child's nutritional status, referred to as an index (42). The widely used indices are length-for-age, weight-for-age, and weight-for-length. Length-for-age provides information on linear growth of a child.

#### 2.2.2.3 Evaluation of anthropometric indices using z-scores: length-for-age z-scores

The anthropometric indices can be calculated either as z-scores, percentiles, or percentage of median, to enable comparison of an individual with a reference population (43). Z-scores are particularly useful to avoid spurious interpretations of correlations in length and weight (44).

The means of the z-scores for length-for-age, weight-for-length, and weight-for-age are used as summary statistics when describing the nutritional status of children in a population, often compared to the WHO growth standards (43). WHO growth standards were developed to demonstrate the full genetic growth potential of healthy, breast-fed children living in optimal environments. To allow for global applicability, the data were analysed from six study sites (Brazil, Ghana, India, Norway, Oman and the USA) across five continents, using rigorous statistical methods (41).

#### 2.2.2.4 Nutritional conditions identified using anthropometric indices: stunting

Three indices: length-for-age; weight-for-age; and weight-for-length are used to identify three nutritional conditions: stunting, underweight, and wasting, respectively. Children with LAZ <-2 standard deviations (SD) from the median of the WHO growth standards are classified as moderately stunted, whereas children with LAZ <-3 SD from the median of the WHO growth standards are classified as severely stunted (41). However, negative mean LAZ of a population demonstrates a downward shift of the entire distribution, suggesting that most children in the population, not only those who are categorized as stunted, are affected (45).

### 2.2.3 Global prevalence and trends of stunting in children

Globally, 151 million children under 5 years of age were considered stunted (LAZ <-2) in 2017, and more than 30% of them lived in Africa (1). However, the number of children experiencing suboptimal growth in LMICs is believed to be significantly larger since the mean LAZ in these populations are usually below zero (45). In addition, some children present with more than one form of malnutrition (e.g. stunting and wasting) but the estimates for these combined conditions are currently unavailable (1).

Although globally there has been a reduction in stunting in children under 5 years of age from 32.6% (198 million children) in 2000 to 22.2% (151 million children) in 2017, there exist large disparities within/between regions/sub-regions. For example, Asia managed to reduce stunting from 38.2% to 23.3% while Africa only reduced stunting from 38.0% to 30.3% during the same time period. The African region is evidently lagging behind in efforts to reach the 2025 targets set by the World Health Assembly and the SDGs to reduce stunting by 40% (4,46).

### 2.2.4 Timing of linear growth faltering

In LMICs, growth faltering typically starts in utero, and continues to two years of age, hence the first 1000 days are known as the optimal “window of opportunity” for growth promoting interventions (40,47,48). Within the 1000 days, the period from 6 months to 24 months is very critical because rapid development of the brain and body is expected to occur while at the same time, the children are introduced to complementary foods which, in LMICs, are usually contaminated and poor in both quality and quantity. The children also begin to crawl and are exposed to various pathogens at the time they are losing immunity from maternal antibodies while their own immunity is nascent (5,49).

### 2.2.5 Linear growth faltering in the context of Malawi

In the past two decades, Malawi has made strides in reducing the rate of stunting in children under 5 years of age by nearly 20 percentage points (50), with the greatest decrease reported between 2010 (47%) and 2016 (37%) (51). However, the prevailing rates of stunting are still very high based on the WHO-UNICEF

threshold of 30% (53, Figure 1). In the same population, the prevalence of wasting is only 3%, which is within the global low threshold of less than 5% (51,52). Malawi therefore has a population of children who are generally very short but not thin.

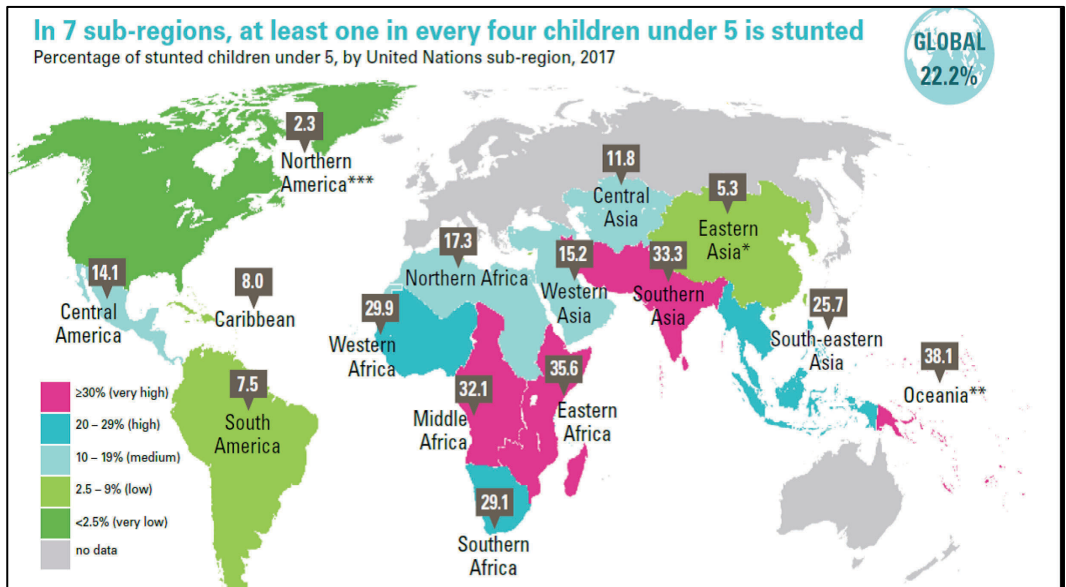
Similar to global estimates, most of the linear growth faltering in Malawian children occurs between 6 months and 24 months which coincides with high incidence of infectious diseases (53).

## 2.2.6 Child development

Childhood development is the acquisition of a range of skills including cognitive, language, and socio-emotional as part of the normal maturation process (37). Child development is assessed using standardized tools that measure achievement of milestones through testing and evaluating the child's ability to perform a series of tasks (54). These assessments serve either as screening tests or tests to evaluate the child's abilities (55). Aggregation of individual assessments creates population-based estimates which can be compared to the WHO Child Growth Standards (56).

In the first 1000 days, impairments in brain development can potentially cause permanent damage to growth, hence the optimal "window of opportunity" for improving child development (48). In LMICs, 249 million (43%) of the children under 5 years of age are believed to be at risk of not reaching their developmental potential, with children from Sub-Saharan Africa being the most affected (57). The full extent of the problem is difficult to estimate because of paucity of reliable measurement standards for child development, as well as context-specific data for children in LMICs (57).

**Figure 1.** Percentage of stunted children under 5, by United Nations sub-region, 2017



Source: UNICEF / WHO / World Bank Group Joint Child Malnutrition Estimates (1).

## 2.3 Malaria

### 2.3.1 Definition

Malaria is an infectious disease caused by protozoan parasite *Plasmodium*. Malaria in humans is mainly caused by four different species of *Plasmodium*: *P. falciparum*; *P. malariae*; *P. ovale*; and *P. vivax*, with occasional infections by *P. knowlesi* (58).

### 2.3.2 Assessment of malaria

Traditionally, malaria was diagnosed and treated symptomatically using presence of fever because microscopy tests were only available in hospitals with laboratory facilities. From 2010, the WHO recommended malaria confirmation by rapid diagnostic test (RDT) or microscopy test before administering antimalarials (59).

Malaria RDT uses lateral flow immune-chromatography to detect histidine-rich protein 2 (HRP2) for *Plasmodium* species. Malaria RDT has increased access to confirmatory tests in underserved populations because of its simplicity to use. Inability of some RDT kits to distinguish new infections from recently treated infections and poor sensitivity to detect other *Plasmodium* species are some of its limitations (59).

Malaria microscopy uses Giemsa staining and light microscopy to identify the *Plasmodium* presence, species and density. With a well-trained reader, light microscopy provides accurate parasite counting, distinguishes new infections and species of malaria parasites. However, the skill-set and equipment required for microscopy is often not available in LMICs where malaria is endemic. Worse still, in these countries, most malaria patients are treated at home or within the community rendering microscopy less useful (59).

Definitive diagnostic tests for malaria exist based on nucleic-acid amplification techniques (e.g. loop-mediated isothermal amplification or polymerase chain reaction (PCR), but these hitherto exist for research purposes (59).

### 2.3.3 Global prevalence and trends of malaria in children

Malaria is one of the most serious public health problems in the world, with 3.4 billion people in 92 countries estimated to be at risk of being infected with malaria and developing disease (60, Figure 2). An estimated 219 million cases and 435,000 deaths reported in 2017, 90% of which occurred in the African Region. Of great concern is that between 2010 and 2017, there has been no significant reduction in global malaria incidence, with some countries in Africa reporting an increase in incidence (61).

Globally, malaria contributes to 1.8% of the disability-adjusted life years (DALYs) while in sub-Saharan Africa, malaria is responsible for 11.5% of the DALYs in children under 5 years of age. With more than 70% of the malaria deaths occurring in this age group, malaria is the fourth leading cause of death among children in this region. Simply put, malaria is believed to kill a child every two minutes in Africa (6,62).

### 2.3.4 Timing of malaria infections

Children aged < 6 months may experience few or no malaria episodes due to protection by maternal antibodies, circulating foetal haemoglobin, and minimal exposure to mosquito bites (63–66). With age, the risk for malaria changes in severity and mortality, peaking at 6–24 months (61), which coincides with the peak of linear growth faltering. Thereafter, the risk for severe malaria and death declines as humoral immunity improves following repeated exposures to parasites. In malaria-endemic areas, this immunity protects against symptomatic malaria but not against infection (67), hence older children (5–15 years) have highest malaria parasitaemia while children under 5 years of age suffer more severe disease (11,68,69).

### 2.3.5 Malaria in the context of Malawi

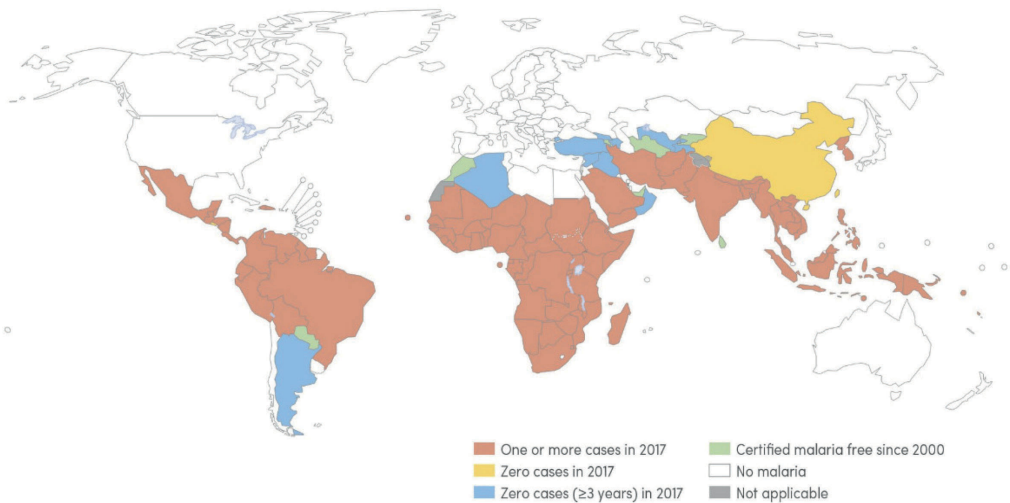
Malawi is one of the malaria-endemic countries with around 6.5 million malaria cases reported in 2016. Of these, 2.7 million cases (41.5%) occurred in children under 5 years of age, with an incidence rate of 976 new cases per 1000 children and prevalence of malaria (confirmed by microscopy) of 24.3% (70). Malaria is

responsible for 40% of the deaths in Malawian children in this age group (71). *Plasmodium falciparum* is the most dominant species and causes about 98% of all malaria infections with highest transmission rates occurring between November and April (rainy season), especially in low-lying and high temperature areas (70,71).

In 2013, Malawi rolled out the testing of all malaria cases using RDTs. However, by 2016, malaria tests were done on 76.9% of all suspected cases, and antimalarial drugs were administered to 86.1% of all suspected malaria cases, indicating the persistent challenges in diagnosing and treating malaria, especially by the health surveillance assistants (HSAs) when implementing the integrated management of childhood illness (IMCI) protocols (70).

**Figure 2.** Global distribution of malaria

**Countries with indigenous cases in 2000 and their status by 2017** Countries with zero indigenous cases over at least the past 3 consecutive years are considered to be malaria free. All countries in the WHO European Region reported zero indigenous cases in 2016 and again in 2017. In 2017, both China and El Salvador reported zero indigenous cases. *Source: WHO database.*



Source: World Malaria Report 2018 (60).



## 2.4 The role of linear growth faltering in malaria epidemiology

More than one third of the stunted children live in Africa, where the burden of parasitic infections such as malaria is also very high: 90% of the infections and deaths due to malaria occur in Africa (1,5,61). In malaria-endemic countries, a small proportion of children suffer repeated malaria infections, and are responsible for most of the malaria cases (72–74). The risk factors for the repeated malaria infections in these children are not fully understood, believed to be genetic (73), behavioural (75), and environmental (74). Other authors believe linear growth faltering could be a risk factor for malaria because of the geographic overlap of stunting and malaria and the synergistic interactions between nutrition and infections (7–9).

Undernutrition in children below 2 years of age is associated with reduction in leptin hormone and reduced antibody-mediated antimalarial immunity (76–79). The vicious cycle of undernutrition and infection further weakens the immune response, making children susceptible to repeated infections (80,81). In addition, a capacity load model developed to demonstrate the impact of early undernutrition on later health suggests that undernutrition in children leads to depletion of the metabolic capacity, predisposing to further infections (82).

These mechanisms suggest that theoretically, stunting may play a role in increasing the risk of malaria infections, which has also been reported in several studies. In the Gambia, a short (20 weeks follow-up) study of 487 children under 5 years of age reported an increased risk of malaria associated with stunting (crude RR = 1.35; 95% CI 1.08–1.69) (7). In Kenya, a cross-sectional study of 1,862 children under 3 years of age reported that undernourished children had more malaria episodes (8), while in Uganda, a one-year cohort study involving 351 children under 2.5 years of age found that stunting was associated with increased incidence of malaria parasitaemia (9).

However, other studies of similar designs have reported no association between stunting and malaria (83–86) and one study reported a protective effect of stunting on malaria in 136 children aged 10–120 months in Papua New Guinea – though the biological mechanisms for this finding were not fully explained (87).

Hence, several reviews on this topic have determined that the role of stunting in malaria epidemiology is still inconclusive; attributed to the heterogeneity in the study populations, malaria parasite species, and host-parasite relationship which warrant further studies (10–12).

## 2.5 The role of malaria in growth faltering

High burden of infections is one of the nutrition-specific indicators associated with stunting (88). Just like stunting may predispose children to infections through immune dysfunction, infections may predispose children to growth faltering through the same pathway (Figure 1). Repeated infections result in sustained high levels of cytokines, increasing the metabolic demand and suppressing appetite through satiety hormones: ghrelin and leptin (89). High concentration of circulating cytokines may also suppress the release of insulin-like growth factors (IGF) (78,90). Immune system activation may also affect the concentration of nutrients (e.g. iron, vitamin A and zinc), restricting the availability of these nutrients for growth (91).

All common childhood infections probably contribute to growth faltering as the body's adaptive mechanism to prioritize survival at the expense of growth (91). However, the role of malaria in growth faltering is still controversial. While some studies reported an increased risk of stunting associated with malaria, others have reported no association, limiting the drawing of conclusions on the effect of malaria on stunting. For instance, in a short (3 months follow-up) study of 165 Zambian children aged <2 years, presence of infections including *falciparum* malaria was associated with short-term linear growth faltering (92). In Peru, a 3.5 years' follow-up study reported that in 442 children aged <6 years *vivax* malaria had a larger effect on growth compared to diarrhoea (93). In Ghana, a study aimed to estimate the causal effect between malaria and stunting, by combining methods of Mendelian randomization and matching on 884 children aged <2 years, reported an increased risk of stunting by 32% associated with malaria (94). However, a 2 years' follow-up study of 340 children under 5 years of age in Kenya did not find any association (83). In rural Malawi, treatment of malaria and other infections with monthly sulfadoxine-pyrimethamine and 2 doses of azithromycin (AZI-SP) during pregnancy reduced the incidence and prevalence of childhood stunting, while monthly treatment with sulfadoxine-pyrimethamine alone did not show similar effect (95). Hence a systematic review on this topic reported that the evidence of

malaria as a determinant of stunting is inconclusive owing to the paucity and heterogeneity of the available literature, as well as the ethical requirement to immediately treat all children diagnosed with malaria (23).

## 2.6 The role of nutrition supplementation in malaria epidemiology

Consumption of complementary foods that are dominated by staple foods poor in nutrient density and micronutrient bioavailability is considered one of the contributing factors for high rates of stunting in children in LMIC (24). To improve the quality of complementary foods, home fortification with products such as LNS has been explored as one of the novel strategies (25).

However, LNS is fortified with iron, and like with other iron-containing supplements, there are concerns of the potential increase in the risk of malaria in children in malaria-endemic areas following findings from a few studies. In a randomised, placebo-controlled trial in Zanzibar, iron supplementation to 24,076 children under 3 years of age increased the risk of malaria infection and deaths in children who were iron-replete (31). In Pakistan, a randomised trial involving 256 urban and rural clusters, provision of iron-containing (MMN) powders daily to 2,746 children between 6 and 18 months of age was associated with increased risk of diarrhoea and reported chest in-drawing in children (29). Excess iron is believed to promote proliferation of bacteria and *Plasmodium* parasites (35,96,97).

Consequently, oral iron supplementation is not recommended in children in malaria-endemic countries unless effective malaria control interventions are certain to be provided (28). However, this evidence is contentious because while the studies cited above have reported harmful effects, some studies suggest that iron-containing home fortificants (e.g. multiple micronutrient powders) are safe (98,99) and may even reduce morbidity (100).

Reports from the few studies on LNS provision suggest that it is safe (15,32–34), but the evidence is not conclusive because those studies had either a relatively short duration i.e. 6 months or less, or did not have sufficient power because of small sample sizes (35,36).

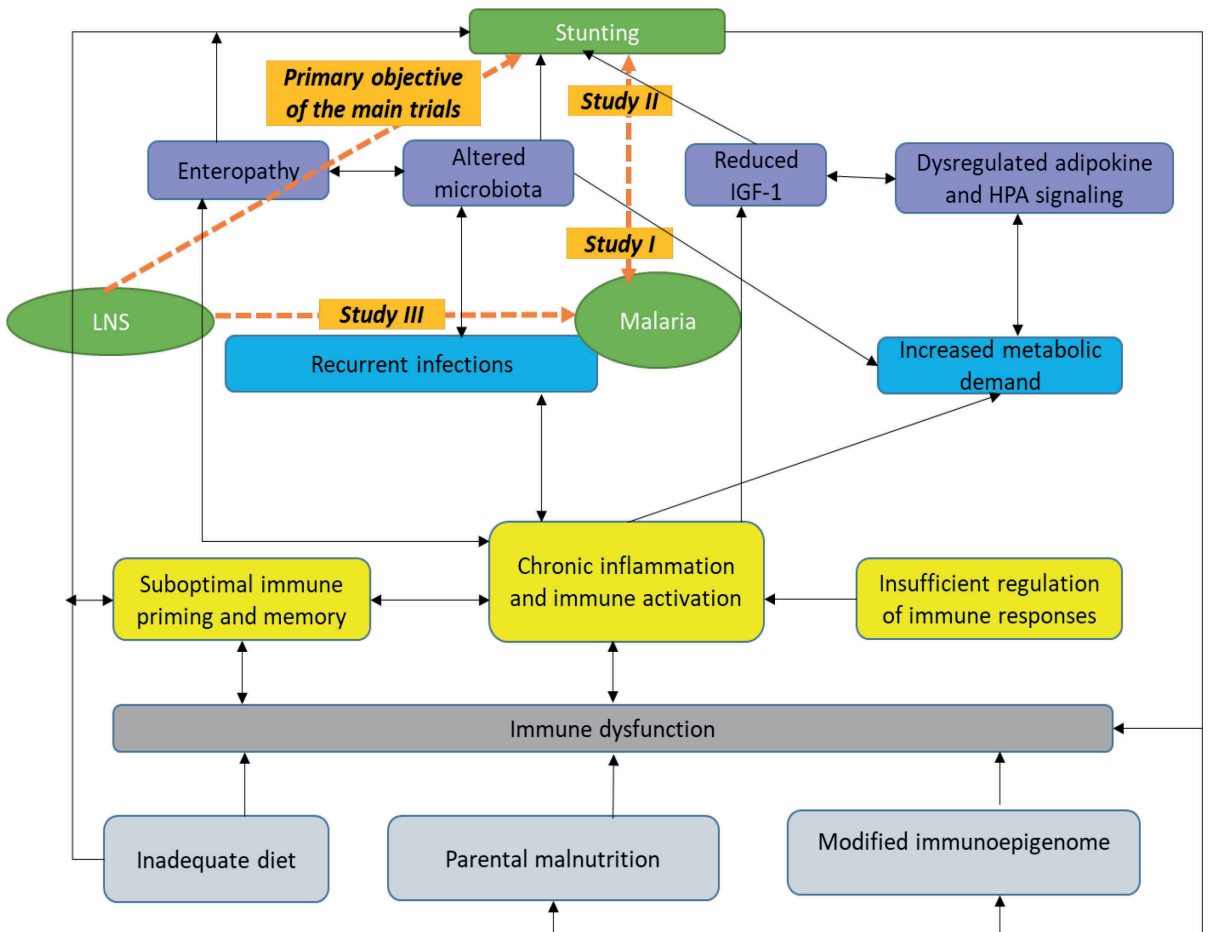
## 2.7 Justification of the present study

The literature review revealed that high incidence of malaria and high prevalence of linear growth faltering overlap geographically, affecting the same age-groups (children aged 6–24 months). A small number of children are responsible for most of the malaria infections, but the predisposing factors are not fully understood, with some authors believing linear growth faltering plays a role. The geographic overlap between stunting and malaria suggests malaria may be associated with linear growth faltering. Home fortificants such as LNS, aimed to promote growth, may also increase risk of malaria in children in malaria-endemic countries.

Therefore, it is possible that children who have faltered linear growth also suffer from repeated malaria infections, but the evidence is inconclusive. It is also possible that malaria may be associated with linear growth faltering rendering nutrition interventions ineffective, but the evidence is scant. Finally, it is likely that LNS may not increase the risk of malaria, but the evidence of safety is limited.

With this background, we formulated the concept of the present study, which was later modified and adapted from the review of the possible links between stunting and malaria through the immune dysfunction pathway (78) (Figure 3)

**Figure 3.** Conceptual diagram of the potential associations between nutrition supplementation, malaria and linear growth faltering



*Conceptual diagram showing the possible links between stunting and malaria through the immune dysfunction pathway, and the potential role of nutritional supplements in malaria-endemic settings. Adapted from Bourke et al, 2016 (78). Cells highlighted in BLUE represent the stages in the original pathway that were the focus of this study. Cells in GREEN represent the specific variables for our study, and their theoretical relationship connected by dotted arrows which generated the three study aims.*

*Abbreviations: HPA, hypothalamus–pituitary–adrenal axis; IGF-1, insulin-like growth factor 1; LNS, lipid-based nutrient supplements*

### 3 AIMS

The aim of this study was to determine the associations between malaria infection and linear growth faltering among infants and young children. The safety of iron-containing LNS in a setting where malaria is endemic was also determined. The specific objectives were:

1. To test the hypothesis that linear growth faltering predicts malaria incidence and prevalence in infants and young children (Study I).
2. To test the hypothesis that malaria incidence is positively associated with linear growth faltering and development in infants and young children (Study II).
3. To determine whether long-term provision of iron-containing LNS increases the risk of malaria in infants and young children living in a malaria-endemic setting (Study III).

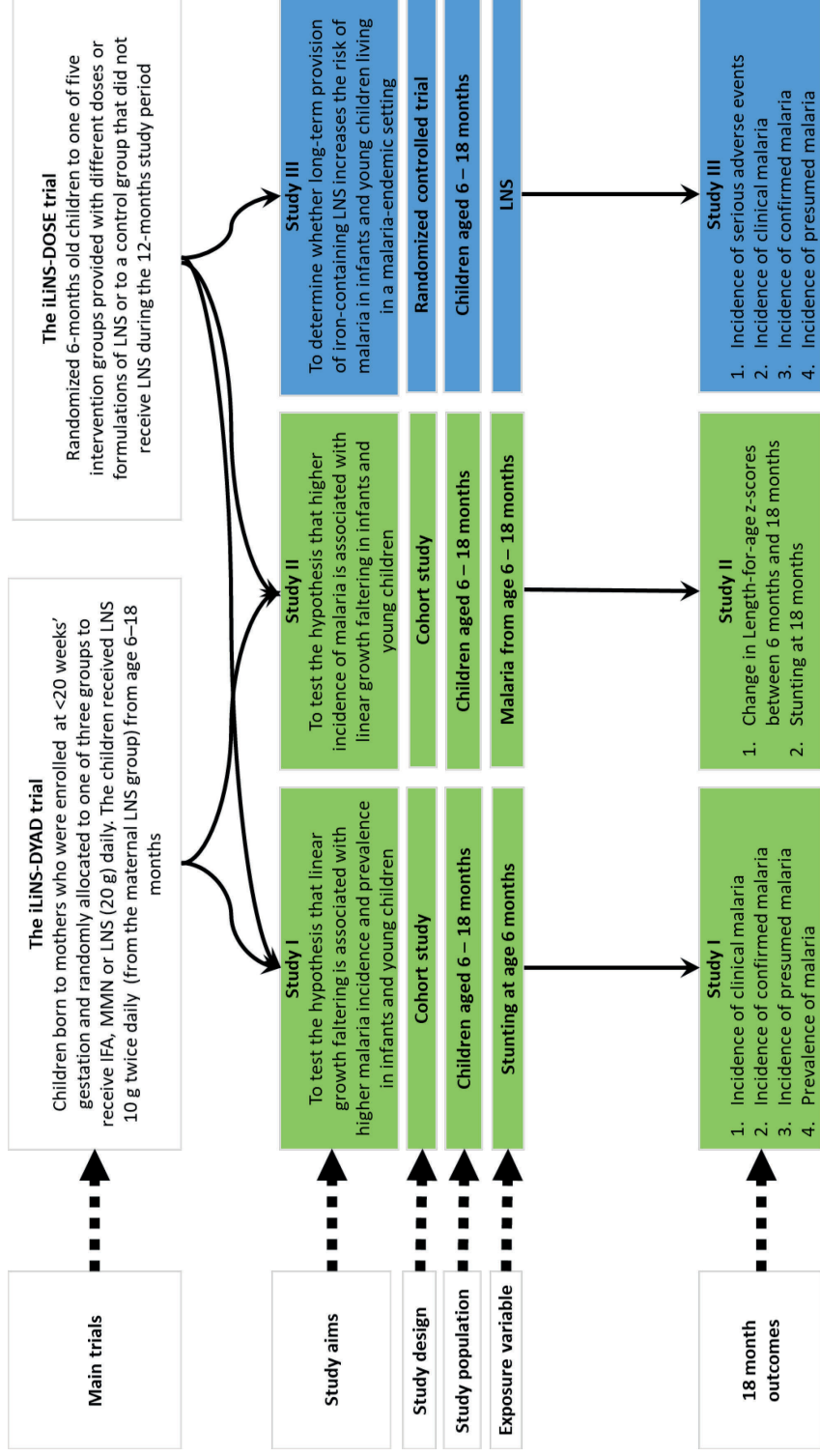
## 4 METHODS

### 4.1 Approach to the study

The three study aims were addressed using data from the International Lipid-based Nutrient Supplements Project (iLiNS) which implemented the iLiNS-DOSE and iLiNS-DYAD trials: two large community-based randomized nutrition intervention trials in Malawi. Details of the design, randomization and enrolment for the two trials are presented in the main outcome papers (101,102).

The overall study design for this thesis is summarized in Figure 4. For study aims I and II, the data were pooled from the two trials while for study aim III, the data was drawn from the iLiNS-DOSE trial only.

**Figure 4.** Overall design of the study



*Illustration of the design of the studies which used data from the two main trials: iLiNS-DOSE and iLiNS-DYAD-M. The green boxes represent studies that used data from the both trials (Studies I and II), while the blue boxes represent study III that used data from iLiNS-DOSE only. Abbreviations: IFA, iron and folic acid; LNS, lipid-based nutrient supplements; MMN, multiple micronutrients.*



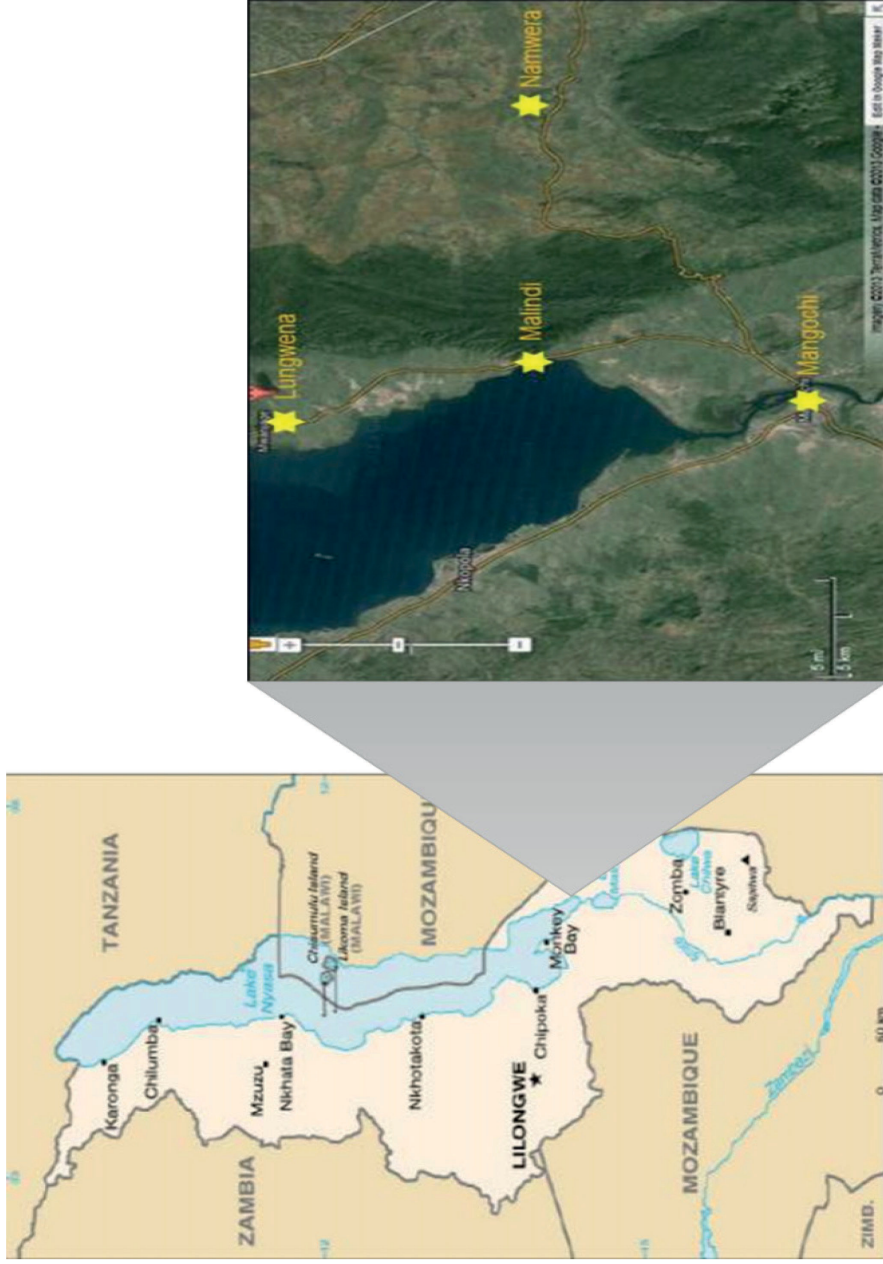
## 4.2 Study setting and participants

### 4.2.1 Study area

The study was conducted in Mangochi district, one of the 29 districts in Malawi, located in the country's south-eastern region. Malawi is one of the LMICs in the SSA, neighbours with Tanzania in the north, Zambia in the west, and Mozambique in the south and east (Figure 5).

Mangochi district was selected as study site because of its poor demographic indicators (Table 1) and convenience. The College of Medicine established a research site in Mangochi district which has helped establish and maintain close relationship with the communities within the catchment areas, facilitating easy implementation of research studies. Before this study, the lead researchers have had ongoing child health-related research projects in the district for almost 15 years.

Figure 5. Map of the study area



At the time of conducting the study (2009 – 2014), Malawi had an estimated population of 13.9 million people, 85% of which lived in rural community and subsisted on small scale farming (103,104). With a per capita gross domestic product (GDP) of 479 United States Dollars (USD), Malawi is one of the low-income countries and majority of the people live below the poverty line (<USD 1.90 per day) (105). The tropical climate comprising a warm, wet season (November to April), a cool, dry cold season (May to August), and a hot, dry season (September to October) favours the proliferation of *Anopheles* mosquitoes which transmit *Plasmodium* parasites (106).

The Malawi demographic and health survey (MDHS) results at the time of conducting this study showed that Mangochi district had one of the worst health indicators for children under 5 years of age in Malawi: 88.5% were reported to have problems accessing health care; 76.4% received vaccinations by 24 months; and 34.2% were reported to have slept under insecticide-treated mosquito bed net (ITN). The prevalence of fever, diarrhoea and acute respiratory infections (ARI) was 26.0%, 11.1% and 5.9%, respectively. The prevalence of stunting, underweight, and wasting was 48.3%, 15.9%, and 5.9% respectively (104).

Within Mangochi district, the study was conducted in four health facilities: Namwera, Malindi, Lungwena and Mangochi, with a total catchment population of 180,000 that mainly subsisted on farming and fishing. Mangochi site was more urban than the other three sites. Two of the study sites (Lungwena and Malindi) are low-lying along the eastern shore of Lake Malawi at an altitude of ~485 metres above sea level. Mangochi site is also low-lying at a similar altitude (~485 metres) but traversed by the Shire River (the largest river in Malawi). In contrast, Namwera lies at the top of Namwera Hills, bordering Mozambique, at an altitude of ~900 metres above sea level and is far from the large water bodies. Namwera experienced higher rainfall and cooler temperatures than the other three study sites (107,108).

The Malawi health care system is largely managed by the public sector, free of charge and divided into three levels of health care delivery: primary, secondary and tertiary. The primary level care (village clinics and health centres) provides preventive and outpatient services through health surveillance assistants, nurses and medical assistants. The secondary level care provides preventive, curative outpatient and inpatient care led by medical officers. Tertiary level care provides

specialized inpatient services. A few semi-private health facilities also exist, owned by religious organizations providing primary and secondary care.

At the primary health care level, the current IMCI strategy is implemented, aimed to provide simplified assessment and treatment for sick children using a syndromic approach (109). At both primary and secondary health care levels, growth monitoring and promotion clinics exist, aimed to provide health and nutrition surveillance and treatment, and provide linkage to existing nutrition programmes (110). During the study period, Lungwena and Namwera provided services at primary health care level while Malindi and Mangochi provided services at secondary health care level.

Table 1 shows the key health, demographic, geographic and socio-economic indicators for Malawi, compared, where applicable, with Mangochi district where the study was conducted.

Table 1. Basic demographic, socio-economic, geographic and health indicators for Malawi and Mangochi

Indicator	Measure <sup>1</sup>	
	Mangochi	Malawi
Total area (km <sup>2</sup> )	6,273	118,484
Total population (2010 estimate)	855,663	13,947,592
Population density (per km <sup>2</sup> )	136	117
Total fertility rate <sup>2</sup>	7.0	5.7
Per capita gross domestic product (USD)	n.a.	458
Proportion reporting problems in accessing health care (%)	88.5%	82.1%
Women literacy rate <sup>3</sup> (%)	51.5%	67.6%
HIV prevalence (15-49 years, %)	n.a.	10.6%
Life expectancy (years)	50.2 – 53.7	51.0 – 54.0
Maternal mortality ratio (per 100,000 live births)	n.a.	675
Infant mortality rate (per 1,000 live births)	n.a.	66
Under-five mortality rate (per 1,000 live births)	n.a.	112
Proportion children who received basic vaccinations by 24 months (%)	76.4%	80.9%
Proportion children who slept under ITN (%)	34.2%	35.2%
Proportion children < 5 years stunted (%)	48.3%	47.1%
Proportion children < 5 years underweight (%)	15.9%	12.8%
Proportion children < 5 years wasted (%)	5.9%	4.0%
Proportion infants < 6 months exclusively breastfed (%)	n.a.	71.4%
Prevalence of fever <sup>4</sup> in children < 5 years (%)	26.0%	34.5%
Prevalence of diarrhoea in children < 5 years (%)	11.1%	17.5%
Prevalence of ARI symptoms <sup>5</sup> in children < 5 years (%)	5.9%	6.8%
Annual average rainfall (2009 – 2015, mm)	725 – 1,133	685 – 2,500
Annual average temperatures (2009 – 2015, min-max degrees Celsius)	19.1 - 31	4 - 37

ARI, acute respiratory infection; HIV, human immune deficiency virus; ITN, insecticide treated mosquito bed net

<sup>1</sup>Values are n, proportions, or ranges

<sup>2</sup>Average number of children per woman

<sup>3</sup>Women aged 15–49 who completed secondary school (or those who didn't but could read all or part of a sentence when asked)

<sup>4</sup>Proxy for malaria

<sup>5</sup>Symptoms of ARI (cough accompanied by short, rapid breathing) are considered a proxy for pneumonia

n.a. = data not available

Sources: National Statistical Office (NSO) and ICF Macro, 2011; National Statistical Office (NSO), 2008; Malawi Meteorological Department ([www.metmalawi.com](http://www.metmalawi.com)).

## 4.2.2 Study participants

The study participants were children enrolled in the iLiNS-DOSE and iLiNS-DYAD-M trials.

In the iLiNS-DOSE trial, we identified potential participants in the catchment areas through community surveys and invited them to the study clinic for further eligibility assessment. Children aged 5.50 to 6.49 months, whose guardians signed informed consent and planned to be available during the entire study follow-up were considered eligible. Exclusion criteria included any severe illness warranting hospital referral, weight-for-length z-score  $< -2.0$ , bipedal oedema, haemoglobin  $< 50\text{g/L}$ , history of peanut allergy or concurrent participation in another clinical trial.

In the iLiNS-DYAD-M trial, the study sample comprised children born to mothers who were enrolled in the maternal cohort with the following characteristics at enrolment: pregnancy of  $< 20$  completed gestation weeks confirmed by ultrasound scan, permanent resident in catchment area, and provided informed consent. Exclusion criteria were age  $< 15$  years, chronic health condition requiring regular medical attention, severe illness that warranted hospital referral, history of allergy to peanuts, history of anaphylaxis, pregnancy complications, earlier participation in the iLiNS-DYAD-M trial (during a previous pregnancy), or concurrent participation in other clinical trials.

The iLiNS-DOSE trial recruited participants from two facilities (Mangochi and Namwera). The iLiNS-DYAD-M trial recruited participants from four health facilities (Namwera, Malindi, Lungwena and Mangochi), but children from Namwera were not followed up intensively, i.e. they belonged to a “simplified follow-up” group, with no morbidity visits after birth, but were examined at 6 and 18 months of age, to assess their growth.

## 4.2.3 Nutrition supplements

In the iLiNS-DOSE trial, children were randomly assigned to six groups to receive a daily dose of either 10 g, 20 g or 40 g milk-containing LNS or 20 g or 40 g milk-free LNS and a control group which did not receive LNS during the one-year

follow-up. These LNS doses were designed to provide about 55–241 kcal per day and meet the recommended daily allowance of 22 micronutrients, with all the LNS doses containing similar micronutrient concentrations. The milk-free formulations had lower protein content compared to the milk-containing LNS. The nutrient contents of the five different doses of LNS are summarized in Table 2.

In the iLiNS-DYAD-M trial, pregnant mothers were randomly assigned to receive either iron and folic acid (IFA), multiple micronutrients (MMN) or 20 g of LNS daily. After delivery, women in the IFA group received placebo tablets, while MMN and LNS supplementation was continued up to 6 months postpartum. Children of mothers in the LNS group also received LNS 10 g twice daily from 6 months to 18 months. Both LNS products contained the same micronutrients as the MMN capsules plus 4 additional minerals, protein, and fat, and their daily dose also provided 118 kcal of energy. The nutrient compositions of the supplements are summarized in Table 3.

In both studies, the LNS was produced and packed by Nutriset S.A.S, Malaunay, France. The ingredients were soybean oil, dry skimmed milk powder, peanut paste, premade micronutrient mixture, and sugar. Maltodextrine was used as substitute for milk in the milk-free LNS. The data collectors delivered supplements fortnightly to each participant's home from enrolment until the children were aged 18 months. At each visit, the data collectors counted and collected any unused supplement sachets from the participants.

All children received the routine growth monitoring and promotion services, including vaccinations according to the national Expanded Program on Immunization (EPI) schedule at the time. Following the Malawi public health system, free medical treatment was available to all children at the health facilities in the study areas.

Table 2. Nutrient and energy contents of the food supplements provided in the iLiNS-DOSE trial

<b>Nutrient/Intervention</b>	<b>10g milk LNS</b>	<b>20g milk LNS</b>	<b>20g no-milk LNS</b>	<b>40g milk LNS</b>	<b>40g no-milk LNS</b>
Daily ration (g)	10	20	20	40	40
Total energy (kcal)	55	117	117	241	241
Protein (g)	1.3	2.5	1.0	5.0	2.0
Fat (g)	4.7	9.5	9.4	18.9	18.8
Linoleic acid (g)	2.22	4.44	4.44	8.88	8.88
$\alpha$ -Linolenic acid (g)	0.29	0.58	0.58	1.16	1.16
Vitamin A ( $\mu$ g RE)	400	400	400	400	400
Vitamin C (mg)	30	30	30	30	30
Vitamin B1(mg)	0.3	0.3	0.3	0.3	0.3
Vitamin B2 (mg)	0.4	0.4	0.4	0.4	0.4
Niacin (mg)	4	4	4	4	4
Folic acid ( $\mu$ g)	80	80	80	80	80
Pantothenic acid (mg)	1.8	1.8	1.8	1.8	1.8
Vitamin B6 (mg)	0.3	0.3	0.3	0.3	0.3
Vitamin B12 ( $\mu$ g)	0.5	0.5	0.5	0.5	0.5
Vitamin D (IU)	200	200	200	200	200
Vitamin E (mg)	6	6	6	6	6
Vitamin K ( $\mu$ g)	30	30	30	30	30
Iron <sup>1</sup> (mg)	6	6	6	6	6
Zinc (mg)	8	8	8	8	8
Cu (mg)	0.34	0.34	0.34	0.34	0.34
Calcium (mg)	240	240	240	240	240
Phosphorus (mg)	208	208	208	208	208
Potassium (mg)	265	265	265	265	265
Magnesium (mg)	50	50	50	50	50
Selenium ( $\mu$ g)	20	20	20	20	20
Iodine ( $\mu$ g)	90	90	90	90	90
Manganese (mg)	1.2	1.2	1.2	1.2	1.2
Phytate (mg)	28	56	56	112	112

<sup>1</sup>Encapsulated ferrous sulphate



Table 3. Nutrient and energy contents of the food supplements provided in the iLiNS-DYAD-M trial

<b>Nutrient/Intervention</b>	<b>IFA tablet</b>	<b>MMN tablet</b>	<b>LNS P&amp;L</b>	<b>LNS 20 g</b>
Recipient	Mothers, pregnancy only	Mothers, P&L	Mothers, P&L	Infants aged 6 to 18 months
Daily ration	1 tablet	1 tablet	20 g	20 g
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
$\alpha$ -Linolenic acid (g)	0	0	0.59	0.58
Vitamin A ( $\mu$ g RE)	0	800	800	400
Vitamin C (mg)	0	100	100	30
Vitamin B <sub>1</sub> (mg)	0	2.8	2.8	0.3
Vitamin B <sub>2</sub> (mg)	0	2.8	2.8	0.4
Niacin (mg)	0	36	36	4
Folic acid ( $\mu$ g)	400	400	400	80
Pantothenic acid (mg)	0	7	7	1.8
Vitamin B <sub>6</sub> (mg)	0	3.8	3.8	0.3
Vitamin B <sub>12</sub> ( $\mu$ g)	0	5.2	5.2	0.5
Vitamin D (IU)	0	400	400	200
Vitamin E (mg)	0	20	20	0.5
Vitamin K ( $\mu$ g)	0	45	45	200
Iron <sup>1</sup> (mg)	60	20	20	6
Zinc (mg)	0	30	30	8
Cu (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 ( $\mu$ g)	0	130	130	20
Iodine ( $\mu$ g)	0	250	250	90
Manganese (mg)	0	2.6	2.6	1.2

IFA, iron and folic acid; LNS, lipid-based nutrient supplements; MMN, multiple micronutrients; P&L, pregnancy and lactation.

<sup>1</sup>Encapsulated ferrous sulphate

## 4.3 Data collection

### 4.3.1 Enrolment and follow-up

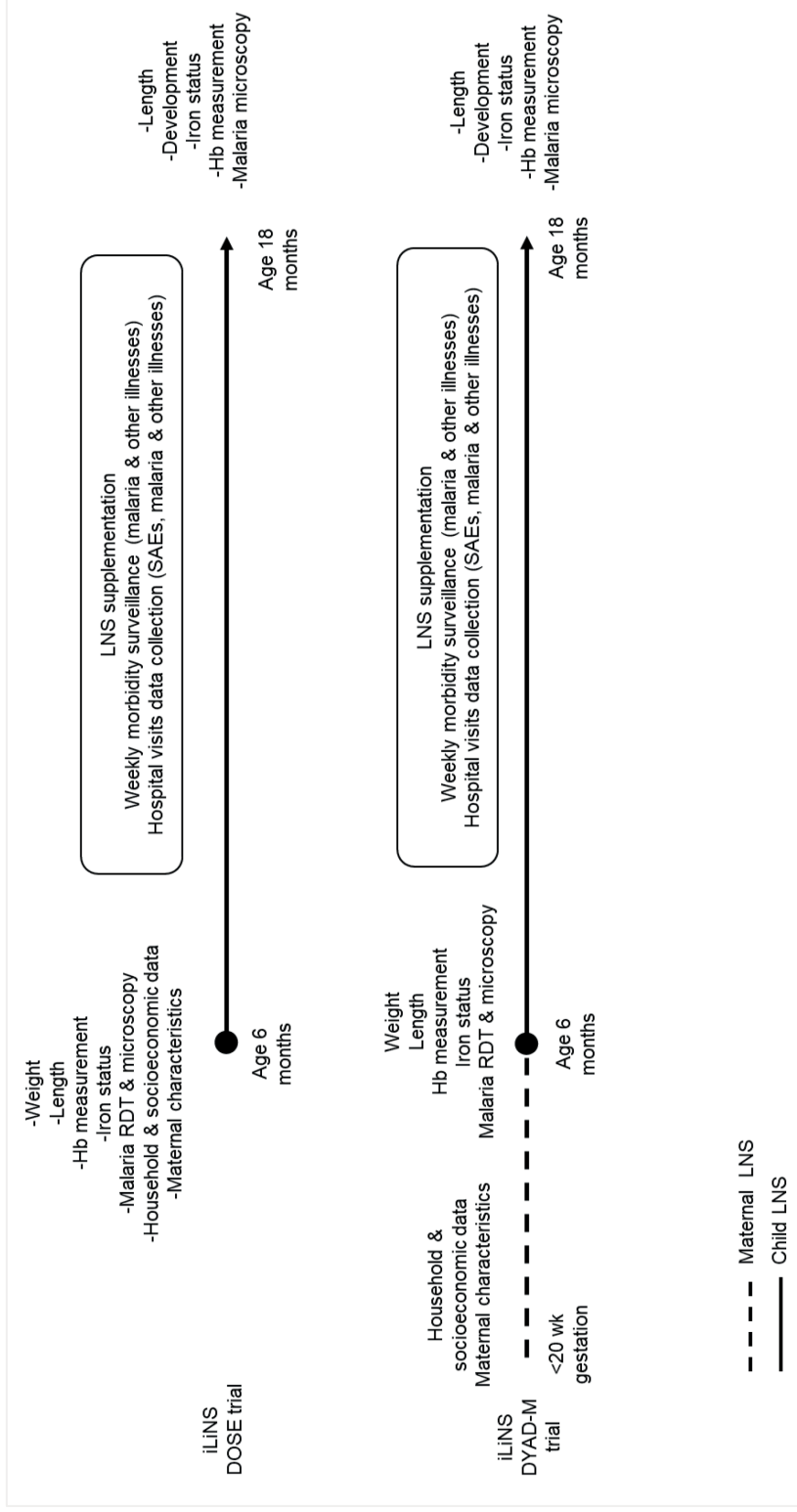
Enrolment of children in the iLiNS-DOSE trial was conducted between November 2009 and May 2011. The group allocation for each participant was sealed in an individual opaque randomization envelope and stored in a locked cabinet until use. When the guardian consented to let her infant participate and the infant met all the enrolment criteria, the guardian was asked to choose and open one randomization envelope from a block of six unused envelopes.

Enrolment of pregnant mothers in the iLiNS-DYAD-M trial was conducted between February 2011 and August 2012. A researcher not involved with the trial created individual randomization slips and packed them in sealed, numbered, opaque randomization envelopes that were stored in numerical order. Eligible pregnant mothers were asked to select one of the envelopes in a pile, which indicated her participant identification number and group allocation.

In both trials, the participant identification number and group allocation were recorded in a logbook and on the participant's identification card.

Research assistants visited the children's homes every week from 6 months to 18 months to collect information on morbidity and supplement use. The follow-up and data collection procedures are schematically presented in Figure 3.

**Figure 6.** Participant follow-up and data collection



Abbreviations: Hb, haemoglobin; LNS, lipid-based nutrient supplements; RDT, rapid diagnostic test; SAE, serious adverse event.

### 4.3.2 Anthropometric assessments

Anthropometric assessments were done at 6 months and 18 months. Study anthropometrists measured the infant's length with a high-quality length board (Harpenden Infantometer; Holtain Limited, Crosswell, United Kingdom) and recorded it to the nearest 1 mm. They weighed unclothed infants with electronic infant weighing scale (SECA 735; Seca GmbH & Co, Hamburg, Germany), recording to the nearest 10 g. Anthropometric indices (weight-for-age z-scores [WAZ], length-for-age z-scores [LAZ], and weight-for-length z-scores [WLZ]) were calculated using WHO Child Growth Standards (2010 STATA igrowup package) (41).

### 4.3.3 Developmental assessments

Research assistants assessed fine and gross motor development at 18 months using the Kilifi Developmental Inventory (KDI) (111). Language development was assessed using the MacArthur-Bates Communicative Development Inventory (112) adapted for local languages (Chichewa and Chiyao), and 18-months socio-emotional development was assessed using the Profile of Social and Emotional Development (PSED) (113).

The research assistant rated the child's mood during KDI assessment as positive (smiling/laughing) or not positive (crying/inconsolable, changeable/mood swings, or no visible emotions). Child's interaction with the research assistant 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 the assessor). Child's activity level during the KDI was rated as positive (active and maintains interest) or not positive (unarousable, sleepy and can hardly be awakened, sleepy but easily awakened, does not spontaneously engage in activity, and awake but loses interest). The KDI, vocabulary, and PSED scores showed high inter-rater agreement and moderate to high test-retest reliability in this study setting (114,115).

#### 4.3.4 Morbidity assessments

At the start of the study, the author of this thesis trained research assistants to collect morbidity data through interviewing the guardians about the child's health in the previous seven days using a structured questionnaire. The information was complemented by a picture calendar filled out by the guardians daily to aid memory of their children's morbidity status (Appendix 1). This was designed to minimize problems of recall associated with community morbidity assessments (116,117). The usefulness of maternal interviews to collect data on child morbidity has been validated elsewhere (118,119). The research assistants referred all cases of presumed malaria (presence of fever) to the nearby health facility for malaria RDT and treatment with artemether/lumefantrine, the nationally recommended antimalarial drug. The children were followed throughout the year, covering periods of both high and low malaria transmission.

The author of this thesis trained facility health workers to collect data on non-scheduled visits made to health facility when the child was sick. The information included clinic visits (non-scheduled), clinical diagnoses, malaria RDT, hospitalizations, and deaths. For deaths occurring at home, the information on the causes of death was collected by a verbal autopsy method, previously validated in the study area (120). Records of all hospitalizations and deaths were reviewed by a study physician (the author of this thesis) and serious adverse events were reported to members of the data and safety monitoring board (DSMB) within 48 hours of occurrence.

During the study visits at 6 months and 18 months, all children had malaria microscopy tests done. Study nurses obtained blood smears from all children at the time of the blood sampling for biochemical assessment. Laboratory technicians stained the smears with 2% Giemsa for 30 minutes and reviewed all thick samples to determine the malaria parasite count and determined the parasite densities by counting the number of asexual parasites per 200 white blood cells (WBCs) and assuming a WBC count of 8,000/ $\mu$ l.

At 6 months, study nurses tested the children for malaria using rapid test kit (Clearview Malarial Combo, Alere, South Africa).

#### 4.3.5 Other variables

During the study visits at 6 months and 18 months, study nurses collected 5-7 ml of blood by venipuncture using a 23-gauge needle into 7.5 ml evacuated, trace element-free polyethylene tubes containing lithium heparin (CB 300 LH, Sarstedt AG & Co, Nümbrecht, Germany). The blood tube was immediately inverted 10 times to mix the heparin anticoagulant with the blood to prevent clotting. A small aliquot of the whole blood was pipetted out and used to analyse haemoglobin (Hb) on the Hemocue 201+ system (Hemocue, Ängelholm, Sweden). The tube containing the remaining whole blood was then placed in an insulated cooler with ice packs and processed within 2 hours of collection. Trained laboratory staff then aliquoted whole blood into microcuvettes and measured zinc protoporphyrin (ZPP) concentration from unwashed venous blood sample using a hematofluorometer (206D, AVIV Biomedical Inc., Lakewood, NJ, USA).

During the study visit at 6 months, research assistants interviewed the mothers to obtain household level information including assets, number of children, maternal education (years spent in school) and age. Household food security was assessed using Household Food Insecurity Access Scale (HFIAS) (121). Use of ITNs was assessed by asking the guardians the number of days that the child slept under a mosquito bed net during the week preceding the interview.

#### 4.3.6 Quality assurance

The study team ensured the data were of high quality by providing the data collectors with written standard operating procedures, visit guides and step-by-step instructions on how to complete individual morbidity forms. In addition, 10% of the home visits were quality controlled by a data monitor responsible for a team of 5–7 data collectors who repeated and verified that morbidity visits and supplement delivery were done according to protocol. If problems were identified, a repeat training for the data collectors was provided. The anthropometric assessments were done only by trained personnel whose measurement reliability was verified at the start of the study and thereafter at 6-months intervals with methods adapted from the procedures used in the WHO Multicentre Growth Reference Study (41). Each measurement was taken and recorded in triplicate. The anthropometrists calibrated all equipment with standard weights and length rods daily.

For the LNS delivery, single masked procedures were used; i.e., field workers who delivered the supplements knew which mothers were receiving LNS, and the participants were advised not to disclose information about their supplements to anyone other than an iLiNS study team member. The data collectors who performed the anthropometric assessments or other outcomes were not aware of group allocation. All researchers (including the author of this thesis) responsible for the data cleaning remained blind to the trial code, until the database was considered fully cleaned.

## 4.4 Statistical approach

### 4.4.1 Sample size calculation

The sample size calculations were based on the primary objectives of the main trials. In the iLiNS-DOSE trial the primary objective was to test the hypothesis of non-inferiority of LNS without milk on change in length-for-age z-score as compared to LNS containing milk. Assuming a SD for the change in LAZ of 1.0 z-score units, a pre-determined non-inferiority margin of 0.25 z-score units and an estimated 15% attrition rate, a sample size of 320 / group was estimated to provide the trial with 90% power and 95% confidence (one-sided test) to discard an inferiority null-hypothesis.

In the iLiNS-DYAD-M trial, the primary objective was to test the hypothesis that provision of LNS to mothers in pregnancy and lactation and to their infants from 6 months to 18 months would promote infant and child growth. Assuming an effect size of at least 0.3 (difference between groups, divided by the pooled SD) for each continuous outcome, a power of 80%, and a 2-sided type I error rate of 5%. This would have required 216 participants per group, for a total of 648 subjects. Allowing for up to 25% attrition rate, the target sample size was 864 subjects.

### 4.4.2 Data management and definition of variables

The data were recorded on paper forms and double-entered using Microsoft® Access, REDCap™ and OpenText™ TeleForm. Typographical errors, extreme observations, and discrepancies were resolved prior to data analysis. The

subsections below describe how the data outputs were manipulated to create predictor and outcome variables.

#### 4.4.2.1 Anthropometric indices

WAZ, LAZ, and WLZ were calculated based on the WHO Child Growth Standards (41) and values below -2.0 were considered indicative of underweight, stunting and wasting, respectively. Change in LAZ for each child was calculated as the difference between LAZ at 18 months and LAZ at 6 months.

The use of stunting (LAZ < -2) as a threshold to classify children who are nutritionally worse off in epidemiologic research has its limitations because currently there is no evidence that the risk of death or poor development in children becomes worse below this threshold, hence more information is derived from the use of other metrics such as slope of the change in mean LAZ with time (45).

#### 4.4.2.2 Developmental milestones

From the child development data at 18 months, fine motor scores were calculated as the sum of 34 KDI fine motor items, each scored 0 or 1. Gross motor scores were calculated as the sum of 35 KDI gross motor items, each scored 0 or 1 (111). Vocabulary score was calculated as the maternal-reported child expressive vocabulary out of the 100-word checklist. For these outcomes, moderate to severe delay was defined as the bottom 25% of the sample. The socio-emotional score was calculated as the sum of 19 PSED items. Moderate to severe delay was defined as the top 25% of the sample (a higher score indicates less advanced socio-emotional development).

#### 4.4.2.3 Guardian-reported morbidity episodes

Using data from guardian-reported morbidity symptoms, diagnoses of presumed malaria, gastroenteritis and ARI were derived from a combination of symptoms reported on one or more days. To ensure the diagnoses were mutually exclusive, we created an algorithm whereby any fever with a diarrhoea episode (three or more loose stools in 24 h) or bloody stools was categorized as diarrhoea; any fever in the



presence of any respiratory symptoms (cough, rapid or difficult breathing and nasal discharge) was categorized as ARI. Presumed malaria was defined as any fever episode in the absence of diarrhoea or respiratory symptoms.

An episode of presumed malaria, ARI or diarrhoea was defined as the period starting from the day the child had the symptoms when preceded by at least 2 days of no symptoms or no data. The episode was considered to end on the last day the child had the symptoms which was then followed by at least 2 symptom-free days. Incidence of presumed malaria, ARI or diarrhoea for each child from 6 months to 18 months was calculated as total episodes / total follow-up years at risk.

#### 4.4.2.4 Non-scheduled visits

These were visits made by the participants to any health facility because of illness. At each non-scheduled visit, a diagnosis of either malaria (clinical or confirmed), gastroenteritis, ARI or other illnesses was made by health workers.

#### 4.4.2.5 Clinical malaria episodes

A diagnosis of clinical malaria was taken from the malaria diagnosis made by health worker in the absence of a diagnostic test at the non-scheduled visit. An episode of clinical malaria was defined as any point when a diagnosis of clinical malaria was made.

#### 4.4.2.6 Confirmed malaria episodes

A diagnosis of confirmed malaria was taken from the malaria diagnosis made by health worker confirmed by a positive malaria RDT. An episode of RDT-confirmed malaria was defined as any point when a diagnosis of RDT-confirmed malaria was made, occurring at least 28 days after a previously treated RDT-confirmed malaria episode (59).

Malaria cases identified at the non-scheduled visits (clinical malaria and RDT-confirmed malaria) comprise passive case detection (PCD) and can be used to gauge the burden of illness caused by malaria relative to all other common causes

of child illness. PCD data largely depend on the accuracy of the diagnostic test and quality of the source data and assumes that all infections are hospital-treated. As such, PCD data are less reliable in malaria-endemic settings because: other causes of febrile illness are often misclassified and inappropriately treated as malaria; self-treatment results in underreporting which masks the true burden of malaria in the community; and naturally acquired immunity leaves most malaria infections unnoticed and untreated at the health facilities (122).

#### 4.4.2.7 Malaria parasitaemia

Malaria parasitaemia was defined as evidence of asexual parasite on a slide viewed through 200 high-power microscopy fields. Because the tests were done in all children regardless of symptoms, this variable measured the prevalence of parasitaemia independently of fever and therefore can be qualified as active case survey (ACS). The ACS may be useful in estimating risk of infection in populations where parasitaemia without fever is common, e.g. in malaria-endemic settings (122).

#### 4.4.2.8 Serious adverse events (SAEs)

SAEs were defined as hospitalizations or deaths, according to the United States Department of Health and Human Services Office for Human Research Protections (123).

#### 4.4.2.9 Biochemical status

Anaemia at 6 months was defined as blood Hb concentration <105 g/L (124), anaemia at 18 months was defined as blood Hb concentration <110 g/L (125). Iron deficiency at 6 months and 18 months was defined as whole blood ZPP >70 µmol/mole haeme (126).

#### 4.4.2.10 Household and socio-economic variables

HFIAS z-scores were generated by summing the value of responses to nine questions regarding food insecurity (See Appendix 2): the higher the score, the higher the degree of food insecurity in the last four weeks (121).

Household asset index<sup>3</sup> was defined as the principal components score based on baseline ownership of a set of assets and household quality: the higher the score, the better the living conditions (127).

Number of children under 5 was defined as number of children under 5 years of age who were part of the participant's household on the day of enrolment.

Study site was defined based on the clinics where the two studies were conducted (i.e. Namwera, Mangochi, Malindi, and Lungwena).

## 4.5 Data analysis

All data management and analyses were done using Stata version 14 (Stata-Corp, College Station, TX, USA).

Negative binomial regression was applied to assess the association of LAZ (independent variable) with the incidence of malaria (dependent variable), and Poisson regression (with a robust variance) to assess the association of LAZ with prevalence of malaria parasitaemia (128) (Study aim I).

Ordinary least squares regression was used to assess the association between malaria incidence and each of the continuous outcomes; and modified Poisson regression (with a robust error variance) for each of the binary outcomes (129) (Study aim II).

A non-inferiority margin of 20% was set to determine if the risk of malaria in the intervention groups (LNS) was clinically significantly different from the risk of malaria in the control group. Generalised linear modelling (log-binomial family)

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<sup>3</sup>The variables used in generating household asset scores were: house quality (burnt bricks, type of roofing material, floor tiles, water source, type of toilet, energy source) and ownership.

was used to estimate and compare the risk of SAEs between the intervention groups and the control group, reported risk ratios (RR) and 95% confidence intervals (CI).

Negative binomial regression was used to compare the incidences of malaria outcomes between the intervention groups and the control group, reported as incidence rate ratios (IRR) and 95% CI. If the 95% CIs of the RR or IRR for an intervention group compared to the control group fell entirely below 1.20, non-inferiority was conclusive, but not otherwise (Study aim III).

In all analyses, multivariate models were constructed by including all theoretically relevant variables collected at age 6 months. The following variables were included in the models: child sex, LAZ, WLZ, Hb, iron status, maternal education, maternal age, HFIA, household assets, number of children under 5 years of age in the household, incidence of other morbidities (diarrhoea and ARI), and whether the child received an intervention (LNS) or not. All results are reported as RR, IRR or prevalence ratios [PR] and their 95% confidence intervals (95% CI) at  $p=0.05$ .

Statistical methods are explained in detail in the individual articles.

## 4.6 Ethical Approval

The studies were conducted according to Good Clinical Practice and the ethical standards of the Declaration of Helsinki. The protocols were approved by two ethics committees: the College of Medicine Research and Ethics Committee, University of Malawi; and the Ethics Committee of Pirkanmaa Hospital District, Finland. The guardians provided an informed consent form before enrolment. The trials were registered at clinical trial registry ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) with the registration IDs of NCT00945698 and NCT01239693. Independent DSMBs monitored the incidence of suspected SAEs.

## 5 RESULTS

### 5.1 Enrolment and success of follow-up

The iLiNS-DOSE trial was conducted from November 2009 to June 2012. Out of 2,136 infants who came to the study clinics for assessment, 1,932 (90.4%) were enrolled and randomized into six study groups. The group allocation, reasons for exclusion and loss to follow-up, and final cohort which was used for Study III are shown in **Figure 1 of Article III**.

The iLiNS-DYAD-M trial was conducted from February 2011 to April 2015. The study staff approached a total of 9,310 pregnant mothers, from whom 1,391 (14.9%) were enrolled in the trial and were randomly assigned to one of the three intervention groups. Of these, 869 mothers were assigned to the complete intervention, i.e. their children were followed up to 18 months. A total of 793 children (including five sets of twins) were born to the mothers; these children represent the sample for the present study (we excluded the children born to the remaining 522 mothers who were assigned to the pregnancy intervention only).

The final cohorts for Study I and Study II which combined children from both iLiNS-DOSE and iLiNS-DYAD-M are shown in **Figure 1 of Article I** and **Figure 1 of Article II**, respectively.

### 5.2 Background information

The total sample obtained from the two trials at 6 months was 2,657 children (1,932 children from the iLiNS-DOSE trial and 725 children from the iLiNS-DYAD-M trial). However, 96 children were dropped due to missing morbidity data due to loss to follow-up due to death or withdrawal from the study before morbidity visits were done; thus 2,561 children were included in the final pooled sample.

Table 4 shows the characteristics of the children at 6 months. The mean (SD) age of the children was 5.9 (0.3) months and their mean (SD) LAZ, WAZ and WLZ z-

scores were -1.4 (1.1), -0.7 (1.2) and 0.3 (1.1) respectively. The median (25th, 75th centile) ZPP concentration was 85 (63, 131)  $\mu\text{mole/mole haeme}$  and the proportion of children with iron deficiency was 66.2%. The mean (SD) Hb concentration was 103.5 (15.9) g/L while the proportion of children with anaemia was 51.5%. The reported mosquito bed net utilization by children was 81.1%.

Table 4. Participant characteristics at 6 months

Variable	iLiNS-DOSE <sup>a</sup>	iLiNS-DYAD-M <sup>a</sup>	Pooled data <sup>a</sup>
Number of children <sup>b</sup>	1,928	633	2,561
Mean (SD) weight, kg	7.0 (1.0)	7.3 (1.0)	7.1 (1.0)
Mean (SD) length, cm	63.4 (2.4)	64.2 (2.6)	63.6 (2.5)
Mean (SD) length-for-age z-score	-1.4 (1.0)	-1.2 (1.1)	-1.4 (1.1)
Mean (SD) weight-for-length z-score	0.3 (1.1)	0.4 (1.1)	0.3 (1.1)
Mean (SD) weight-for-age z-score	-0.7 (1.1)	-0.5 (1.2)	-0.7 (1.2)
Mean (SD) haemoglobin, g/L	103.6 (16)	103.7 (15)	103.6 (15)
Proportion of boys n (%)	974 (50.5%)	301 (47.6%)	1,275 (49.6%)
Proportion with LAZ < -2 score n (%)	561 (29.1%)	148 (23.4%)	709 (27.4%)
Proportion with WLZ < -2 score n (%)	35 (1.8%)	13 (2.0%)	48 (1.8%)
Proportion with WAZ < -2 score n (%)	268 (13.9%)	49 (7.7%)	317 (12.2%)
Proportion with haemoglobin <105 g/L n (%)	976 (50.6%)	344 (54.4%)	1,320 (51.7%)
Proportion with ZPP >70 µmole/mole haeme <sup>c</sup> n (%)	1,325 (68.7%)	442 (69.8%)	1,767 (69.0%)
Proportion with malaria by RDT n (%)	322 (16.7%)	61 (9.7%)	383 (14.5%)
Proportion with malaria by microscopy n (%)	83 (4.3%)	33 (5.2%)	116 (4.5%)
Mean (SD) maternal age (years)	26.1 (6.2)	25.1 (6.0)	25.8 (6.1)
Mean (SD) maternal education, completed years	4.7 (3.6)	3.8 (3.5)	4.4 (3.6)
Mean (SD) HFIAS	6.5 (6.0)	5.0 (4.3)	6.0 (5.6)
Mean (SD) asset score	0.001 (1.0)	-0.005 (1.0)	-0.001 (1.0)
Mean (SD) number of children under 5 years of age in each household	1.7 (0.7)	1.5 (0.7)	1.6 (0.7)
Proportion who slept under ITN daily <sup>d</sup> n (%)	1,533 (79.5%)	541 (85.4%)	2,074 (81.1%)
Proportion who received LNS intervention n (%)	1,610 (83.5%)	205 (32.4%)	1,815 (68.7%)
<b>Study site:</b>			
Mangochi	1,483	191	1,664
Namwera	445	0	445
Malindi	0	119	119
Lungwena	0	323	323

HFIAS, Household Food Insecurity Access Scale; ITN, insecticide-treated mosquito bed net; LAZ, length-for-age z-score; LNS, lipid-based nutrient supplement; RDT, rapid diagnostic test; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score; ZPP, zinc protoporphyrin

<sup>a</sup>Values are n, mean (SD) or proportions

<sup>b</sup>Children who had morbidity data from 6 months to 18 months

<sup>c</sup>Measured from unwashed venous blood

<sup>d</sup>A week prior to the date of visit



## 5.3 Descriptive statistics

### 5.3.1 Linear growth and development

Between 6 months and 18 months, the children gained, on average, 12.9 (2.1) cm in length but there was a decline in mean (SD) LAZ from -1.4 (1.1) at 6 months to -1.9 (1.1) at 18 months. The prevalence of severe stunting (LAZ <-3) and moderate to severe stunting (LAZ <-2) increased from 6.1% to 12.9% and 26.7% to 41.2% respectively. The prevalence of moderate to severe underweight (WAZ <-2) increased from 12.2% to 16.4%, whereas the prevalence of moderate to severe wasting (WLZ <-2) increased from 1.8% to 4.9% between 6 months and 18 months. The mean (SD) scores for fine motor, gross motor, language and PSED were 20.9 (2.2), 17.3 (2.6), 26.2 (5.0) and 16.2 (5.4) respectively.

### 5.3.2 Malaria morbidity

The children in the two trials contributed 2,478.6 years of follow-up, i.e. the mean (SD) duration of follow-up was 344 (73) days / child. A total of 28,032 morbidity episodes were reported during the home visits. Of these, 15,586 (55.6%) were ARI, 9,419 (33.6%) were diarrhoea, 2,719 (9.7%) were malaria, and 308 (1.1%) were minor conditions. The mean (SD) incidence of all illnesses combined was 11.6 (13.1) episodes per child year, of which 7.8 (8.0) were episodes of ARI, 2.7 (2.7) were episodes of diarrhoea, and 1.1 (1.6) were episodes of presumed malaria.

On average, the children made 5.5 visits to the hospital due to illness during the one-year follow-up. Of these, 1.7 were due to malaria (categorized as clinical malaria, mean (SD) incidence: 0.4 (0.8) and confirmed malaria, mean (SD) incidence: 1.3 (2.0) episodes per child year). The prevalence of malaria confirmed by microscopy increased from 4.7% to 9.6% between 6 months and 18 months.

During the 12-month follow-up, 45% of the children did not report any episode of malaria, 29% reported one episode and 26% reported >1 malaria episodes. The children who reported >1 malaria episodes were responsible for 71.4% of all malaria episodes. The pattern was similar across all definitions of malaria.

## 5.4 The association of linear growth faltering at 6 months with subsequent 12-month malaria morbidity (Study I)

Of the 2,725 children enrolled in the two study cohorts, 2,561 (94%) had data for the primary outcome, incidence of presumed malaria (1,928 children from the iLiNS-DOSE trial and 633 children from the iLiNS-DYAD-M trial). These were included in the final analysis for study aim I (**Figure 1 of Article I**).

Figure 5 shows the association of LAZ with malaria in different definitions. When considered across the ranges of severities in linear growth faltering, there appears to be a risk gradient in incidence of RDT-confirmed malaria and prevalence of malaria parasitaemia. The severely stunted children (LAZ <-3) had the highest incidence of malaria; the incidence/prevalence decreased with progressive increase in LAZ. However, these associations were not statistically significant when adjusted for other predictors<sup>4</sup> (IRR = 1.03, 95% CI = 0.98 to 1.09, p = 0.394 for presumed malaria; IRR = 0.98, 95% CI = 0.89 to 1.07, p = 0.703 for clinical malaria, and IRR = 1.00, 95% CI = 0.94 to 1.07, p = 0.882 for confirmed malaria).

When stratified by stunting at 6 months, children who were stunted had higher mean incidence of clinical malaria from 6 months to 18 months, higher mean incidence of RDT-confirmed malaria from 6 to 18 months (Figure 6), and higher prevalence of malaria parasitaemia at 18 months (Figure 7), although these did not reach statistical significance (IRR = 1.13, 95% CI = 0.61 to 2.09, p = 0.707 for presumed malaria incidence; IRR = 1.14, 95% CI = 0.92 to 1.39, p = 0.233 for clinical malaria incidence; IRR = 1.02, 95% CI = 0.88 to 1.18, p = 0.789 for confirmed malaria incidence; and PR = 1.10, 95% CI = 0.93 to 1.37, p = 0.712).

### *Independent predictors of malaria*

Independent predictors for the different malaria outcomes are presented in Table 5

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<sup>4</sup>Adjusted for the following factors at 6 months: sex of the child, haemoglobin concentration, iron status, month of birth, daily use of insecticide-treated bed nets, distance to health facility, study site, asset scores, maternal age, maternal education, children under 5 years of age, household food insecurity access scale, and whether the child received the study intervention (lipid-based nutrient supplements) or not.

Table 5. Independent predictors of incidence and prevalence of malaria in multivariate analysis<sup>1</sup>

	Incidence of 'presumed' malaria (N=2561)		Incidence of clinical malaria (N=2497)		Incidence of confirmed malaria (N=2497)		Prevalence of malaria parasitaemia at age 18mo (N=1662)	
	IRR <sup>2</sup> (95% CI)	p-value	IRR <sup>2</sup> (95% CI)	p-value	IRR <sup>2</sup> (95% CI)	p-value	PR <sup>3</sup> (95% CI)	p-value
<i>Child factors at age 6 months</i>								
Haemoglobin (g/L)	1.00 (0.99 to 1.01)	0.529	0.99 (0.98 to 1.00)	0.016	1.00 (0.99 to 1.01)	0.845	0.98 (0.97 to 0.99)	0.001
Iron deficiency <sup>4</sup>	1.04 (0.91 to 1.18)	0.587	1.03 (0.83 to 1.28)	0.773	1.20 (1.04 to 1.39)	0.012	2.11 (1.24 to 3.57)	0.006
<i>Maternal factors at enrollment</i>								
Maternal age	1.01 (1.00 to 1.02)	0.011	0.99 (0.97 to 1.01)	0.149	1.00 (0.98 to 1.01)	0.200	0.97 (0.94 to 0.99)	0.033
Maternal education	0.99 (0.97 to 1.01)	0.427	1.00 (0.98 to 1.03)	0.783	0.97 (0.95 to 0.99)	0.024	0.90 (0.85 to 0.96)	0.001
<i>Household factors at enrollment</i>								
Household Food Insecurity Access Score	1.00 (0.99 to 1.01)	0.809	1.02 (1.01 to 1.04)	0.022	1.01 (0.99 to 1.02)	0.065	1.02 (0.99 to 1.05)	0.184
Asset scores	0.86 (0.80 to 0.93)	<0.001	0.94 (0.83 to 1.07)	0.343	0.73 (0.64 to 0.83)	<0.001	0.92 (0.63 to 1.34)	0.666
<i>Environmental factors</i>								
LNS intervention (vs control)	1.00 (0.88 to 1.14)	0.983	1.11 (0.89 to 1.37)	0.343	1.02 (0.86 to 1.22)	0.783	1.07 (0.70 to 1.62)	0.765
Study site	Reference		Reference		Reference		Reference	
Mangochi	0.94 (0.81 to 1.09)	0.457	1.49 (1.20 to 1.85)	<0.001	1.73 (1.50 to 1.99)	<0.001	1.49 (0.99 to 2.24)	0.054
Namwera	1.12 (0.88 to 1.42)	0.337	0.45 (0.27 to 0.73)	0.001	0.58 (0.43 to 0.78)	<0.001	0.74 (0.29 to 1.88)	0.531
Malindi	0.91 (0.75 to 1.09)	0.303	0.24 (0.14 to 0.41)	<0.001	0.33 (0.25 to 0.44)	<0.001	0.65 (0.36 to 1.19)	0.163
Lungwena								

CI: confidence interval; IRR: incidence rate ratio; LNS: lipid-based nutrient supplements; PR: prevalence ratio; ZPP: zinc protoporphyrin

<sup>1</sup> Other factors included in the models but not significant were: sex of the child, birth month, number of children in household, and daily use of insecticide-treated bed nets

<sup>2</sup> Incidence rate ratio, obtained using negative binomial regression. The IRR represents the rate of change in incidence of malaria (for each 1-unit higher in the continuous predictors or for each group compared to the reference group in the categorical predictors), adjusted for other variables

<sup>3</sup> Prevalence ratio, obtained using a modified Poisson regression (with a robust variance estimator) (129). The PR represents the rate of change in prevalence of malaria parasitaemia (for each 1-unit higher in the continuous predictors or for each group compared to the reference group in the categorical predictors), adjusted for other variables

<sup>4</sup> ZPP >70 µmole/mole heme (127).

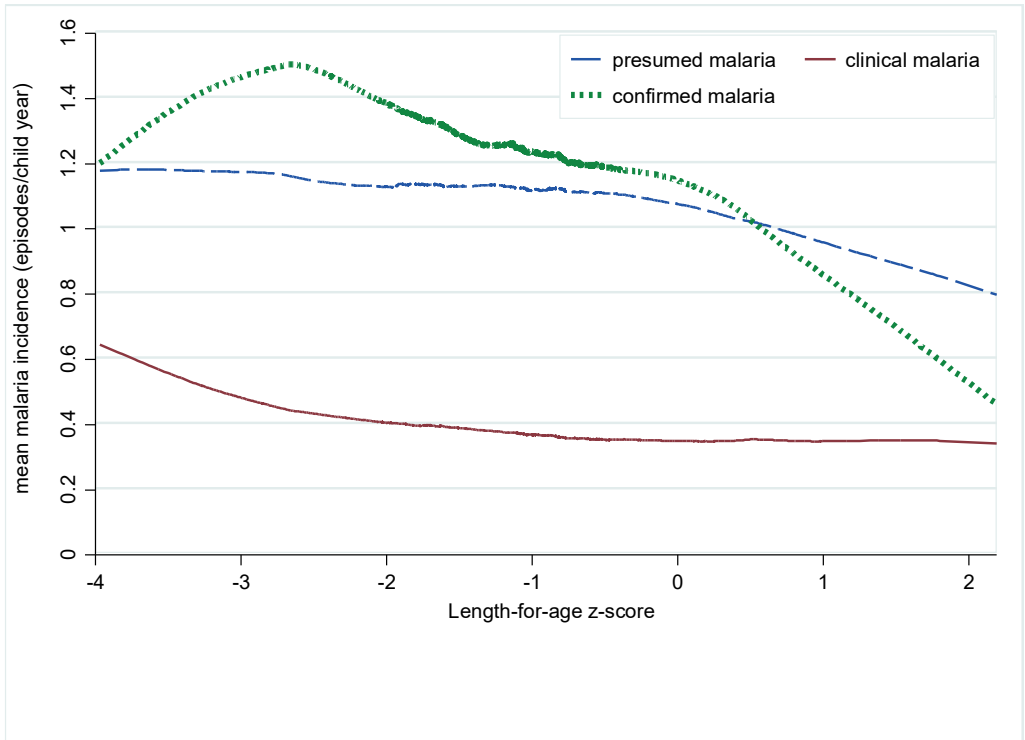
Incidence of presumed malaria from 6 to 18 months was predicted by maternal age (IRR = 1.01, 95% CI = 1.00 to 1.02, p = 0.011) and asset score (IRR = 0.86, 95% CI = 0.80 to 0.93, p <0.001) at 6 months.

Incidence of clinical malaria from 6 to 18 months was predicted by Hb concentration at 6 months (IRR = 0.99, 95% CI = 0.98 to 1.00, p = 0.016) and HFIAS at 6 months (IRR = 1.02, 95% CI = 1.01 to 1.04, p = 0.022). In addition, the incidence of clinical malaria significantly varied across the study sites, with Namwera site recording higher incidence of clinical malaria compared to Mangochi (IRR = 1.49, 95% CI = 1.20 to 1.85, p <0.001), while the Malindi and Lungwena had lower incidence of clinical malaria compared to Mangochi (IRR = 0.45, 95% CI = 0.27 to 0.73, p = 0.001 and IRR = 0.24, 95% CI = 0.14 to 0.41, p <0.001, respectively).

Incidence of confirmed malaria from 6 to 18 months was predicted by iron deficiency at 6 months (IRR = 1.20, 95% CI = 1.04 to 1.39, p = 0.012), maternal education (IRR = 0.97, 95% CI = 0.95 to 0.99, p = 0.024), and asset score at 6 months (IRR = 0.73, 95% CI = 0.64 to 0.83, p <0.001). There was also a significant variation in the incidence of confirmed malaria across the study sites: children from Namwera had higher incidence of confirmed malaria compared to Mangochi (IRR = 1.73, 95% CI = 1.50 to 1.99, p <0.001), while Malindi and Lungwena children had lower incidence of confirmed malaria compared to Mangochi (IRR = 0.58, 95% CI = 0.43 to 0.78, p <0.001 and IRR = 0.33, 95% CI = 0.25 to 0.44, p <0.001, respectively).

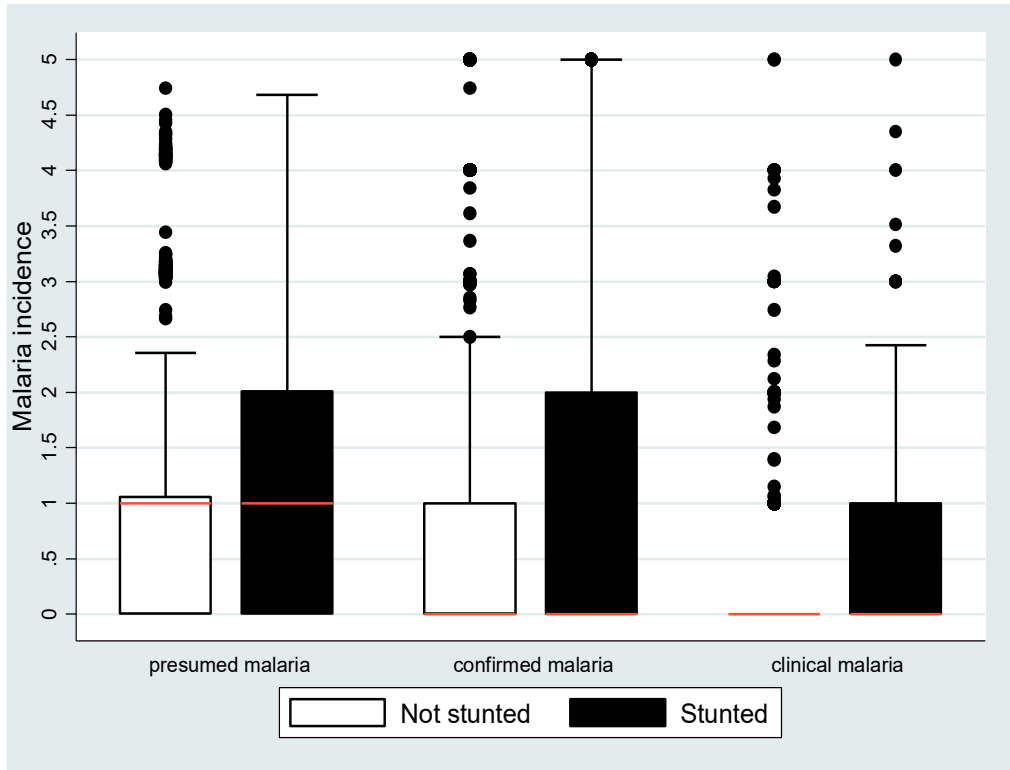
Malaria parasitaemia prevalence was predicted by WLZ at 6 months (PR = 0.80, 95% CI = 0.67 to 0.94, p = 0.007), Hb concentration at 6 months (PR = 0.98, 95% CI = 0.97 to 0.99, p = 0.001), iron deficiency at 6 months (PR = 2.11, 95% CI = 1.24 to 3.57, p = 0.006), maternal age (PR = 0.97, 95% CI = 0.94 to 0.99, p = 0.033), and maternal education (PR = 0.90, 95% CI = 0.85 to 0.96, p = 0.001).

**Figure 7.** Association of length-for-age z-score at age 6 months with incidence of malaria from age 6 months to 18 months for children in the iLiNS-DOSE and iLiNS-DYAD-M trials, adjusted for other predictors<sup>5</sup>. The graphs are LOWESS-smoothed.



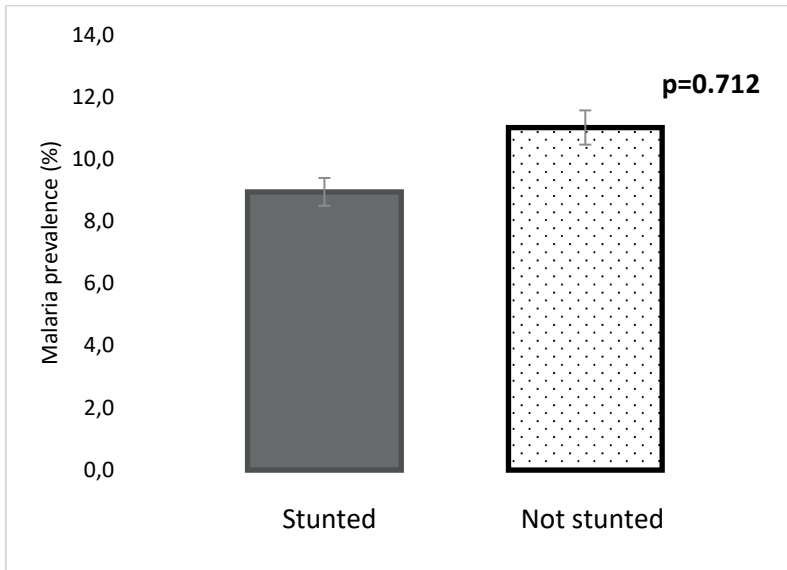
<sup>5</sup>Adjusted for the following factors at 6 months: sex of the child, haemoglobin concentration, iron status, month of birth, use of insecticide treated bed nets, distance to health facility, study site, asset scores, maternal age, maternal education, children under 5 years of age, household food insecurity access scale, and whether the child received the study intervention (lipid-based nutrient supplements) or not.

**Figure 8.** Mean incidence of presumed malaria, clinical malaria and confirmed malaria (by rapid diagnostic test) from age 6 months to 18 months for children in the iLiNS-DOSE and iLiNS-DYAD-M trials by stunting status at age 6 months



*Box plot showing the incidence of presumed, confirmed, or clinical malaria in children who were stunted at 6 months (black boxes) compared to children who were not stunted (clear boxes). Incidence (defined as number of episodes/child years of follow-up). Summarized by median, interquartile range and outliers.*

**Figure 9.** Prevalence of malaria parasitaemia at age 18 months by stunting status at age 6 months for children in the iLiNS-DOSE and iLiNS-DYAD-M trials



*Graph showing the prevalence of malaria parasitaemia (confirmed by microscopy) in children who were stunted at 6 months (grey bar) compared to children who were not stunted (dotted bar). Prevalence was defined as number of children with positive microscopy/total number of children tested at 18 months. P-value calculated using modified Poisson regression (with a robust error variance).*

## 5.5 Association of malaria morbidity with linear growth faltering and child development (Study II)

Of the 2,725 children enrolled in the two study cohorts, 2,016 (74%) had length measured both at 6 months and 18 months (1,417 children from the iLiNS DOSE trial and 599 children from the iLiNS DYAD-M trial). These were included in the final analysis for study aim II (**Figure 1 of Article II**).

The mean (SD) change in LAZ from 6 months to 18 months was -0.44 (0.77). **Figure 8** shows an increase in change in LAZ with increasing incidence of presumed malaria incidence, although not statistically significant in multivariate analysis ( $\beta = -0.02$ , 95% CI = -0.04 to 0.01,  $p = 0.069$ ). Similarly, there was no significant association of clinical malaria incidence ( $\beta = 0.04$ , 95% CI = -0.08 to 0.15,  $p = 0.531$ ) or confirmed malaria incidence ( $\beta = -0.01$ , 95% CI = -0.03 to 0.02,  $p = 0.569$ ) with change in LAZ from 6 months to 18 months.

The proportion of children who were stunted increased from 27.4% at 6 months to 41.5% at 18 months. Presumed malaria incidence was significantly associated with a higher proportion of children with stunting at 18 months after adjusting for covariates<sup>6</sup> (RR = 1.04, 95% CI = 1.01 to 1.07,  $p = 0.023$ ). This association was clearer when presumed malaria was categorized by frequency (0, 1, >1 episodes) as shown in **Figure 9**: children with >1 malaria episodes from 6 months to 18 months had higher risk of stunting at 18 months compared to children with zero malaria episodes (RR = 1.39, 95% CI = 1.13 to 1.70,  $p = 0.002$ ). There were no significant associations of clinical malaria incidence (RR = 1.07, 95% CI = 0.91 to 1.25,  $p = 0.409$ ) or confirmed malaria incidence (RR = 0.99, 95% CI = 0.96 to 1.02,  $p = 0.561$ ) from 6 months to 18 months with stunting at 18 months.

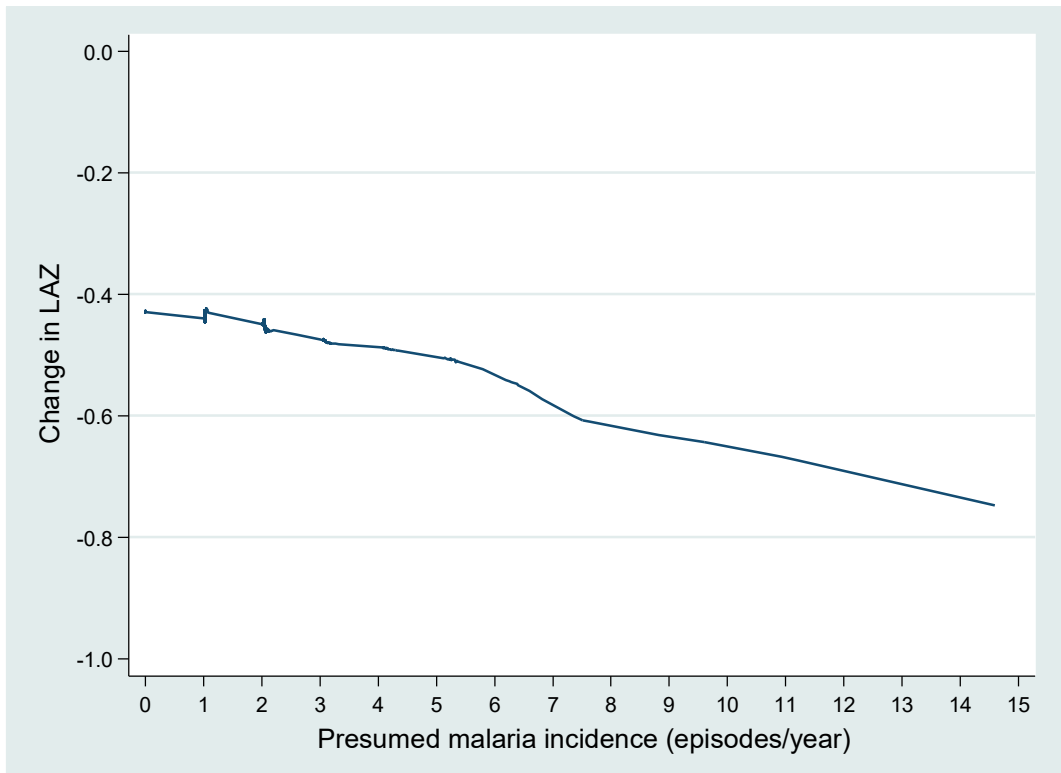
The association of incidence of presumed malaria from 6 months to 18 months with child development at 18 months was significant for PSED scores ( $\beta = -0.21$ ; 95% CI = 0.39 to -0.03;  $p = 0.041$ ), but not for the other domains of child development (**Table 4 and Table 5 of article II**).

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<sup>6</sup>Adjusted for the following factors at 6 months: sex of the child, stunting, weight-for-length z-score, haemoglobin concentration, iron status, household food insecurity access scale, maternal education, incidence of diarrhoea and acute respiratory infections from 6 months to 18 months, and whether the child received an intervention (lipid-based nutrient supplements) during the study period.



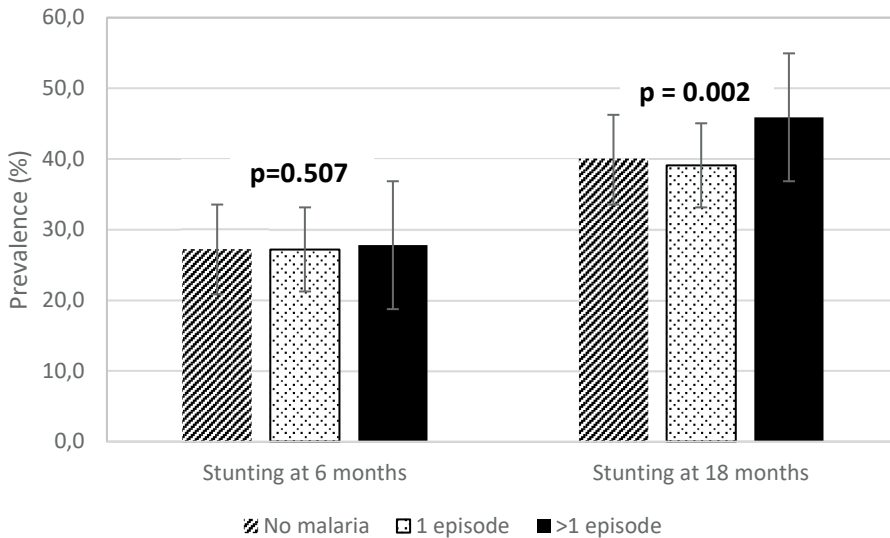
**Figure 10.** Change in length-for-age z-score from age 6 months to 18 months associated with increasing incidence of presumed<sup>7</sup> malaria for children in the iLiNS-DOSE and iLiNS-DYAD-M trials, adjusted for stunting and other variables<sup>8</sup>. The graph is LOWESS-smoothed.



<sup>7</sup> Presumed malaria was defined as any fever episode in the absence of diarrhoea or respiratory symptoms.

<sup>8</sup> Adjusted for the following factors at 6 months: sex of the child, weight-for-length z-score, haemoglobin concentration, iron status, household food insecurity access scale, maternal education, incidence of diarrhoea and acute respiratory infections from 6 months to 18 months, and whether the child received an intervention (lipid-based nutrient supplements) during the study period.

**Figure 11.** The prevalence of stunting at age 18 months by frequency of presumed malaria episodes from age 6 months to 18 months for children in the iLiNS-DOSE and iLiNS-DYAD-M trials



*Graph showing the prevalence of stunting in children at age 6 months and age 18 months, compared across three groups of varying malaria frequencies (from age 6 months to 18 months): 1) children who had no malaria; 2) children who had one malaria episode; 3) children who had more than one malaria episodes during the follow-up period. The group reporting more than one malaria episodes had significantly higher prevalence of stunting at age 18 months. P-values calculated using modified Poisson regression (with a robust error variance). Error bars represent standard error.*

## 5.6 Effect of LNS provision on risk of malaria (Study III)

This study aim was addressed using the data from iLiNS-DOSE trial only, a sample of 1,932 children. At 18 months 1,534 (79.4%) children remained in follow-up (78 children died, and 320 children withdrew before the end of the study). A further 127 children were late for the final visit (excluded from this analysis). Thus, 1,407 (72.8%) were assessed on the final home visit within their scheduled period of two weeks (**Figure 1 of Article III**). There were no intergroup differences either in the mean length of follow-up ( $p=0.974$ ) or the proportion of children who remained in the study until its end ( $p=0.334$ ). Because the malaria outcomes were similar between the 20 g milk LNS and 20 g no-milk LNS groups (data not shown), we collapsed these two groups into one analysis group. Similarly, we combined the 40 g milk LNS and 40 g no-milk LNS groups into another analysis group. Thus, all the results under this aim refer to group comparisons by daily ration of LNS (0, 10 g, 20 g, or 40 g), irrespective of the milk content in the LNS. The results are summarized in **Table 5**.

A total of 271 SAEs were recorded among the children: 78 were deaths (4.0% of the enrolled children) and 193 were hospitalizations. Compared to the control group, the 95% CI for the RR of experiencing an SAE was entirely below 1.20 (suggestive of non-inferiority) in the 10 g LNS group, entirely below 1.00 (suggestive of a protective effect) in the 20 g LNS group and ranged from 0.66 to 1.25 (inconclusive) in the 40 g LNS group. The 95% CI for the RR of hospitalizations was entirely below 1.20 (suggestive of non-inferiority) in the 10 g and 20 g LNS groups and ranged from 0.57 to 1.22 (inconclusive) in the 40 g LNS group (Table 5). The 95% CI for the risk of death was entirely below 1.20 (suggestive of non-inferiority) in the 20 g LNS groups and ranged from 0.42 to 1.79 and 0.58 to 1.92 (inconclusive) in the 10 g and 40 g LNS groups respectively (Table 5).

Among the children in the iLiNS-DOSE trial, we identified 19,690 morbidity episodes from the home visits data, 9.7% of which were due to presumed malaria. Compared to the control group, the 95% CIs of the IRRs for presumed malaria were entirely below 1.20 (suggestive of non-inferiority) in all the intervention groups (Table 5). The children made 9,034 hospital visits due to illnesses, each child

making on average 5.2 hospital visits per year. Compared to the control group, the incidence of RDT-confirmed malaria at the hospital visits was 21% higher in the 40 g LNS group than the control group, but the comparison was inconclusive (Table 5). There was no significant difference in the incidence of clinical malaria in any of the LNS groups, compared to the control group (Table 5).

Finally, all five intervention groups were combined into one group to compare all children who received LNS to those who did not. In this comparison, the 95% CIs of the IRRs for all malaria outcomes were entirely below 1.20 (suggestive of non-inferiority). The 95% CI for the risk of death was too wide to draw any conclusions (Figure 6).

Table 6. Comparisons of the incidences of serious adverse events, presumed malaria, clinical malaria and RDT-confirmed malaria, according to intervention

Variable	Results by study group						Comparison between the groups				
	Control (n=320) Follow-up years=296.7		10g LNS (n=321) Follow-up years=294.9		20g LNS (n=645) Follow-up years=595.8		40g LNS (n=646) Follow-up years=601.9		10g LNS vs Control	20g LNS vs Control	40g LNS vs Control
	Total cases	Proportion or incidence <sup>1</sup>	Total cases	Proportion or incidence <sup>1</sup>	Total cases	Proportion or incidence <sup>1</sup>	Total cases	Proportion or incidence <sup>1</sup>	RR (95%CI) <sup>2</sup> or IRR (95%CI) <sup>3</sup>	RR (95%CI) <sup>2</sup> or IRR (95%CI) <sup>3</sup>	RR (95%CI) <sup>2</sup> or IRR (95%CI) <sup>3</sup>
Children who experienced any SAE	50	15.6%	36	11.2%	68	10.5%	92	14.2%	0.71 (0.48 to 1.07)	<b>0.67</b> (0.48 to 0.95)	0.91 (0.66 to 1.25)
Children who were hospitalized	38	11.9%	25	7.8%	53	8.2%	64	9.9%	0.66 (0.41 to 1.06)	0.69 (0.47 to 1.03)	0.83 (0.57 to 1.22)
Children who died	15	4.7%	13	4.1%	18	2.8%	32	5.0%	0.86 (0.42 to 1.79)	0.60 (0.30 to 1.17)	1.05 (0.58 to 1.92)
Presumed malaria	345	1.6	322	1.5	657	1.5	687	1.6	0.97 (0.81 to 1.16)	0.96 (0.83 to 1.12)	0.99 (0.85 to 1.16)
Hospital visits	1412	4.8	1417	4.8	2988	5.0	3217	5.3	1.02 (0.89 to 1.18)	1.05 (0.93 to 1.19)	<b>1.13</b> ( <b>1.00 to 1.27</b> )
Clinical malaria	185	0.6	174	0.6	418	0.7	409	0.7	0.94 (0.74 to 1.20)	1.12 (0.92 to 1.37)	1.08 (0.89 to 1.33)
RDT-Confirmed malaria	385	1.3	418	1.4	834	1.4	941	1.6	1.10 (0.88 to 1.37)	1.08 (0.89 to 1.31)	<b>1.21</b> ( <b>1.00 to 1.46</b> )

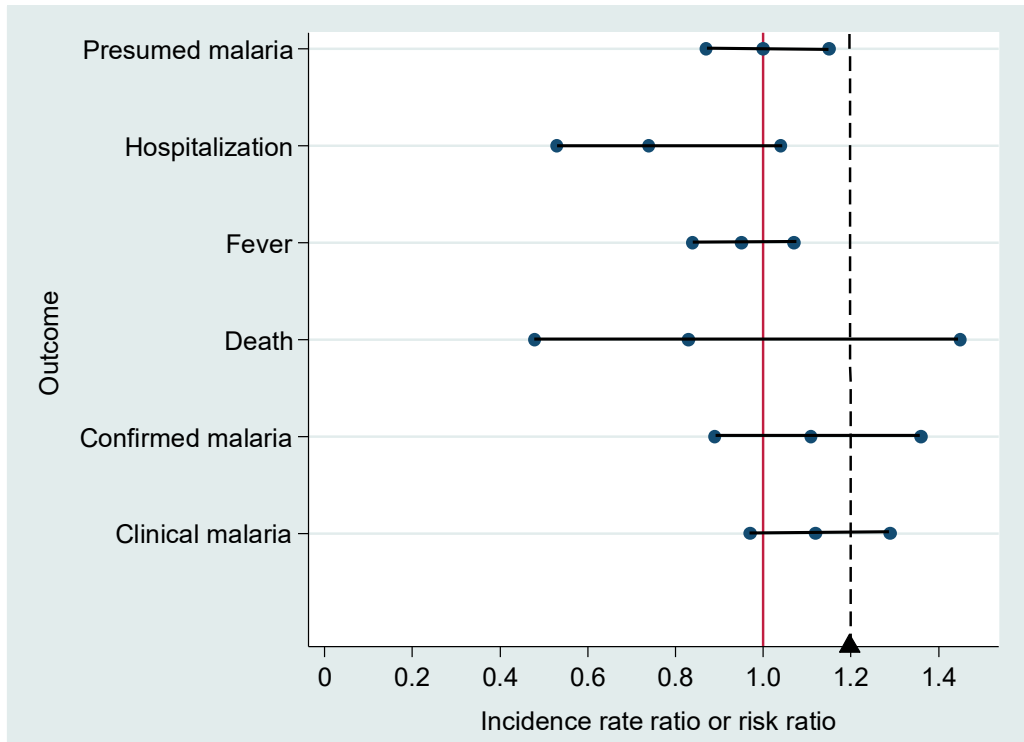
IRR, incidence rate ratio; LNS, lipid-based nutrient supplements; RR, risk ratio; SAE, serious adverse events; RDT, rapid diagnostic test

<sup>1</sup>Incidence = number of episodes or visits/child/year of follow-up

<sup>2</sup>Obtained using generalized linear modelling (log-binomial family).

<sup>3</sup>Obtained using negative binomial regression. Statistically significant results in bold.

**Figure 12.** Effect of lipid-based nutrient supplements provision on malaria-related outcomes for children in the iLiNS-DOSE trial only



All intervention groups receiving lipid-based nutrient supplements (LNS) were pooled together and compared with the control group, with results (●) reported as incidence rate ratio (IRR) or risk ratio (RR) and their 95% CI (represented by horizontal lines). The vertical dashed line represents the margin of non-inferiority. The solid line represents the null effect. None of the point estimates and their 95% CI fell entirely above the non-inferiority margin (1.20; ▲). All the IRR/RR and their corresponding 95% CI ought to fit to the left area of the non-inferiority margin line to conclude that LNS was at least as safe as no supplement i.e. to conclude non-inferiority. We conclude that LNS does not increase morbidity.

## 6 DISCUSSION

This community-based prospective cohort study was aimed at determining the associations between malaria and linear growth faltering; both conditions highly prevalent in LMICs. The study further determined the safety of providing iron-containing lipid-based nutrient supplements to children living in settings where malaria is endemic amidst the concerns of increased risk of infectious disease morbidity associated with iron provision.

The first hypothesis was that lower LAZ at 6 months would be associated with a higher incidence of malaria from 6 months to 18 months and higher prevalence of malaria parasitaemia at 18 months. This hypothesis was not supported by the study findings because in a sample of 2,561 Malawian children, LAZ at 6 months was not associated with incidence of malaria from 6 months to 18 months, nor prevalence of malaria parasitaemia at 18 months.

The second hypothesis was that linear growth and development would be poorer in children with higher incidence of malaria. In a sample of 2,016 Malawian children aged 6–18 months, incidence of presumed malaria was positively associated with risk of stunting (i.e. one additional episode of presumed malaria per year was associated with 4% higher risk of stunting). Incidence of presumed malaria was negatively associated with socio-emotional scores (i.e. one additional episode of presumed malaria per year was associated with a reduction in PSED scores by 0.21), suggesting that children with higher malaria incidence have fewer socio-emotional problems, possibly because malaria causes lethargy and inactivity which may manifest as fewer behavioural problems. However, the overall finding can be deemed inconclusive because presumed malaria was not associated with change in LAZ, nor other domains of child development.

The third hypothesis was that long-term supplementation with LNS would not increase malaria morbidity in children. This hypothesis was supported by the study findings because in a sample of 1,932 Malawian children aged 6–18 months, one-year provision of LNS was not associated with excess hospitalizations, deaths or

malaria incidence when all intervention groups were combined. However, the incidence of RDT-confirmed malaria was 21% higher in the 40 g LNS group compared to the control group whereas children in the 10 g and 20 g LNS groups had malaria incidence similar to the control group.

This chapter discusses how these findings compare with the prevailing scientific discourse on this topic. The strengths and weaknesses of the study are discussed first, followed by the main findings in the order of the study aims.

## 6.1 Strengths and weaknesses of the study

Three main strengths of the study were: the longitudinal design; the frequent home morbidity data collection; and the large sample size.

The longitudinal study design helped to determine the temporality of the associations because: malaria outcomes were obtained from 6 months to 18 months after identifying the predictors at 6 months; and malaria is less common in children < 6 months due to the protective effect of foetal haemoglobin and maternal antibodies (64,65,130). Thus, the use of anthropometric status at 6 months as a potential predictor of malaria minimized the problem of reverse causation. The weekly morbidity visits complemented by a daily pictorial calendar minimized problems of recall associated with home morbidity assessments (116,117). The iLiNS-DOSE and iLiNS-DYAD-M trials had large sample sizes and pooling of data from the two trials for study aims I and II further enhanced the power of the study to detect any significant associations.

The study had several weaknesses. Presumptive diagnosis of malaria may have overestimated the malaria episodes due to an overlap in the symptoms of malaria, diarrhoea and ARI (131), resulting in misclassification. Although RDTs were recommended by the WHO in 2010 (59), they were not yet rolled out nationwide by the time of conducting this study. Hence, presumptive malaria diagnosis was commonly used not only in this study but also in national prevalence surveys (104,132), following IMCI guidelines (133,134).

Although presumptive diagnosis of malaria might have caused misclassification bias, additional analysis using more specific definitions of malaria such as malaria



confirmed by RDT at hospital visits showed similar results. Of note is that RDT-confirmed malaria may have underestimated the outcome because: 23% of the children were treated for malaria at the hospital without RDT confirmation; and home treatment is quite common in this population therefore children who had malaria episodes but received home treatment or no treatment and did not present to the hospitals were missed. These are generally considered as limitations of passive case detection (122). Presumptive diagnosis and treatment of malaria is hitherto common especially by the lower cadres of health care providers, posing risk of poor management, treatment failure, and under-reporting (70).

Research assistants referred all suspected malaria cases to a clinic for treatment. The active surveillance may have resulted in over-reporting of morbidity at the home visits whereby only 44% of the morbidity episodes were treated at the hospital. This discrepancy could also be due to missing data from hospital visits because the hospital data were filled by non-study staff. Loss of data was minimized by daily follow-up of admitted children by study staff, regular training, and mentoring the non-study staff on accurate data collection.

The active surveillance may also have led to prompt treatment and reduction in incidence and severity of malaria, resulting in fewer hospital visits as well as attenuating the strength of the associations in our findings. Unmeasured confounders (e.g. genetic variations) may also have influenced the strength of association between malaria and linear growth faltering, which can be minimized by mendelian randomization (94). Confounding was minimized by adjusting for known biological, socio-economic and household factors in all analyses. The random allocation of participants and blinding of the investigators to the treatment groups for study III further reduced confounding.

Considering the strengths of the study and the efforts to address its weaknesses, the author believes the overall findings are valid and generalizable to the study population

## 6.2 The association of linear growth faltering at 6 months with subsequent 12-month malaria morbidity

High rates of growth faltering and malaria overlap geographically and greatly affect the same age groups, but studies on the associations between the two conditions have so far reported conflicting findings. The findings of this study suggest there is no association of linear growth faltering at 6 months with malaria in the subsequent 12-month period, which is similar to a few previous studies (83–86), suggesting that stunting may be of little significance in malaria epidemiology.

These findings are, however, different from those reported in Gambia, Kenya, and Uganda. In Gambia, a prospective study of children under 5 years of age reported an increased risk of malaria associated with stunting (crude RR = 1.35; 95% CI, 1.08–1.69) (7). The follow-up was very short (20 weeks), only crude RR were reported therefore confounders such as socio-economic and household factors were not accounted for. In Kenya, stunting in children aged 0–36 months was associated with an increased odds of malaria parasitaemia (odds ratio = 1.98,  $P < 0.0001$ ) (8). The cross-sectional design of this study suggests the observed association may have been due to reverse causation. In Uganda, mild stunting (IRR = 1.24, 95% CI 1.06–1.46) and moderate-severe stunting (IRR = 1.24, 95% CI 1.03–1.48) in children aged  $<2.5$  years were associated with increased incidence of malaria parasitaemia (9). However, it was not clear whether exposure assessment preceded the outcomes, therefore the temporal relationship between stunting and risk of malaria was difficult to ascertain.

In this study, 26% of the children reported frequent malaria episodes and were responsible for the majority of the malaria infections. Knowledge of this over-dispersion in malaria infections is important because interventions targeted at this core group could be the most effective (135). Further analysis showed that children's WLZ at 6 months was a significant predictor of subsequent malaria episodes, i.e. children with better WLZ had fewer malaria episodes (data not shown), raising a question whether there is value in malaria screening targeted to children who are wasted. A mathematical model by Lakkam and Wein demonstrated that targeted malaria interventions to underweight children has potential to reduce malaria morbidity and mortality in malaria hypoendemic or mesoendemic settings (136).

In this setting, socio-economic factors such as household asset scores, maternal education, and food insecurity were significantly associated with malaria. These factors may be more important than anthropometric status in malaria epidemiology but were not the primary interest of the study and therefore warrant further exploration. There were also variations in hospital-diagnosed malaria by study site, with the Namwera site reporting higher incidences of clinical and RDT-confirmed malaria compared to the other three study sites. Namwera site receives high rainfall which promotes mosquito breeding, but the high altitude and cool temperatures should theoretically limit malaria transmission. This finding requires further exploration.

### 6.3 Association of malaria morbidity with linear growth faltering and child development

The association of malaria with linear growth was contradictory in this study: there was significant association of malaria with stunting (dichotomous outcome) but not with change in LAZ (continuous outcome). The lack of association of malaria with linear growth faltering has been reported in other studies (83,137–139). Similar to this study, the previous studies provided active malaria diagnosis and treatment, which may have attenuated the strength of the association.

The significant association of malaria with stunting has also been reported in other studies, attributed to the body's immune reactivation leading to anorexia, vomiting, and a catabolic state in response to infections (92-94). Children living in settings where infectious diseases are frequent and complementary food is of poor quality often fail to achieve catch up growth after illness episodes, and if these episodes are too frequent, might increase the risk of stunting (140).

The contradictory findings in this study where malaria was significantly associated with stunting but not change in LAZ may be due to the differences in the information these outcomes provide. Change in LAZ indicates the growth rate between two time-points and therefore provides more information about linear growth faltering than stunting status assessed at one time-point (45,141). Therefore, it is possible that there exists no association between malaria and linear growth faltering (based on change in LAZ), or the association is very weak. It is also possible that the association of malaria with linear growth faltering is only seen in children in

the left end of the curve (i.e. LAZ<-2), hence the significant association of malaria with stunting but not change in LAZ. In contrast, diarrhoea incidence was positively associated with both change in LAZ and risk of stunting, suggesting an entire leftward shift in LAZ (data not shown).

## 6.4 Effect of LNS provision on risk of malaria

Controversy continues among health policy makers on the perceived risk of malaria associated with iron provision in malaria-endemic settings, particularly among the iron-replete children. Iron deficiency anaemia is highly prevalent, but iron supplementation targeted to the iron-deficient may not be feasible because of problems of screening. Hence, the WHO does not recommend routine iron supplementation unless provided alongside malaria control interventions (28).

The mechanisms of the adverse effects of iron in infectious disease morbidity are not fully understood. Two possible pathways are proposed: a large dose of ingested iron raises plasma non-transferrin-bound iron concentration which may facilitate hepatic entry of malaria sporozoites; and iron may promote growth of gut microorganisms, and impair gut immune response, leading to pathogen sequestration into the systemic circulation (35,96,97).

The findings that provision of 10 g and 20 g LNS was not associated with higher malaria morbidity are similar to the previous LNS studies (15,32–34), attributed to the quantity and mode of delivery of iron (25).

These findings are different from other intervention studies (29–31) which reported increases in malaria-related hospitalizations and deaths, other infections and intestinal inflammation associated with iron supplements or iron-containing MMN provision.

Several reasons could explain the differences. The dose of iron provided in this study was less than half (6 mg/day) of that provided in the other studies (12.5mg/day). Guardians were advised that the LNS should be eaten on two or more occasions during the day, to limit the amount of iron ingested in a single meal (25). Increased risk of malaria morbidity has been reported in iron-replete but not iron-deficient children (31,142). Therefore, it is possible that the high prevalence of iron

deficiency (66.2%) together with the reported high utilization of ITNs (81.1%) in this sample may have offered some protection against malaria. Lastly, the intensive morbidity surveillance and treatment may have improved the overall health of the children and attenuated any possible adverse effects of LNS.

The finding that the 40 g LNS/d dose may have been associated with excess malaria-related hospital visits is puzzling, especially given that the 10 g and 20 g LNS/d doses were conclusively not associated with increased malaria. Although the lower limit of the confidence interval was below the set non-inferiority margin of 1.20, the point estimate of 1.21 in the 40 g LNS/d group suggest the risk for malaria in this group was clinically significantly higher. However, there is no clear biological explanation for this finding because the dose of iron was intended to be similar in all the LNS groups. In the maternal supplementation arm of the iLiNS-DYAD-M trial, provision of MMN or LNS to the pregnant women was associated with an increased incidence of dental caries (143), suggesting perhaps the safety of LNS is dependent on specific populations and doses.

## 7 SCIENTIFIC CONCLUSIONS

1. In rural Malawi, where both stunting and malaria are common, LAZ at 6 months does not predict malaria incidence from 6 months to 18 months nor prevalence of malaria at 18 months.
2. In this population of children aged 6–18 months, with active disease surveillance and early treatment, higher incidence of malaria is not associated with change in LAZ in infants and young children but may increase the risk of stunting and reduce socio-emotional development at 18 months.
3. Long term provision of iron-containing LNS at certain doses does not increase the risk of malaria when provided to infants and young children living in malaria-endemic settings where iron deficiency and mosquito bed net utilization is high, and malaria surveillance and treatment is available.

## 8 PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH NEEDS

This study has demonstrated that linear growth faltering at 6 months does not predict malaria infections from 6 months to 18 months. Therefore, malaria screening targeted at children with low LAZ may not be a viable malaria control strategy. In contrast, the study findings showed that WLZ at 6 months is associated with subsequent malaria infections, hence malaria screening in children found with low WLZ at the growth monitoring and promotion clinics can add value in malaria control efforts, but this requires further exploration.

The study also highlights two issues that may be important in the malaria control: there is over-dispersion of malaria infections where a minority of children bears the major burden of repeated malaria attacks; and there exist significant site (micro-geographic) variations in malaria distribution. These were not primary outcomes of this study therefore were not explored further. Additional studies are required to identify factors for such malaria distribution which can be useful to policy makers and implementers to prioritize the limited resources for malaria control to target vulnerable subpopulations at country level.

Although the findings show that malaria is not associated with linear growth faltering in infants and young children from 6 months to 18 months, the fact that the children received active disease surveillance and early treatment perhaps supports the notion of incorporating disease prevention and control in programs that promote child growth and development. However, the finding that despite the surveillance and treatment, malaria increased the risk of stunting and reduced socio-emotional development at 18 months, suggests that disease surveillance and treatment alone are not adequate. Children who suffer repeated malaria infections may require close follow-up and support at home and at growth monitoring and promotion clinics to enhance prevention and early detection of undernutrition.

This study has demonstrated that at certain doses, LNS supplementation does not increase malaria infections, hospitalizations or deaths in malaria-endemic setting where iron deficiency and ITN utilization are high, and malaria surveillance and treatment is available. Hence iron-containing LNS can be safely provided in such settings. This evidence can assist policy makers when developing guidelines for prevention of iron deficiency anaemia through routine supplementation in children under 5 years of age living in malaria-endemic settings. However, further studies are required to determine the optimal dose of iron in food fortificants that can reduce iron deficiency anaemia in children under in 5 years of age living in settings where iron deficiency is high.

This study has highlighted several areas that require further research such as:

- A study aimed at evaluating the value of screening children with low WLZ for malaria at the growth monitoring and promotion clinics to facilitate early treatment and prevent severe malaria and its complications.
- A study aimed at determining the predisposing factors in children who suffer repeated malaria infections, focussing on suspected risk factors that were not assessed in this study such as HIV infection, polyparasitism, vitamin A and zinc deficiency, and genetic variations including haemoglobinopathies.
- Considering that children living in higher altitude and far from water bodies (Namwera) reported more malaria-related hospitalizations than those in lower altitude, this unexpected finding in the microepidemiology of malaria needs further exploration using more sensitive tools such as geospatial mapping and use of big data analytical algorithms.
- A study aimed at determining the optimal dose of iron in LNS to reduce iron deficiency anaemia without adverse effects, taking into account the country-specific prevalence of iron deficiency anaemia and infectious diseases in children under 5 years of age.



## 9 ACKNOWLEDGEMENTS

This study was conducted by an international team of fine researchers from the University of Malawi College of Medicine, Department of Public Health, School of Public Health, Blantyre, Malawi, the Tampere University, Faculty of Medicine and Life Sciences, Center for Child Health Research, Tampere, Finland, and University of California, Davis, California, USA. The study was funded through a generous grant from the Bill & Melinda Gates Foundation to the University of California, Davis, USA. I completed epidemiology courses at the University of Tampere School of Public Health, through the International Postgraduate Programme in Epidemiology (IPPE) and nutrition courses at the Program for International and Community Nutrition (PICN) at University of California, Davis, USA through a study grant from Forgy Foundation.

Throughout my studies, I received support from numerous marvelous people, and while I desire to express my sincere gratitude to them all, I realize it is virtually impossible to individually acknowledge them all. I would like to single out the following great scientific minds and personalities:

Professor Per Ashorn, as my main supervisor, advisor, and mentor. His continuous, persistent and selfless guidance throughout my studies basically determined this thesis. Per's scientific thought process, focus, hard work was instrumental in my developing zeal in community research. His encouragement especially at many times when my enthusiasm was at its lowest ebb was key in producing this work and bringing it to completion. His leading by example, demand for perfection with focus on simplicity is what I aim to emulate in my career as a scientist.

Professor Kenneth Maleta, as my supervisor, advisor, and mentor at the College of Medicine, Malawi, for bringing me into his research team in Mangochi. His immense knowledge in epidemiology and community nutrition and ability to disentangle complex scientific processes always made our meetings pure joy. His faith in me even when I almost gave up on this PhD and his supervisory support in writing my final

two articles taught me invaluable lessons on how to support younger researchers navigate the rough seasons that lurk in the times of every student.

Docent Terhi Tapiainen and Docent Tuula Pelkonen, the official pre-examiners of this thesis, for their honest critique of the content and wonderful suggestions. Their attention to detail made the final thesis more readable than I could have ever done by myself.

Diana Estevez, my colleague at World Health Organization, for being so kind to proofread the final version.

Professor Kathryn Dewey for her impeccable teaching during my time at University of California, Davis and during the manuscripts' preparation and publication. I will forever be grateful for that once-in-a-lifetime chance to study international nutrition at University of California, Davis, USA. The exposure opened my mind and heart to the world of nutrition programs; a catalyst to the new realm of ever-expanding and exciting experiences in public health nutrition.

Professor Yin Bun Cheung, Mrs Lotta Hallamaa and Mr Noel Patson for their practical and thought-provoking comments to statistical methods and simplified approach to interpreting research findings. Their holistic reviews and comments to manuscripts taught me statistics much better than any standard text will ever do.

Professor Matti Salo and Professor Olli Lohi, the follow-up group members for this PhD, for their constructive criticism, expert advice, and measured encouragement which was exactly what I needed when I was trying to find my feet during the developing stages of the PhD process.

The Faculty and staff from the Center for Child Health Research, Faculty of Medicine and Health Technology, Tampere University. Special thanks to Ulla Ashorn; her friendship and support I greatly appreciate. My colleagues Anna Pulakka, Ulla Harjunmaa, Kirsi-Maarit Lehto, for the many interesting discussions we had in Arvo building. I also extend my gratitude to the IPPE and Doctoral Programme in Public Health staff for introducing me to the field of epidemiology and biostatistics.

The staff and students in the Program for International and Community Nutrition at the University of California Davis: Professors Ken Brown, Sonja Hess, Christine

Stewart, Lindsay Allen, Steve Vosti and Elizabeth Prado; Dr Marjolie Haskell, Dr Souheila Abbeddou, Dorian Signori, Maureen Phelan, Dr Jerome Some, Dr Moses Klevor, Dr Harriet Okronipa; they made my stay in California memorable. I enjoyed the Davis hospitality and simplicity; and how we used to seamlessly delve into heated scientific discussions during braais, potlucks, or over a drink at Wonderbar and University of beer!

All the staff in the International Lipid-based Nutrient Supplements (iLiNS) Project in Lungwena, Namwera, Malindi and Mangochi for their work in collecting the data that led to the production of three scientific articles which were the recipe for this summary. My deep gratitude to the field workers who taught me the nuances of community research, right in the field where the rubber hits the road – skills which are never taught in any standard epidemiology textbook. And the biggest thank you to the unsung heroes of research, the best teachers of clinical research – the children who participated in these clinical trials and the challenging communities they lived in.

To the all-star team in Malawi: Dr John Phuka, Dr Chrissie Thakwalakwa, Dr Charles Mangani, Dr Chiza Kumwenda, Dr Austrida Gondwe, Dr Minyanga Kachale, Dr Thokozani Phiri, Isabel Potani, Allison Daniel, Yankho Kaimila, David Chaima, Kondwani Nyengo, Raphael Chipatala, Nozgechi Phiri, Harmony Phiri, Enita Phiri, Brains Nkhwanzi, John Mbotwa, Misonzi Banda, Zikomo Mbawa, and all members of the College of Medicine Graduate Nutrition Group who in one way or the other shared my joys and pains of plodding the PhD path that is embellished with thorns and thistles!

To my family whose silent encouragement meant approval that I was doing the right thing by pursuing this PhD. And my appreciation for never asking that question every PhD student abhors: ‘when are you finishing...?’

And finally, to my daughter Alaine, whose daily dances, songs, smiles and laughter constantly reminded me that there is happiness and more significant things in life than the crushing disappointment of a non-significant p-value! For the best lesson she taught me, of celebrating every milestone, she deserves the best present I can give: an inspiration to aim higher than her parents, because what’s the excuse for not seeing farther when standing on giants’ shoulders?

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# APPENDICES

## 1. Pictorial Calendar

*iLiNS-DYAD trial: Form 26* (version 2011-06-11)













### Picture calendar for child morbidity, chewa

Participant Code (Participant): \_\_\_\_\_

#### 1. Visit information

- 1.1 Number (code) of visit .....
- 1.4 Date of first day of data collection (day when calendar is left at home)  
    |   | 20
- 1.5 Date of last day of data collection (day preceding next home visit)  
    |   | 20

#### 2. LNS use and morbidity during the follow-up period

Tsiku la msabata Day of the week	Write in day (circle Friday)								
Mwana anadya Chiponde Child ate LNS Form 27, Q2.4									
Mwana anali bwino Child was well									
Anachepetsa zichitochito/ kaseweredw Reduced activity Form 27, q2.5									
Analibe chilakolako chofuna kudya Poor appetite Form 27, q2.6									
Kutsegula mmimba Diarhoea (mark number) Form 27, q2.7, q2.8									
Kusanza Vomiting Form 27, q2.9									
Kutantha thupi Fever Form 27, q2.10									
Kutsokomola Cough Form 27, q2.11, 2.12, 2.13									
Kutuluka mamina Nasal discharge Form 27, q2.14									
Kuonana ndi a chipatala koyamba Health visit 1 Form 27, q2.15-2.21									

## Appendix 2. Household Food Insecurity Access Scale (HFIAS) generic questions

Each of the questions in the following table is asked with a recall period of four weeks (30 days). The respondent is first asked an occurrence question – that is, whether the condition in the question happened at all in the past four weeks (yes or no). If the respondent answers “yes” to an occurrence question, a frequency-of-occurrence question is asked to determine whether the condition happened rarely (once or twice), sometimes (three to ten times) or often (more than ten times) in the past four weeks.

### Example:

1. In the past four weeks, did you worry that your household would not have enough food?

0 = No (skip to Q2)

1 = Yes

1.a. How often did this happen?

1 = Rarely (once or twice in the past four weeks)

2 = Sometimes (three to ten times in the past four weeks)

3 = Often (more than ten times in the past four weeks)

No.	Occurrence Questions
1.	In the past four weeks, did you worry that your household would not have enough food?
2.	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?
3.	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?
4.	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?
5.	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?
6.	In the past four weeks, did you or any household member have to eat fewer meals in a day because there was not enough food?
7.	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?
8.	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?
9.	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?

# ORIGINAL PUBLICATIONS



# PUBLICATION

I

**Does anthropometric status at 6 months predict the over-dispersion of malaria infections in children aged 6–18 months? A prospective cohort study**

Jaden Bendabenda, Noel Patson, Lotta Hallamaa, Ulla Ashorn, Kathryn G. Dewey, Per Ashorn & Kenneth Maleta

Malaria Journal volume 18, 143 (2019)

<https://doi.org/10.1186/s12936-019-2778-y>

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RESEARCH

Open Access



# Does anthropometric status at 6 months predict the over-dispersion of malaria infections in children aged 6–18 months? A prospective cohort study

Jaden Bendabenda<sup>1,2</sup>, Noel Patson<sup>1,4</sup>, Lotta Hallamaa<sup>2</sup>, Ulla Ashorn<sup>2</sup>, Kathryn G. Dewey<sup>3</sup>, Per Ashorn<sup>2</sup> and Kenneth Maleta<sup>1\*</sup>

## Abstract

**Background:** In malaria-endemic settings, a small proportion of children suffer repeated malaria infections, contributing to most of the malaria cases, yet underlying factors are not fully understood. This study was aimed to determine whether undernutrition predicts this over-dispersion of malaria infections in children aged 6–18 months in settings of high malaria and undernutrition prevalence.

**Methods:** Prospective cohort study, conducted in Mangochi, Malawi. Six-months-old infants were enrolled and had length-for-age z-scores (LAZ), weight-for-age z-scores (WAZ), and weight-for-length z-scores (WLZ) assessed. Data were collected for 'presumed', clinical, and rapid diagnostic test (RDT)-confirmed malaria until 18 months. Malaria microscopy was done at 6 and 18 months. Negative binomial regression was used for malaria incidence and modified Poisson regression for malaria prevalence.

**Results:** Of the 2723 children enrolled, 2561 (94%) had anthropometry and malaria data. The mean (standard deviation [SD]) of LAZ, WAZ, and WLZ at 6 months were  $-1.4$  (1.1),  $-0.7$  (1.2), and  $0.3$  (1.1), respectively. The mean (SD) incidences of 'presumed', clinical, and RDT-confirmed malaria from 6 to 18 months were: 1.1 (1.6), 0.4 (0.8), and 1.3 (2.0) episodes/year, respectively. Prevalence of malaria parasitaemia was 4.8% at 6 months and 9.6% at 18 months. Higher WLZ at 6 months was associated with lower prevalence of malaria parasitaemia at 18 months (prevalence ratio [PR] = 0.80, 95% confidence interval [CI] 0.67 to 0.94,  $p=0.007$ ), but not with incidences of 'presumed' malaria (incidence rate ratio [IRR] = 0.97, 95% CI 0.92 to 1.02,  $p=0.190$ ), clinical malaria (IRR = 1.03, 95% CI 0.94 to 1.12,  $p=0.571$ ), RDT-confirmed malaria (IRR = 1.00, 95% CI 0.94 to 1.06,  $p=0.950$ ). LAZ and WAZ at 6 months were not associated with malaria outcomes. Household assets, maternal education, and food insecurity were significantly associated with malaria. There were significant variations in hospital-diagnosed malaria by study site.

**Conclusion:** In children aged 6–18 months living in malaria-endemic settings, LAZ, WAZ, and WLZ do not predict malaria incidence. However, WLZ may be associated with prevalence of malaria. Socio-economic and micro-geographic factors may explain the variations in malaria, but these require further study.

*Trial registration* NCT00945698. Registered July 24, 2009, <https://clinicaltrials.gov/ct2/show/NCT00945698>, NCT01239693. Registered Nov 11, 2010, <https://clinicaltrials.gov/ct2/show/NCT01239693>

**Keywords:** Children, iLiNS studies, Infections, Malaria, Over-dispersion, Stunting, Undernutrition, Wasting

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## Background

Malaria is one of the most serious public health problems in the world, with an estimated 216 million cases and 445,000 deaths reported in 2016, and approximately 90% of the cases and deaths occurring in the African Region [1]. Malawi is one of the malaria-hyperendemic countries in sub-Saharan Africa, with around 4 million malaria cases reported annually [2]. In malaria-hyperendemic countries, it is assumed that virtually all exposed individuals would suffer a malaria episode by early childhood [3]. However, several studies have shown that in these settings, only a small proportion of children suffer repeated malaria infections, and these children are responsible for most of the malaria cases [4–6], an over-dispersion known as the ‘20/80’ rule [7].

The suggested underlying factors for this over-dispersion in malaria infections are varied, and include for instance genetic [5], behavioural [8] and environmental factors [6]. Other studies suggest that undernutrition plays an important role in malaria epidemiology because of the synergistic interactions between nutrition and infections [9–11]. However, several reviews on the relationship between undernutrition and malaria determined that the current evidence is inconclusive; attributed to the heterogeneity in the study populations, malaria parasite species, and host-parasite relationship [12–14]. Hence the need for further understanding of the role of undernutrition in malaria epidemiology.

The International Lipid-based Nutrient Supplements (iLiNS) Project DOSE and DYAD studies were randomized controlled trials conducted in Malawi to study the impact of lipid-based nutrient supplements (LNS) on growth of children [15, 16]. Analysis of longitudinal malaria data in the two studies showed that 39% of the infants and young children aged 6 to 18 months did not report any malaria episode in the one-year study period; only 30.7% reported more than one episode of ‘presumed’ malaria but these were responsible for 73.7% of the ‘presumed’ malaria episodes [17]. The aim of the current analysis was to further investigate the distribution of malaria in children in these cohorts and determine whether this distribution is predicted by anthropometric status at 6 months. The hypothesis was that lower length for age z-scores (LAZ), weight-for-age z-scores (WAZ), and lower weight for length z-scores (WLZ) at 6 months will be associated with a higher incidence of malaria from 6 to 18 months, and higher prevalence of malaria at 18 months.

## Methods

### Study setting

The iLiNS-DOSE and iLiNS-DYAD-M studies were conducted in four facilities: one public district hospital

(Mangochi), one mission hospital (Malindi), and two rural public health centres (Lungwena and Namwera) in Mangochi District, Southern Malawi. The total catchment population of 180,000 largely subsisted on farming and fishing. Mangochi site is low-lying at an altitude of ~485 m above sea level, but traversed by the Shire River (the largest river in Malawi). Two of the study sites (Lungwena and Malindi) are also low-lying with the similar altitude along the eastern shore of Lake Malawi. In contrast, Namwera lies at the top of Namwera Hills, bordering Mozambique, at an altitude of ~900 m above sea level and is far from the large water bodies. Namwera experienced higher rainfall and cooler temperatures than the other three study sites [18, 19].

In Malawian children aged <5 years, the prevalence of malaria (by microscopy), diarrhoea and acute respiratory infections were 24.3%, 22% and 5%, respectively, with seasonal fluctuations [2, 20]. The sub-tropical climate comprising a warm, wet season from November to April, a cool, dry winter season from May to August, and a hot, dry season from September to October [21] is favourable for the *Anopheles* mosquitoes which transmit *Plasmodium* parasites. *Plasmodium falciparum* is the most dominant and causes about 98% of all malaria infections in Malawi. Malaria transmission occurs throughout the year with highest transmission rates occurring between October and April (rainy season), mainly in low-lying and high temperature areas.

### Study design, data collection and ethics statement

The data for this analysis were taken from the iLiNS-DOSE and iLiNS-DYAD-M studies—two large community-based randomized controlled trials conducted in rural Malawi.

In the iLiNS-DOSE study, 6-months old children were randomly allocated to one of five intervention groups provided with different doses or formulations of LNS or to a control group that did not receive LNS during the 12-month study period, between November 2009 and May 2012. In the iLiNS-DYAD-M study, pregnant women <20 weeks’ gestation were randomly allocated to one of three groups to receive iron and folic acid (IFA), multiple micronutrients (MMN) or a small-quantity (20 g) of LNS daily. After delivery, women in the IFA group received placebo tablets, while MMN and LNS supplementation was continued up to 6 months postpartum. Children of mothers in the LNS group also received LNS 10 g twice daily from 6 to 18 months. This study was conducted from February 2011 to April 2015. Details of design, randomization and enrolment for the two studies can be found in the main outcome papers [15, 16].

In both studies, research assistants visited the children’s homes every week from age 6 to 18 months to interview

the guardians about the child's health in the previous 7 days using a structured questionnaire. The information was complemented by a picture calendar filled out by the guardians daily to aid memory of their children's morbidity status. These were done to minimise problems of recall associated with community morbidity assessments [22, 23]. The use of maternal interviews to collect data on child morbidity has been validated in previous studies [24, 25]. The research assistants referred all cases of fever to the nearby health facility for a malaria rapid diagnostic test (RDT) and treatment with artemether/lumefantrine, the nationally recommended anti-malarial drug. The children were followed throughout the year, covering periods of both high and low malaria transmission.

Facility health workers were trained to collect data on clinical diagnosis, RDT, and/or malaria microscopy results whenever the child was treated at the health facility. In addition, all children had malaria microscopy tests done during the scheduled study clinic visits at age 6 months and 18 months. Blood smears were obtained from all children at the time of the blood sampling for biochemical assessments, stained with 2% Giemsa for 30 min. All thick slides were reviewed by two microscopists using a high-power microscope to determine the presence of malaria parasites. Discrepant readings were resolved by a third reviewer. Malaria RDT was also done at 6 months scheduled clinic visit using rapid test kit (Clearview Malarial Combo, Alere, South Africa).

Anthropometric assessments were done at 6 months and 18 months. Study anthropometrists measured the infant's length with a high-quality length board (Harpenden Infantometer; Holtain Limited) and recorded it to the nearest 1 mm. They weighed unclothed infants with electronic infant weighing scale (SECA 735; Seca GmbH & Co), recording to the nearest 10 g. The anthropometrists were trained and their measurement reliability was verified at the start of the study and at 6-months intervals thereafter with methods adapted from the procedures used in the WHO Multicentre Growth Reference Study [26]. The anthropometrists calibrated all equipment with standard weights and length rods daily.

Study nurses collected 5–7 mL of blood by venepuncture using a 23-gauge needle into 7.5 mL evacuated, trace element-free polyethylene tubes containing lithium heparin (Sarstedt Monovette, NH4-heparin, Sarstedt Inc., Newton, NC, USA). The blood tube was immediately inverted 10 times to mix the heparin anticoagulant with the blood to prevent clotting. A small aliquot of the whole blood was pipetted out and used to analyse haemoglobin (Hb) on the Hemocue 201+ system (Hemocue, Brea, CA, USA). The tube containing the remaining whole blood was then placed in an insulated cooler with ice packs and processed within 2 h of collection. Trained laboratory

staff then aliquoted whole blood into microcuvettes and measured zinc protoporphyrin (ZPP) concentration from unwashed venous blood sample using a haematofluorometer (206D, AVIV Biomedical Inc., Lakewood, NJ, USA).

At enrolment, research assistants interviewed the mothers to obtain household level information including assets, number of children, maternal education (years spent in school) and maternal age. Household food security was assessed using Household Food Insecurity Access Scale (HFIAS) [27]. Use of insecticide treated bed nets (ITNs) was assessed by asking the guardians the number of days that the child slept under a bed net during the week preceding the interview at 6 months. Site was defined based on the clinics where the two studies were conducted (i.e. Namwera, Mangochi, Malindi, and Lungwena).

## Definition of the predictors and the outcomes

### Outcome variables

The primary outcome for this analysis was the incidence of 'presumed' malaria, derived from the weekly morbidity data. 'Presumed' malaria diagnosis was defined as history of fever either reported by the guardians or tympanic temperature  $\geq 38$  °C measured by the research assistants during the home visit. An episode of 'presumed' malaria was defined as the period starting from the day the child had malaria symptoms, preceded by at least 2 days of no symptoms or no data. The episode ended on the last day the child had malaria symptoms, if followed by at least 2 symptom-free days. Fever episodes accompanied by respiratory signs or diarrhoea were excluded from the malaria diagnosis.

Secondary outcomes included (1) incidence of clinical malaria, taken from the malaria diagnosis made by the health worker in the absence of a diagnostic test whenever the child visited a health facility, (2) incidence of confirmed malaria, taken from the malaria diagnosis made by the health worker confirmed by a positive RDT, and (3) prevalence of malaria parasitaemia derived from the malaria microscopy results at age 18 months. Malaria incidence was calculated as total episodes of malaria/total child years at risk; malaria parasitaemia prevalence was calculated as the proportion with a positive malaria parasite slide.

### Predictor variables

Length for age z-scores (LAZ), weight-for-age z-scores (WAZ), and weight for length z-scores (WLZ) were calculated from the anthropometry data using WHO growth standards [26]. Iron deficiency at age 6 months was defined as whole blood ZPP > 70  $\mu\text{mol/mol}$  haem [28]. HFIAS z-scores were generated by summing the

value of responses to nine questions regarding food insecurity: the higher the score, the higher degree of food insecurity in the last 4 weeks [27]. Household asset scores were defined as the principal components score based on baseline ownership of a set of assets and household quality: the higher the score, the better the living conditions. Number of children aged <5 years was defined as number of children below the age of five who were part of the participant's household at 6 months.

### Statistical analysis

All children who had malaria data at any point from age 6 to 18 months were included in the analysis. Negative binomial regression was used to assess the association of LAZ, WAZ, and WLZ (independent variables) with the incidence of malaria (dependent variable), and Poisson regression (with a robust variance estimator) [29] to assess the association of LAZ, WAZ, and WLZ with prevalence of malaria parasitaemia.

To study the independent effect of various predictors, multivariate regression models were constructed that included potential predictors collected at 6 months. The following were potential predictors: household asset scores, maternal age (centred around the mean), and education, number of children aged <5 years, HFIA score, whether the child received the study intervention (LNS) or not, adjusted for the additional control group [MMN] in iLiNS-DYAD, sex of the child, Hb concentration, iron status, month of birth, daily use of ITNs, distance to health facility and study site. Collinearity among the predictors was tested using the `collin stata` command. If the predictors were highly collinear ( $>0.5$ ), the one that was less strongly associated with the outcomes was dropped.

The results are reported as incidence rate ratios [IRR] or prevalence ratio [PR] and their 95% confidence intervals (95% CI) at  $p=0.05$ . Robust standard errors were computed to adjust for correlation of recurrent malaria episodes in a single child.

Other potential predictors were considered including immunization status, markers of inflammation (C-reactive protein and alpha1-acid glycoprotein concentrations) at 6 months, maternal and child malaria immunity, and maternal HIV status. However, these variables were available only from a subsample of the two studies, hence were eventually dropped from the final models to maximize the sample size. Furthermore, these variables showed little effect on the model during sensitivity analysis. Stata version 14 (Stata Corp, Texas, USA) was used for the main analyses.

## Results

### Study population

Of the 2723 children enrolled in the two study cohorts, 2561 (94%) had data for the primary outcome (1928

children from the iLiNS DOSE study and 633 children from the iLiNS DYAD-M study). These were included in the final analysis (Fig. 1). At age 6 months, the mean (SD) length-for-age z-scores (LAZ), weight-for-age z-scores (WAZ), and weight-for-length z-scores (WLZ) were  $-1.4$  (1.1),  $-0.7$  (1.2), and  $0.3$  (1.1) respectively. The proportions of children who were stunted, underweight wasted were 28.3%, 12.9%, and 2.0%, respectively. Further characteristics of these children at age 6 months are summarized in Table 1.

### Incidence and prevalence of malaria

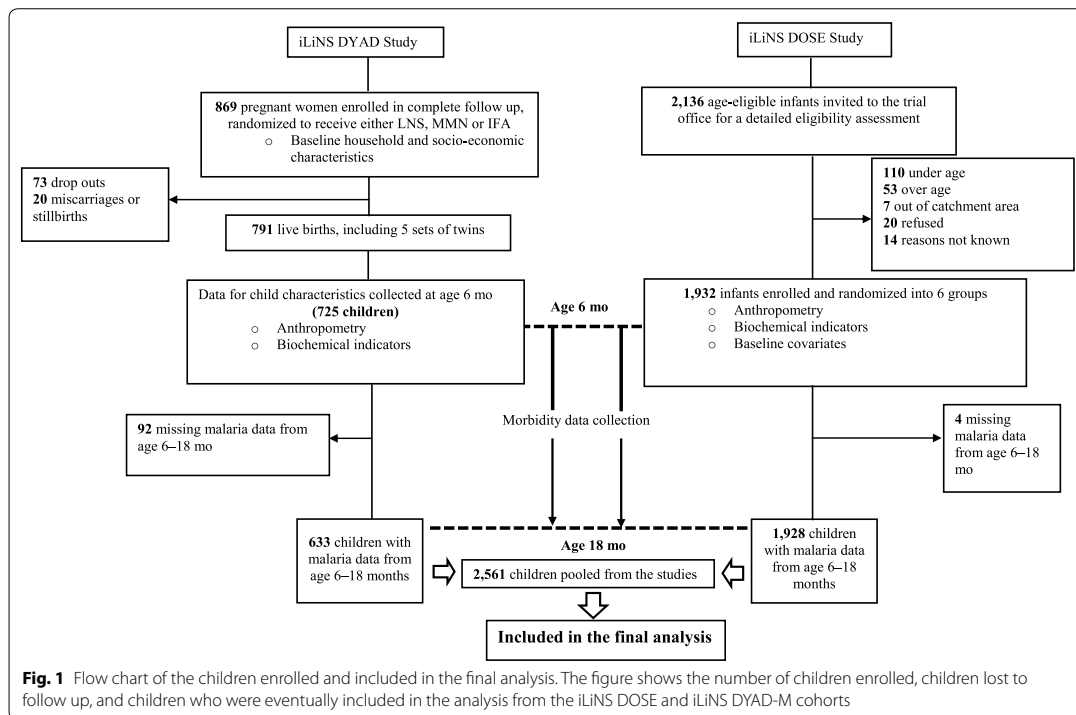
The children contributed 2405.6 child years of follow up, i.e. the mean (SD) duration of follow up was 344 (73) days/child. During the home visits, 27,340 morbidity episodes were reported, 9.3% (2549/27,340) of which were episodes of 'presumed' malaria. The rest were due to: ARI, 53.8% (14,708/27,340); diarrhoea, 23% (6282/27,340) and minor conditions, 13.9% (3801/27,340).

Of the total morbidity episodes identified at the home visits, 44% (12,048/27,340) were reported and treated at a health facility, 32.5% (3917/12,048) of which were treated for malaria, 76.8% (3007/3917) of them confirmed by RDT.

From the home visits data, the mean (SD) incidence of all illnesses combined was 16.2 (13.1) episodes per child year. The mean (SD) incidence of 'presumed' malaria was 1.1 (1.6) episodes per child year. The mean (SD) incidence of acute respiratory infections was 7.8 (8.0) episodes per child year and the mean (SD) incidence of diarrhoea was 2.7 (2.7) episodes per child year. Conversely, the national data show that diarrhoea incidence is higher than ARI incidence in children under-five, probably because the age ranges are slightly different. The national data do not provide specific incidences for children under 18 months.

From the hospital visits data, the mean (SD) incidences of clinical malaria and confirmed malaria were, respectively, 0.4 (0.8) and 1.3 (2.0) episodes per child year (i.e. the mean (SD) incidence of all malaria at hospital visits was 1.7 (2.4) episodes per child year).

The prevalence of malaria parasitaemia (by microscopy) increased from 4.8% at age 6 months to 9.6% at age 18 months. During the 12-month follow up period, 45.1% (1156/2561) of the children included in this analysis did not report any episode of malaria, 28.4% (728/2561) reported one episode and 26.4% (677/2561) reported > 1 malaria episodes. The children who reported > 1 malaria episodes were responsible for 71.4% (1821/2549) of all malaria episodes reported in the two studies. These findings were similar across the different definitions of malaria.



#### Association of LAZ at 6 months with malaria from 6 to 18 months

In bivariate analysis, a higher LAZ at 6 months was associated with a lower incidence of clinical malaria and lower incidence of confirmed malaria from 6 to 18 months (Table 2, unadjusted), and lower prevalence of malaria parasitaemia at 18 months (Table 3, unadjusted), but not incidence of 'presumed' malaria (Table 2, unadjusted). When adjusted for other predictors, these associations were no longer evident (Tables 2, 3, adjusted, and 4).

#### Association of WLZ at 6 months with malaria from 6 to 18 months

There was no association between WLZ at 6 months and the incidence of 'presumed' malaria, clinical malaria or confirmed malaria from 6 to 18 months, in either bivariate or multivariate analyses (Table 2). In multivariate analysis, higher WLZ at 6 months was associated with lower prevalence of malaria parasitaemia at 18 months, adjusted for other predictors (i.e. 1 SD higher WLZ at 6 months was associated with 20% decrease in prevalence of malaria parasitaemia at 18 months) (Tables 3, 4).

#### Association of WAZ at 6 months with malaria from 6 to 18 months

In bivariate analysis, a higher WAZ at 6 months was associated with lower incidence of confirmed malaria from 6 to 18 months (Table 2, unadjusted), and lower prevalence of malaria parasitaemia at 18 months (Tables 3, unadjusted, 4), but not incidence of 'presumed' malaria nor incidence of clinical malaria (Table 2, unadjusted). When adjusted for other predictors, these associations were no longer evident (Tables 2, 3, adjusted and 4).

#### Independent predictors of malaria

Independent predictors that were significantly associated with malaria outcomes are presented in Tables 3, 4. Incidence of 'presumed' malaria from 6 to 18 months was independently associated with maternal age and asset score at 6 months (i.e. each year higher in maternal age was associated with 1% increase in incidence of 'presumed' malaria; and 1 SD higher asset score was associated with 14% decrease in incidence of 'presumed' malaria).

Incidence of clinical malaria from 6 to 18 months was independently associated with Hb concentration and HFAS at 6 months (i.e. each g/L higher Hb at 6 months

**Table 1 Participant characteristics at age 6 months**

	Mean (SD) or n (%)	N
<i>Child factors</i>		
Mean (SD) weight-for-length z-score	0.3 (1.1)	2561
Mean (SD) weight-for-age z-score	− 0.7 (1.2)	2561
Mean (SD) length-for-age z-score	− 1.4 (1.1)	2561
Mean (SD) haemoglobin, g/L	104 (16)	2523
Proportion of boys	1265 (49.4%)	2561
Proportion wasted	50 (2.0%)	2561
Proportion underweight	330 (12.9%)	2561
Proportion stunted	725 (28.3%)	2561
Proportion with haemoglobin < 105 g/L	1334 (51.5%)	2523
Proportion with ZPP > 70 $\mu$ mol/mole haem <sup>a</sup>	1567 (66.1%)	2371
Proportion with malaria positive by RDT	349 (14.6%)	2389
<i>Maternal factors</i>		
Mean (SD) maternal age (years)	25.8 (6.2)	2265
Mean (SD) maternal education, completed years	4.4 (3.6)	2425
<i>Socio-economic and household factors</i>		
Mean (SD) Household Food Insecurity Access Score	6.1 (5.6)	2128
Mean (SD) asset score	− 0.02 (0.99)	2179
Mean (SD) number of children aged < 5 years in the household	1.6 (0.7)	2149
Proportion who slept under ITN daily <sup>b</sup>	2065 (81.1%)	2546
<i>Environmental factors</i>		
Proportion who received LNS intervention	1804 (70.6%)	2556
<i>Study site</i>		
Mangochi	1667 (65.2%)	2561
Namwera	445 (17.4%)	2561
Malindi	120 (4.7%)	2561
Lungwena	324 (12.7%)	2561

ITN, insecticide-treated bed nets; LNS, lipid-based nutrient supplement; RDT, malaria antigen rapid diagnostic test; ZPP, zinc protoporphyrin

<sup>a</sup> Measured from unwashed venous blood

<sup>b</sup> A week prior to the day of assessment

was associated with 1% decrease in incidence of clinical malaria; and 1-unit higher HFIAS was associated with 2% increase in incidence of clinical malaria). Higher HFIAS represents higher degree of food insecurity. There was also a significant variation in the incidence of clinical malaria between the health facilities (study sites), with Namwera site reporting higher incidence of clinical malaria compared to Mangochi, while the other two sites (Malindi and Lungwena) had lower incidence of clinical malaria compared to Mangochi.

Incidence of confirmed malaria from 6 to 18 months was independently associated with iron deficiency, maternal education and asset score at 6 months (i.e. iron deficiency at 6 months was associated with 20% higher incidence of confirmed malaria; each year higher in maternal schooling was associated with 3% decrease in incidence of confirmed malaria; 1 SD higher asset score was associated with 27% decrease in incidence of

confirmed malaria). There was also a significant variation in the incidence of confirmed malaria between the study sites with Namwera site reporting higher incidence of confirmed malaria compared to Mangochi, while the other two sites (Malindi and Lungwena) reported lower incidence of confirmed malaria compared to Mangochi.

Independent predictors of malaria parasitaemia prevalence were WLZ, maternal age, maternal education, Hb concentration, and iron deficiency (i.e. 1 SD higher WLZ was associated with 20% decrease in prevalence of malaria parasitaemia (see Tables 3, 4); each year higher in maternal age was associated with 3% decrease in prevalence of malaria parasitaemia; each additional year in maternal schooling was associated with 10% decrease in prevalence of malaria parasitaemia; each g/L higher Hb at 6 months was associated with 2% decrease in prevalence of malaria parasitaemia; and iron deficiency at

**Table 2 Association of length-for-age, weight-for-length, and weight-for-age z-scores at age 6 months with malaria incidence from age 6 months to 18 months**

Predictor at age 6 months	Incidence of 'presumed' malaria (N = 2561)			Incidence of clinical malaria (N = 2497)			Incidence of confirmed malaria (N = 2497)					
	Unadjusted		Adjusted <sup>a</sup>	Unadjusted		Adjusted <sup>a</sup>	Unadjusted		Adjusted <sup>a</sup>			
	IRR <sup>b</sup> (95% CI)	p-value	IRR <sup>b</sup> (95% CI)	p-value	IRR <sup>b</sup> (95% CI)	p-value	IRR <sup>b</sup> (95% CI)	p-value	IRR <sup>b</sup> (95% CI)	p-value		
LAZ	0.98 (0.94 to 1.02)	0.370	1.03 (0.98 to 1.09)	0.394	0.91 (0.85 to 0.98)	0.011	0.98 (0.89 to 1.07)	0.703	0.92 (0.87 to 0.97)	0.003	1.00 (0.94 to 1.07)	0.882
WLZ	0.96 (0.92 to 1.01)	0.098	0.97 (0.92 to 1.02)	0.190	0.99 (0.95 to 1.04)	0.762	1.03 (0.94 to 1.12)	0.571	0.99 (0.94 to 1.04)	0.623	1.00 (0.94 to 1.06)	0.950
WAZ	0.97 (0.93 to 1.01)	0.094	1.10 (0.50 to 2.42)	0.810	0.95 (0.88 to 1.01)	0.093	1.07 (0.29 to 3.91)	0.916	0.95 (0.90 to 0.99)	0.030	1.46 (0.48 to 4.40)	0.506

CI: confidence interval; IRR: incidence rate ratio; LAZ: length-for-age z-score; WLZ: weight-for-length z-score

<sup>a</sup> Adjusted for the following factors at age 6 months: sex of the child, haemoglobin concentration, iron status, month of birth, daily use of insecticide-treated bed nets, distance to health facility, study site, asset scores, maternal age, maternal education, children < 5 years, HFIAs score, and whether the child received the study intervention (LNS) or not

<sup>b</sup> Incidence rate ratio, obtained using negative binomial regression. The IRR represents the rate of change in incidence of malaria for each 1-SD higher LAZ, WAZ, or WLZ



**Table 3 Association of length-for-age, weight-for-length, and weight-for-age z-scores at age 6 months with prevalence of malaria parasitaemia at age 18 months**

	Prevalence of malaria parasitaemia at age 18 months (N = 1662) <sup>a</sup>			
	Unadjusted		Adjusted <sup>b</sup>	
	PR <sup>c</sup> (95% CI)	p-value	PR <sup>c</sup> (95% CI)	p-value
<i>Predictor at age 6 months</i>				
LAZ	0.82 (0.72 to 0.93)	0.003	1.11 (0.93 to 1.33)	0.259
WLZ	1.01 (0.88 to 1.16)	0.877	0.80 (0.67 to 0.94)	0.007
WAZ	0.85 (0.75 to 0.96)	0.007	0.88 (0.75 to 1.03)	0.115

CI: confidence interval; LAZ: length-for-age z-score; PR: prevalence ratio; WAZ: weight-for-age z-score; WLZ: weight-for-length z-score

<sup>a</sup> Positive result from malaria microscopy readings

<sup>b</sup> Adjusted for the following factors at age 6 months: sex of the child, haemoglobin concentration, iron status, month of birth, daily use of insecticide-treated bed nets, distance to health facility, study site, asset scores, maternal age, maternal education, children < 5 years, HFIA score, and whether the child received the study intervention (LNS) or not

<sup>c</sup> Prevalence ratio, obtained using a modified Poisson regression (with a robust variance estimator) [29]. The PR represents the rate of change in prevalence of malaria parasitaemia for each 1-SD higher LAZ, WAZ, or WLZ

6 months was associated with 111% higher prevalence of malaria parasitaemia).

## Discussion

The study hypothesis was that lower length for age z-scores (LAZ) and lower weight for length z-scores (WLZ) at 6 months will be associated with a higher incidence of malaria from 6 to 18 months and higher prevalence of malaria parasitaemia at 18 months. In a sample of 2561 Malawian children, LAZ at 6 months was not associated with incidence of malaria from 6 to 18 months, nor prevalence of malaria parasitaemia at 18 months. Higher WLZ at 6 months was associated with lower prevalence of malaria parasitaemia at 18 months for every 1 SD higher WLZ at 6 months), but not with incidence of malaria from 6 to 18 months.

The longitudinal study design helped to determine the temporality of the associations because (1) malaria outcomes were obtained from 6 to 18 months after identifying the predictors at 6 months, and (2) malaria is less

**Table 4 Independent predictors of incidence and prevalence of malaria in multivariate analysis**

	Incidence of 'presumed' malaria (N = 2561)		Incidence of clinical malaria (N = 2497)		Incidence of confirmed malaria (N = 2497)		Prevalence of malaria parasitaemia at age 18 months (N = 1662)	
	IRR <sup>a</sup> (95% CI)	p-value	IRR <sup>a</sup> (95% CI)	p-value	IRR <sup>a</sup> (95% CI)	p-value	PR <sup>b</sup> (95% CI)	p-value
<i>Child factors at age 6 months</i>								
Haemoglobin (g/L)	1.00 (0.99 to 1.01)	0.529	0.99 (0.98 to 1.00)	0.016	1.00 (0.99 to 1.01)	0.845	0.98 (0.97 to 0.99)	0.001
Iron deficiency <sup>c</sup>	1.04 (0.91 to 1.18)	0.587	1.03 (0.83 to 1.28)	0.773	1.20 (1.04 to 1.39)	0.012	2.11 (1.24 to 3.57)	0.006
<i>Maternal factors at enrollment</i>								
Maternal age	1.01 (1.00 to 1.02)	0.011	0.99 (0.97 to 1.01)	0.149	1.00 (0.98 to 1.01)	0.200	0.97 (0.94 to 0.99)	0.033
Maternal education	0.99 (0.97 to 1.01)	0.427	1.00 (0.98 to 1.03)	0.783	0.97 (0.95 to 0.99)	0.024	0.90 (0.85 to 0.96)	0.001
<i>Household factors at enrollment</i>								
Household Food Insecurity Access Score	1.00 (0.99 to 1.01)	0.809	1.02 (1.01 to 1.04)	0.022	1.01 (0.99 to 1.02)	0.065	1.02 (0.99 to 1.05)	0.184
Asset scores	0.86 (0.80 to 0.93)	<0.001	0.94 (0.83 to 1.07)	0.343	0.73 (0.64 to 0.83)	<0.001	0.92 (0.63 to 1.34)	0.666
<i>Environmental factors</i>								
LNS intervention (vs control)	1.00 (0.88 to 1.14)	0.983	1.11 (0.89 to 1.37)	0.343	1.02 (0.86 to 1.22)	0.783	1.07 (0.70 to 1.62)	0.765
<i>Study site</i>								
Mangochi	Reference		Reference		Reference		Reference	
Namwera	0.94 (0.81 to 1.09)	0.457	1.49 (1.20 to 1.85)	<0.001	1.73 (1.50 to 1.99)	<0.001	1.49 (0.99 to 2.24)	0.054
Malindi	1.12 (0.88 to 1.42)	0.337	0.45 (0.27 to 0.73)	0.001	0.58 (0.43 to 0.78)	<0.001	0.74 (0.29 to 1.88)	0.531
Lungwena	0.91 (0.75 to 1.09)	0.303	0.24 (0.14 to 0.41)	<0.001	0.33 (0.25 to 0.44)	<0.001	0.65 (0.36 to 1.19)	0.163

Other factors included in the models but not significant were: sex of the child, birth month, number of children in household, and daily use of insecticide-treated bed nets

CI: confidence interval; IRR: incidence rate ratio; LNS: lipid-based nutrient supplements; PR: prevalence ratio; ZPP: zinc protoporphyrin

<sup>a</sup> Incidence rate ratio, obtained using negative binomial regression. The IRR represents the rate of change in incidence of malaria (for each 1-unit higher in the continuous predictors or for each group compared to the reference group in the categorical predictors), adjusted for other variables

<sup>b</sup> Prevalence ratio, obtained using a modified Poisson regression (with a robust variance estimator) [29]. The PR represents the rate of change in prevalence of malaria parasitaemia (for each 1-unit higher in the continuous predictors or for each group compared to the reference group in the categorical predictors), adjusted for other variables

<sup>c</sup> ZPP > 70 μmol/mol heme [28]



common in children <6 months due to the protective effect of fetal haemoglobin and maternal antibodies [30–32]. Another strength of the study was the enhancement of morbidity recall using a daily pictorial calendar which minimized recall bias associated with home morbidity assessments [22, 23]. The study had a long follow up which allowed assessment of the associations in all seasons, and by pooling data from two large cohorts we ended up with a large sample size with adequate power to detect associations.

The main weakness of the study is the change in the exposure (anthropometric status) during the follow up. There was significant decrease in mean LAZ, WAZ, and WLZ from 6 months to 18 months. Compared to baseline, more children had developed stunting, underweight, and wasting by 18 months (41.5% vs 28.3%, 16.5% vs 12.9%, and 5% vs 2% respectively), indicating that some children became nutritionally worse off during follow up which may have led to misclassification bias.

There is possibility of residual confounding due to unmeasured factors associated with malaria including HIV infection [33], polyparasitism [34], vitamin A and zinc deficiency [35–38], and genetic variations including haemoglobinopathies [39–41].

Presumptive diagnosis of malaria may also have led to overestimating the outcome whereas the use of RDT-confirmed malaria may have led to under-estimating the outcome because (1) 23% of the children were treated for malaria at the hospital without RDT confirmation, and (2) children who had malaria episodes but received home treatment or no treatment and did not present to the hospitals were missed (only 44% of the morbidity episodes were treated at the hospital). However, the consistency of the results across the different malaria definitions suggests that this weakness did not bias the results, and considering the strengths of this study, the conclusions are reasonably valid.

The finding of no association between stuntedness at 6 months and malaria in the subsequent 12-month period is similar to results of previous studies [42–45], which suggests that stunting may be of little significance in malaria epidemiology. These results are however different from those of other studies that reported an increased risk of malaria associated with stunting. In the Gambia, a prospective study of children aged <5 years reported an increased risk of malaria associated with stunting (crude RR = 1.35; 95% CI 1.08–1.69) [9]. The follow up was very short (during the malaria season that lasted only 20 weeks), and although the outcomes were adjusted for age, sex, and ethnicity, crude RR were reported. The authors also did not adjust for socio-economic and household factors therefore could not rule out the influence of these confounders in their results. In

Kenya, stunting in children aged 0–36 months was associated with an increased odds of malaria parasitaemia (odds ratio = 1.98,  $p < 0.0001$ ) [10]. The cross-sectional design of this study suggests the observed association may have been due to reverse causation, i.e. the cumulative deleterious effects of malaria on linear growth. In Uganda, mild stunting (IRR = 1.24, 95% CI 1.06–1.46) and moderate-severe stunting (IRR = 1.24, 95% CI 1.03–1.48) in children aged <2.5 years were associated with increased incidence of malaria parasitaemia [11]. However, although this was a cohort study, it was not clear whether exposure assessment preceded the outcomes, therefore, the temporal relationship between stunting and risk of malaria was difficult to ascertain.

However, a study in Papua New Guinea reported that lower LAZ was associated with lower incidence of malaria in the subsequent one-year period, attributed to increased interferon  $\gamma$  (IFN- $\gamma$ ) response to specific malarial antigens observed in stunted children, although the mechanisms were not fully explained [46]. Malaria varies with age (incidence of malaria decreases whereas prevalence of malaria parasitaemia increases with age) [13, 47, 48], therefore, the wide age range of the study participants (from 10 months to 10 years) makes for difficult comparisons between the studies. In the Papua New Guinea study, the authors noted that interferon- $\gamma$  release increased with age, the prevalence rates of splenomegaly and parasitaemia increased with age, whereas the incidence of malaria decreased with age, further muddling the interpretation of the findings.

In this population, supplementation with LNS did not alter malaria antibody acquisition [49], which was attributed to the finding that the nutritional supplements also did not promote infant growth [16]. Further analysis showed no difference in malaria antibody acquisition between the stunted and non-stunted children at 6 months (data not shown), supporting the null findings.

In this study, higher WLZ at 6 months was associated with lower prevalence of malaria parasitaemia at 18 months, when adjusted for other predictors. Wasting is associated with low levels of leptin through depletion of fat mass [50]. Low levels of leptin can lead to reduced immune response [51], resulting in increased risk of malaria associated with wasting. So far, the evidence of association (increased or decreased risk) of malaria with WLZ has mainly come from cross-sectional studies [10, 52, 53]. A recent systematic review determined that the relationship between wasting and risk of malaria is hitherto inconclusive with most longitudinal studies reporting no association [54]. This association was not conclusive and may be due to chance (e.g., because of multiple testing [55]), considering that WLZ was

significantly associated with prevalence of malaria parasitaemia only and not with the other malaria outcomes.

In this study, only 30% of the children reported frequent malaria episodes. Knowledge of this over-dispersion in malaria infections is important because interventions targeted at this core group could be the most effective [7]. However, providing routine screening to identify such children is difficult. Platforms such as growth monitoring and promotion clinics (GMP) provide an entry point to preventive and curative health care and have been associated with significant reductions in malnutrition and mortality [56].

Such platforms can be useful for malaria screening especially in malaria hypoendemic or mesoendemic settings where targeting malaria interventions to undernourished children has potential to reduce malaria morbidity and mortality [57]. In this setting, socio-economic factors such as household asset scores, maternal education, and food insecurity were significantly associated with malaria. There were also variations in hospital-diagnosed malaria by study site, with the Namwera site reporting higher incidences of clinical and RDT-confirmed malaria compared to the other three study sites. These factors may be more important than anthropometric status in malaria epidemiology but were not explored further in this analysis.

## Conclusions

In conclusion, in children aged 6 to 18 months living in this malaria-endemic setting, LAZ, WAZ, and WLZ do not predict subsequent malaria incidence when adjusted for other predictors. However, WLZ may be associated with prevalence of malaria parasitaemia. Socio-economic factors and micro-geographic variations may explain most of the over-dispersion in malaria infections in this setting, but these require further study.

## Abbreviations

CI: confidence interval; GMP: growth monitoring and promotion; Hb: hemoglobin; HFIA: household food insecurity access; HIV: human immunodeficiency virus; IFA: iron and folic acid; IMCI: integrated management of childhood illness; iLiNS: international lipid-based nutrient supplements project; ITNs: insecticide-treated bed nets; LAZ: length-for-age z-scores; LNS: lipid-based nutrient supplements; MMN: multiple micronutrients; RDT: malaria rapid diagnostic test; PR: prevalence ratio; SD: standard deviation; WAZ: weight-for-age z-scores; WHO: World Health Organization; WLZ: weight-for-length z-scores; ZPP: zinc protoporphyrin.

## Authors' contributions

The authors' responsibilities were as follows: KM, KGD, UA, and PA designed the study; JB, NP, LH, UA, KGD, PA, and KM conducted the study; JB analysed the data and wrote the paper, with critical input and comments from all other authors; JB and KM had primary responsibility for final content. All authors read and approved the final manuscript.

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## Acknowledgements

The authors thank the study participants, the local communities of Mangochi, the health service staff and their research personnel at the study sites as well as members of the trial's data safety and monitoring board, the iLiNS extended research team and the iLiNS-Project Steering Committee for their contributions in all stages of the study.

## Competing interests

The authors declare that they have no competing interests.

## Availability of data and materials

The datasets used and analysed during this study are available from the corresponding author on reasonable request.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

The study was performed according to International Conference of Harmonization-Good Clinical Practice (ICH-GCP) guidelines and the ethical standards of the Helsinki Declaration. The protocol was reviewed and approved by the Institutional Review Boards of the University of Malawi, College of Medicine (IRB Reference Number P01/09/722) and the Pirkanmaa Hospital District, Finland (IRB Reference Number R09130). At least one guardian signed or thumb-printed an informed consent form before enrolment of each participant. An independent data safety and monitoring board monitored the incidence of suspected serious adverse events during the trial.

## Funding

This publication is funded by a Grant to the University of California, Davis from the Bill & Melinda Gates Foundation. The findings and conclusions contained within the article are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of this manuscript.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 28 February 2019 Accepted: 13 April 2019

Published online: 22 April 2019

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# PUBLICATION II

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BMC Pediatrics volume 18, 396 (2018)  
<https://doi.org/10.1186/s12887-018-1378-2>

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RESEARCH ARTICLE

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# The association of malaria morbidity with linear growth, hemoglobin, iron status, and development in young Malawian children: a prospective cohort study

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## Abstract

**Background:** Although poor complementary feeding is associated with poor child growth, nutrition interventions only have modest impact on child growth, due to high burden of infections. We aimed to assess the association of malaria with linear growth, hemoglobin, iron status, and development in children aged 6–18 months in a setting of high malaria and undernutrition prevalence.

**Methods:** Prospective cohort study, conducted in Mangochi district, Malawi. We enrolled six-months-old infants and collected weekly data for 'presumed' malaria, diarrhea, and acute respiratory infections (ARI) until age 18 months. Change in length-for-age z-scores (LAZ), stunting, hemoglobin, iron status, and development were assessed at age 18 months. We used ordinary least squares regression for continuous outcomes and modified Poisson regression for categorical outcomes.

**Results:** Of the 2723 children enrolled, 2016 (74.0%) had complete measurements. The mean (standard deviation) incidences of 'presumed' malaria, diarrhea, and ARI, respectively were: 1.4 (2.0), 4.6 (10.1), and 8.3 (5.0) episodes/child year. Prevalence of stunting increased from 27.4 to 41.5% from 6 to 18 months. 'Presumed' malaria incidence was associated with higher risk of stunting (risk ratio [RR] = 1.04, 95% confidence interval [CI] = 1.01 to 1.07,  $p = 0.023$ ), anemia (RR = 1.02, 95%CI = 1.00 to 1.04,  $p = 0.014$ ) and better socio-emotional scores ( $B = -0.21$ , 95%CI =  $-0.39$  to  $-0.03$ ,  $p = 0.041$ ), but not with change in LAZ, haemoglobin, iron status or other developmental outcomes. Diarrhea incidence was associated with change in LAZ ( $B = -0.02$ ; 95% CI =  $-0.03$  to  $-0.01$ ;  $p = 0.009$ ), stunting (RR = 1.02; 95% CI = 1.01 to 1.03;  $p = 0.005$ ), and slower motor development. ARI incidence was not associated with any outcome except for poorer socio-emotional scores.

**Conclusion:** In this population of young children living in a malaria-endemic setting, with active surveillance and treatment, 'presumed' malaria is not associated with change in LAZ, hemoglobin, or iron status, but could be associated with stunting and anemia. Diarrhea was more consistently associated with growth than was malaria or ARI. The findings may be different in contexts where active malaria surveillance and treatment is not provided.

**Trial registration:** NCT00945698 (July 24, 2009) and NCT01239693 (November 11, 2010).

**Keywords:** Children, Growth faltering, Malaria, Morbidity, Infections, Stunting, iLiNS studies, Longitudinal studies

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## Introduction

Although poor complementary feeding is associated with poor child growth, many interventions designed to improve complementary foods only have modest impact on growth [1], possibly due to a high burden of infections in children [2, 3]. Studies in which morbidity treatment was integrated with a complementary feeding intervention demonstrated improved linear growth [4] and developmental outcomes in children [5, 6], suggesting the importance of reducing the burden of infections along with improved diet to promote child growth and development.

Longitudinal studies have reported a significant inverse association of diarrhea with growth [7–9]. However, studies on the association of malaria with growth and development have either reported inconsistent results or had cross-sectional designs, which makes it difficult to assess causality or directionality of association [10–13]. This has prevented the inclusion of malaria as a determinant of stunting in the Lives Saved Tool (LiST) model [14].

The International Lipid-based Nutrient Supplements (iLiNS) Project DOSE and DYAD-M studies were randomized controlled trials conducted in Malawi to study the impact of lipid-based nutrient supplements (LNS) on growth of children [15, 16]. The aim of this analysis was to assess the association of malaria with linear growth, hemoglobin, iron status, and child development. Our hypothesis was that linear growth, hemoglobin, iron status, and developmental outcomes at age 18 mo would be poorer in children with higher incidence of malaria from age 6 to 18 mo. We also analyzed the association of diarrhea and acute respiratory infections (ARI) with linear growth, hemoglobin, iron status, and developmental outcomes.

## Methods

### Study setting

The iLiNS-DOSE and iLiNS-DYAD-M studies were conducted in one public district hospital (Mangochi), one mission hospital (St Martins), and two rural public health centers (Lungwena and Namwera) in Mangochi District, Southern Malawi. The total catchment population of 180,000 largely subsisted on farming and fishing. In Malawian children aged <5 years, the prevalence of reported fever (a proxy for malaria), diarrhea and ARI was 29, 22 and 5%, respectively, with seasonal fluctuations [17]. Malaria is endemic in Malawi and the study area has high malaria transmission with high temperature and frequent rainfall from October through April [18].

### Study design and data collection

In the iLiNS-DOSE study, 6-mo old children were randomly allocated to one of five intervention groups provided with different doses or formulations of LNS or to a control group that did not receive LNS during

the 12-mo study period, between November 2009 and May 2012. In the iLiNS-DYAD-M study, pregnant women <20 weeks' gestation were randomly allocated to one of three groups to receive iron and folic acid (IFA), multiple micronutrients (MMN) or a small-quantity (20 g) of LNS daily. After delivery, women in the IFA group received placebo tablets, while MMN and LNS supplementation was continued up to 6 mo postpartum. Children of mothers in the LNS group also received LNS 10 g twice daily from age 6 to 18 mo. This study was conducted from February 2011 to April 2015. Details of study design, randomization and enrolment for the two studies were explained in the main outcome papers [15, 16].

In both studies, research assistants visited the children's homes every week from age 6 to 18 mo to interview the guardians about the child's health in the previous 7 days using a structured questionnaire. The information was complemented by a picture calendar filled out by the guardians daily to aid memory of their children's morbidity status. The use of maternal interviews as a means of collecting data on child morbidity has been validated in previous studies [19, 20]. The research assistants referred all cases of 'presumed' malaria (presence of fever) to the nearby health facility for treatment with lumefantrine/artemether, the nationally recommended antimalarial drug. The children were followed throughout the year, covering periods of both high and low malaria transmission.

Anthropometric measurements were taken at age 6 mo and 18 mo. Study anthropometrists measured the infant's length with a high-quality length board (Harpender Infantometer; Holtain Limited) and recorded it to the nearest 1 mm. They weighed unclothed infants with electronic infant weighing scale (SECA 735; Seca GmbH & Co), recording to the nearest 10 g. The anthropometrists were trained and their measurement reliability was verified at the start of the study and at 6-mo intervals thereafter with methods adapted from the procedures used in the WHO Multicentre Growth Reference Study [21]. The anthropometrists calibrated all equipment with standard weights and length rods daily.

We assessed iron status at age 6 mo and 18 mo by measuring the zinc protoporphyrin (ZPP) concentration in unwashed venous blood sample using a hematofluorometer (206D, AVIV Biomedical Inc., Lakewood, NJ, USA). About 5–7 ml of blood was collected by venepuncture using a 23-gauge needle into 7.5 ml evacuated, trace element-free polyethylene tubes containing lithium heparin (Sarstedt AG & Co, Nümbrecht, Germany). The samples were kept covered in aluminium and away from light, in a refrigerator or on ice, and processed within 2 h of collection. We measured blood hemoglobin (Hb) concentration at age 6 mo and 18 mo from a drop of blood taken from a finger prick and collected in a microcuvette. Hb analysis



was conducted on-site using a Hemo-Cue instrument (Hemocue 201+, HemoCue® AB, Ängelholm, Sweden).

We assessed fine and gross motor development at age 18 mo using the Kilifi Developmental Inventory (KDI) developed in Kenya [22]. Language development was assessed using a 100-word vocabulary checklist by maternal interview based on the MacArthur-Bates Communicative Development Inventory [23] adapted for the local languages, and 18-mo socio-emotional development was assessed using the Profile of Social and Emotional Development (PSED), also developed in Kenya. The child's mood during the KDI assessment was rated as positive (smiling/laughing) or not positive (crying/inconsolable, 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 the assessor). The child's activity level during the KDI was rated as positive (active and maintains interest) or not positive (unarousable, sleepy and can hardly be awakened, sleepy but easily awakened, does not spontaneously engage in activity, and awake but loses interest). The KDI, vocabulary, and PSED scores showed high inter-rater agreement and moderate to high test-retest reliability in this study setting [24, 25].

#### Definition of the predictors and the outcomes

We used a presumptive diagnosis of malaria derived from episodes of fever during the previous week, reported by the guardians. To ensure the diagnoses were mutually exclusive, we created an algorithm whereby any fever with a diarrhea episode (three or more loose stools in 24 h) was categorized as diarrhea; any fever in the presence of any respiratory symptoms (cough, rapid or difficult breathing and nasal discharge) was categorized as ARI. 'Presumed' malaria was defined as any fever episode in the absence of diarrhea and respiratory symptoms.

An episode of 'presumed' malaria, ARI or diarrhea was defined as the period starting from the day the child had the symptoms when preceded by at least 2 days of no symptoms or no data. The episode ended on the last day the child had the symptoms which was then followed by at least 2 symptom-free days. Incidence of 'presumed' malaria, ARI or diarrhea for each child from age 6 to 18 mo was calculated as total episodes / total follow up years at risk.

Longitudinal prevalences of common childhood symptoms (fever, diarrhea, and cough) from age 6 to 18 mo were defined as the number of days with the symptom divided by the total number of days of observation for each child [26].

We calculated age- and sex-standardized anthropometric indices [length-for-age z score (LAZ), weight-for-age z score (WAZ), and weight-for-length z score (WLZ)] based

on the WHO Child Growth Standards [21] and considered values below  $-2.0$  indicative of underweight, stunting and wasting, respectively. Change in LAZ for each child was calculated as the difference between LAZ at age 18 mo and LAZ at age 6 mo.

Iron deficiency at age 6 mo and 18 mo was defined as whole blood ZPP  $>70$   $\mu\text{mol/mole}$  heme [27]. Anemia at age 6 mo was defined as blood Hb concentration  $<105$  g/L [28] while anemia at age 18 mo was defined as blood Hb concentration  $<110$  g/L [29].

From the child development data at age 18 mo, fine motor scores were calculated as the sum of 34 KDI fine motor items, each scored 0 or 1, gross motor scores were calculated as the sum of 35 KDI gross motor items, each scored 0 or 1 [22] and vocabulary score was the maternal-reported child expressive vocabulary out of the 100-word checklist. For these outcomes, moderate to severe delay was defined as the bottom 25% of the sample. The socio-emotional score was calculated as the sum of 19 PSED items. Moderate to severe delay was defined as the top 25% of our sample (a higher score indicates less advanced socio-emotional development).

#### Statistical analysis

We included in the analysis children who had outcomes measured at age 18 mo. For all continuous outcomes, we used ordinary least squares regression to assess the association between malaria incidence and each outcome; and for all binary outcomes, we used modified Poisson regression (with a robust variance estimator) [30].

We first assessed whether the relationship between the predictor and each outcome differed between the two studies. However, the interaction term was not statistically significant indicating that this relationship was not different between the two studies therefore we pooled data from the two cohorts. We then constructed multivariate models to determine which variables independently predicted the outcomes. We included all theoretically relevant variables, regardless of whether they were statistically significant or not after the bivariate analysis. The following variables collected at age 6 mo were included in the models: child sex, LAZ, WLZ, Hb, iron status, maternal education and household food insecurity access (HFIA) score generated by summing the value of responses to nine questions regarding food insecurity [31]. We also included in the models, from age 6 to 18 mo, the incidence of diarrhea and ARI, and whether the child received an intervention (LNS) or not. For the risk of stunting at age 18 mo, we included in the model stunting at age 6 mo (in place of LAZ). In addition, all developmental outcomes were adjusted for the child's mood, activity level, age and interaction with the assessor.

We assessed collinearity among the variables (e.g. LAZ vs WLZ at age 6 mo). If the variables were highly collinear

(> 0.5), we dropped the one that was less strongly associated with the outcomes. We accounted for intracluster correlation due to twins using generalised estimating equations [32].

We also performed exploratory analyses by using frequency of malaria episodes (from age 6 to 18 mo) as a categorical variable (no episode, one episode, and > 1 episodes groups). In addition, we conducted stratified analyses by stunting at age 6 mo. Although we performed bivariate analyses for each individual variable, we will only report the results from multivariate analysis.

We used Stata version 14 (StataCorp, Texas, USA) for all the analyses.

## Results

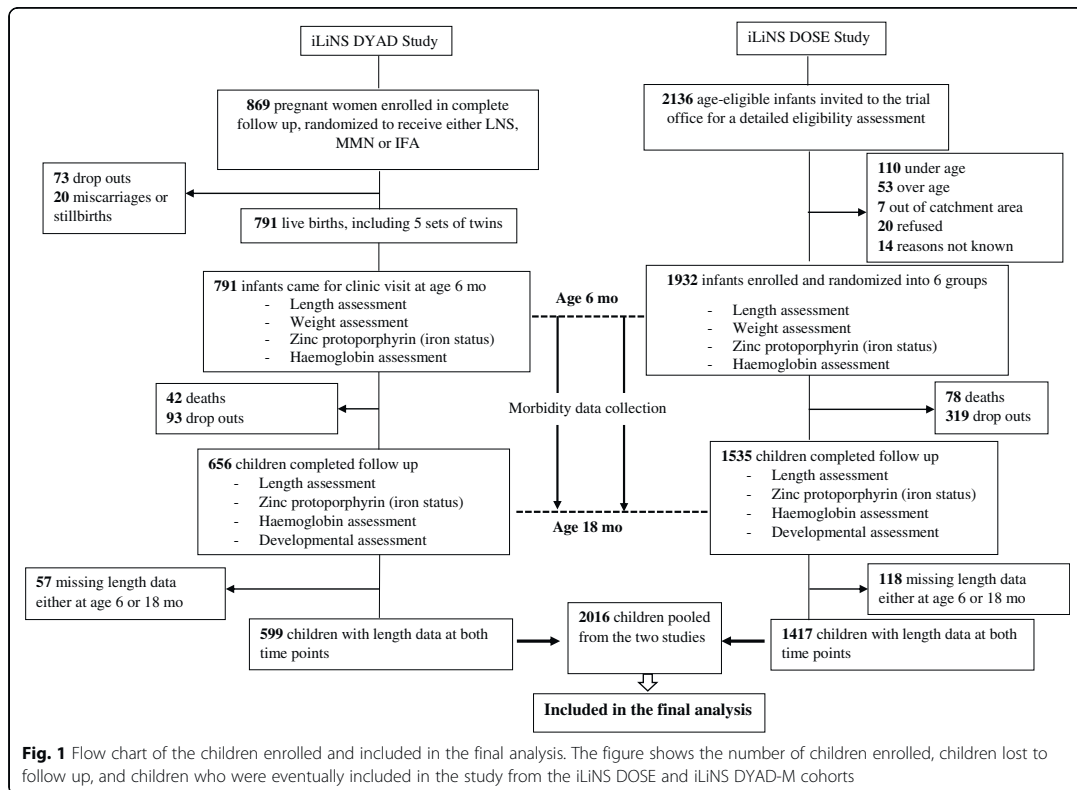
### Baseline characteristics and descriptive statistics

Of the 2723 children enrolled in the two study cohorts, 2016 (74.0%) had length measured both at age 6 mo and 18 mo (1417 children from the iLiNS DOSE study and 599 children from the iLiNS DYAD-M study). These were included in the final analysis (Fig. 1). The characteristics of these children at age 6 mo are summarized in Table 1.

The 2016 children included in the final analysis contributed 1647.9 child years of follow up, i.e. the mean (standard deviation) [SD] duration of follow up was 298 (61) days / child. A total of 24,024 morbidity episodes were reported during the home visits. Of these, 9.7% (2324/24024) were episodes of ‘presumed’ malaria. The rest of the morbidity episodes were due to: acute respiratory infections (ARI), 55.6% (13,360/24024); diarrhea, 33.6% (8083/24024); and minor conditions, 1.1% (257/24024).

Overall, the mean (SD) incidence of all illnesses combined was 14.8 (6.8) episodes per child year. The mean (SD) incidence of ‘presumed’ malaria was 1.4 (2.0) episodes per child year. The mean (SD) incidence of ARI was 8.3 (5.0) episodes per child year and the mean (SD) incidence of diarrhea was 4.6 (10.1) episodes per child year. The longitudinal prevalences of common childhood symptoms (fever, diarrhea, and cough) from age 6 to 18 mo were: 7.5%; 3.4%; and 11.7%, respectively.

During the 12-mo follow up period, 39.0% (787/2016) of the children did not report any episode of ‘presumed’ malaria, 30.3% (611/2016) reported one episode and 30.7% (618/2016) reported > 1 malaria episodes. The children who reported > 1 malaria episodes were responsible for



**Table 1** Participant characteristics at age 6 mo

Variable	DOSE <sup>a</sup>	DYAD-M <sup>b</sup>	Pooled data <sup>a</sup>
Number of children <sup>b</sup>	1417	599	2016
Proportion of boys	50.5%	47.6%	49.6%
Mean (SD) weight, kg	7.0 (1.0)	7.3 (1.0)	7.1 (1.0)
Mean (SD) length, cm	63.4 (2.4)	64.2 (2.6)	63.6 (2.5)
Mean (SD) weight-for-age z-score	-0.7 (1.1)	-0.5 (1.2)	-0.7 (1.2)
Mean (SD) length-for-age z-score	-1.4 (1.0)	-1.2 (1.1)	-1.4 (1.1)
Mean (SD) weight-for-length z-score	0.3 (1.1)	0.4 (1.1)	0.3 (1.1)
Mean (SD) hemoglobin, g/L	103.6 (16.1)	103.7 (15.3)	103.6 (15.9)
Proportion with LAZ < -2 scores	29.1%	23.4%	27.4%
Proportion with Hb < 105 g/L	50.6%	54.4%	51.7%
Proportion with ZPP > 70 $\mu$ mole/mole heme <sup>c</sup>	68.7%	69.8%	69.0%
Proportion with malaria <sup>d</sup>	16.7%	9.7%	14.5%
Mean (SD) maternal education, completed years	4.7 (3.6)	3.8 (3.5)	4.4 (3.6)
Mean (SD) maternal age, years	26.1 (6.2)	25.1 (6.0)	25.8 (6.1)
Mean (SD) Household Food Insecurity Access Score	6.5 (6.0)	5.0 (4.3)	6.0 (5.6)

<sup>a</sup>Values are n, mean (SD) or proportions

<sup>b</sup>Children who had length data at age 6 mo and 18 mo

<sup>c</sup>Measured from unwashed venous blood

<sup>d</sup>Measured by malaria antigen Rapid Diagnosis Test (mRDT)

73.7% (1713/2324) of all 'presumed' malaria episodes reported in the two studies.

At age 18 mo, the mean (SD) length-for-age z-scores (LAZ), weight-for-age z-scores (WAZ) and weight-for-length (WLZ) scores were -1.8 (1.1), -1.0 (1.1) and -0.2 (1.1) respectively. The proportions of children who were stunted, underweight and wasted were 41.5, 16.6 and 5.0%, respectively. The median (25th, 75th centile) zinc protoporphyrin (ZPP) concentration was 74 (51, 114)  $\mu$ mole/mole heme and the proportion with iron deficiency was 54.1%. The mean (SD) hemoglobin (Hb) concentration was 108.5 (15.1) g/L and the proportion with anemia was 50.5%. The mean (SD) scores for fine motor, gross motor, language and Profile of Social and Emotional Development (PSED) were 20.9 (2.2), 17.3 (2.6), 26.2 (5.0) and 16.2 (5.4) respectively.

#### Association of malaria with linear growth

The mean (SD) change in LAZ from age 6 to 18 mo was -0.44 (0.77). In multivariate analysis, there was no association between the incidence of 'presumed' malaria and change in LAZ from age 6 to 18 mo, adjusted for LAZ at age 6 mo ( $B = -0.02$ , 95% CI = -0.04 to 0.01,  $p = 0.069$ ) (Table 2).

The proportion of children who were stunted increased from 27.4% at age 6 mo to 41.5% at age 18 mo. In multivariate analysis, the incidence of 'presumed' malaria was associated with higher risk of stunting at age 18 mo, adjusted for stunting at age 6 mo (RR = 1.04, 95% CI = 1.01 to 1.07,  $p = 0.023$ ) (Table 2). When categorized by frequency

of malaria episodes and adjusted for stunting at age 6 mo, children with > 1 malaria episodes from age 6 to 18 mo had higher risk of stunting at age 18 mo compared to children with zero malaria episodes (RR = 1.39, 95% CI = 1.13 to 1.70,  $p = 0.002$ ).

#### Association of malaria with hemoglobin, anemia and iron status

The incidence of 'presumed' malaria from age 6 to 18 mo was associated with higher risk of anemia at age 18 mo, adjusted for hemoglobin at age 6 mo (RR = -0.12; 95% CI = -0.20 to -0.04;  $p = 0.002$ ) but not with hemoglobin or iron deficiency at age 18 mo (Table 3).

#### Association of malaria with child development

The association of incidence of 'presumed' malaria from age 6 to 18 mo with child development was significant for PSED scores ( $B = -0.21$ ; 95% CI = 0.39 to -0.03;  $p = 0.041$ ), but not for the other domains of child development, adjusted for the covariates listed in the footnotes to Tables 4 and 5.

#### Association of diarrhea and ARI with linear growth, hemoglobin, iron status, and developmental outcomes

In multivariate analysis, incidence of diarrhea from age 6 to 18 mo was associated with change in LAZ from age 6 to 18 mo ( $B = -0.02$ ; 95% CI = -0.03 to -0.01;  $p = 0.009$ ), higher risk of stunting at age 18 mo (RR = 1.02; 95% CI = 1.01 to 1.03;  $p = 0.005$ ) (Table 2), lower gross motor scores

**Table 2** Association of infectious disease morbidity from age 6 to 18 mo with change in LAZ and stunting at age 18 mo

Variables	Mean change in LAZ from age 6 to 18 mo		Stunting at age 18 mo	
	(N = 2016)		(N = 2016)	
	Regression coefficient <sup>a</sup> (95% CI)	P-value	Risk ratio <sup>b</sup> (95% CI)	P-value
Incidence <sup>c</sup> of 'presumed' malaria	-0.02 (-0.04 to 0.01)	0.069	1.04 (1.01 to 1.07)	0.023
> 1 malaria episodes (vs no malaria episode)	-0.04 (-0.12 to 0.04)	0.363	1.23 (1.02 to 1.49)	0.034
Incidence <sup>c</sup> of diarrhea	-0.02 (-0.03 to -0.01)	0.009	1.02 (1.01 to 1.03)	0.005
Incidence <sup>c</sup> of ARI	0.01 (-0.01 to 0.01)	0.614	0.99 (0.98 to 1.00)	0.097
Other predictors <sup>d</sup> :				
Female sex (vs. male)	0.09 (0.02 to 0.16)	0.012	0.92 (0.78 to 1.09)	0.352
LAZ at age 6 mo	-0.27 (-0.30 to -0.24)	< 0.001	Not included in the model	Not included in the model
WLZ at age 6 mo	0.14 (0.11 to 0.17)	< 0.001	0.76 (0.69 to 0.82)	< 0.001
Hemoglobin (g/L) at age 6 mo	0.01 (-0.01 to 0.01)	0.943	0.98 (0.97 to 0.99)	0.009
Stunting at age 6 mo	Not included in the model	Not included in the model	3.02 (2.72 to 3.35)	< 0.001
HFA score	-0.01 (-0.01 to 0.01)	0.155	1.02 (1.00 to 1.03)	0.025

ARI acute respiratory infection, CI confidence interval, HFA household food insecurity access, LAZ length for age z-score, WLZ weight for length z-score

<sup>a</sup>Obtained by ordinary least squares regression

<sup>b</sup>Obtained by modified poisson regression (with a robust variance estimator)

<sup>c</sup>Total episodes/child years at risk

<sup>d</sup>Only predictors that showed statistical significance in any of the multivariate models are presented. Other variables entered in the regression, but not significant in any model, were: iron status at age 6 mo; maternal education; and whether the child received an intervention (LNS) during the study period

at age 18 mo (B = -0.02; 95% CI = -0.03 to -0.01;  $p < 0.001$ ), and higher risk of gross motor delay (RR = 1.01; 95% CI = 1.00 to 1.02;  $p < 0.001$ ) and fine motor delay (RR = 1.01; 95% CI = 1.00 to 1.02;  $p = 0.011$ ) at age 18 mo (Tables 4 and 5).

The incidence of ARI from age 6 to 18 mo was not significantly associated with growth or other outcomes except for PSED scores (B = 0.08; 95% CI = 0.03 to 0.14;  $p = 0.004$ ), and PSED delay (RR = 1.02; 95% CI = 1.00 to 1.04;  $p = 0.025$ ) (Tables 4 and 5).

**Table 3** Association of infectious disease morbidity from age 6 to 18 mo with hemoglobin, anemia and iron deficiency at age 18 mo

Variables	Mean hemoglobin (g/L) at age 18 mo		Anemia <sup>a</sup> at age 18 mo		Iron deficiency <sup>b</sup> at age 18 mo	
	(N = 1157)		(N = 1157)		(N = 1707)	
	Regression coefficient <sup>c</sup> (95% CI)	P-value	Risk ratio <sup>d</sup> (95% CI)	P-value	Risk ratio <sup>d</sup> (95% CI)	P-value
Incidence <sup>e</sup> of 'presumed' malaria	-0.43 (-1.21 to 0.34)	0.273	1.02 (1.00 to 1.04)	0.014	1.01 (0.99 to 1.02)	0.223
> 1 malaria episodes (vs no malaria episode)	-1.80 (-3.74 to 0.14)	0.068	1.14 (1.02 to 1.27)	0.022	1.05 (0.95 to 1.17)	0.304
Incidence <sup>e</sup> of diarrhea	0.01 (-0.06 to 0.08)	0.767	1.00 (0.99 to 1.01)	0.370	1.00 (0.99 to 1.01)	0.776
Incidence <sup>e</sup> of ARI	0.09 (-0.11 to 0.31)	0.392	1.00 (0.99 to 1.01)	0.671	1.00 (0.99 to 1.01)	0.772
Other predictors <sup>f</sup> :						
WLZ at age 6 mo	1.10 (0.21 to 1.99)	0.015	0.98 (0.94 to 1.02)	0.248	0.99 (0.96 to 1.03)	0.811
Hemoglobin (g/L) at age 6 mo	0.21 (0.15 to 0.28)	< 0.001	0.98 (0.97 to 0.99)	< 0.001	1.05 (0.96 to 1.14)	0.283
ZPP > 70 $\mu$ mole/mole heme at age 6 mo	-0.64 (-2.42 to 1.14)	0.483	1.03 (0.92 to 1.15)	0.632	1.95 (1.71 to 2.23)	< 0.001

ARI acute respiratory infection, CI confidence interval, WLZ weight for length z-score, ZPP zinc protoporphyrin

<sup>a</sup>Defined as blood hemoglobin concentration < 110 g/L [29]

<sup>b</sup>Defined as whole blood ZPP > 70  $\mu$ mol/mole heme [27]

<sup>c</sup>Obtained by ordinary least squares regression

<sup>d</sup>Obtained by modified poisson regression (with a robust variance estimator)

<sup>e</sup>Total episodes/child years at risk

<sup>f</sup>Only predictors that showed statistical significance in any of the multivariate models are presented. Other variables entered in the regression, but not significant in any model, were: length for age z-score at age 6 mo; child sex; maternal education; household food insecurity access score; and whether the child received an intervention (lipid-based nutrient supplements) during the study period

**Table 4** Association of infectious disease morbidity from age 6 to 18 mo with developmental scores at age 18 mo

Variables	Mean developmental scores at age 18 mo (N = 2016)							
	Fine motor scores		Gross motor scores		Language scores		PSED scores	
	Regression coefficient <sup>a</sup> (95% CI)	P-value	Regression coefficient <sup>a</sup> (95% CI)	P-value	Regression coefficient <sup>a</sup> (95% CI)	P-value	Regression coefficient <sup>a</sup> (95% CI)	P-value
Incidence <sup>b</sup> of 'presumed' malaria	-0.06 (-0.13 to 0.01)	0.117	0.07 (-0.17 to 0.01)	0.070	-0.01 (-0.09 to 0.06)	0.692	-0.21 (-0.39 to -0.03)	0.041
> 1 malaria episodes (vs no malaria episode)	-0.09 (-0.34 to 0.16)	0.488	-0.17 (-0.45 to 0.12)	0.250	-0.09 (-0.35 to 0.16)	0.460	-0.48 (-1.11 to 0.15)	0.132
Incidence <sup>b</sup> of diarrhea	-0.06 (-0.11 to -0.01)	0.050	-0.02 (-0.03 to -0.01)	<0.001	0.01 (-0.05 to 0.05)	0.984	-0.02 (-0.04 to 0.01)	0.126
Incidence <sup>b</sup> of ARI	0.01 (-0.02 to 0.04)	0.481	0.03 (-0.01 to 0.05)	0.067	0.01 (-0.02 to 0.04)	0.432	0.08 (0.03 to 0.14)	0.004
Other predictors <sup>c</sup>								
Female sex (vs. male)	-0.27 (-0.54 to -0.01)	0.043	-0.67 (-0.94 to -0.39)	<0.001	0.13 (-0.13 to 0.39)	0.315	-1.28 (-1.92 to -0.64)	<0.001
LAZ at age 6 mo	0.20 (0.07 to 0.33)	0.002	0.32 (0.18 to 0.45)	<0.001	0.28 (0.15 to 0.41)	<0.001	0.16 (-0.48 to 0.16)	0.322
HFA score	0.04 (0.02 to 0.06)	0.001	0.01 (-0.01 to 0.03)	0.392	-0.02 (-0.04 to -0.01)	0.044	0.01 (-0.04 to 0.06)	0.719
Child's mood	0.95 (0.70 to 1.19)	<0.001	0.92 (0.64 to 1.19)	<0.001	-0.20 (-0.44 to 0.04)	0.108	-0.75 (-1.36 to -0.14)	0.016
Activity level	0.48 (0.10 to 0.88)	0.014	0.46 (0.02 to 0.90)	0.041	0.39 (-0.01 to 0.78)	0.051	0.94 (-0.04 to 1.92)	0.061

ARI acute respiratory infection; CI confidence interval, HFA household food insecurity access, LAZ length for age z-score, PSED Profile of Social and Emotional Development

<sup>a</sup>Obtained by ordinary least squares regression

<sup>b</sup>Total episodes/child years at risk

<sup>c</sup>Only predictors that showed statistical significance in any of the multivariate models are presented. Other variables entered in the regression, but not significant in any model, were: weight for length z-scores at age 6 mo; hemoglobin concentration; iron status; maternal education; interaction with the assessor during the Kilifi Developmental Inventory (KDI) assessment; and whether the child received an intervention (lipid-based nutrient supplements) during the study period

**Table 5** Association of infectious disease morbidity from 6 to 18 mo with developmental delay at 18 mo

Independent Variables	Developmental delay <sup>a</sup>				PSED delay			
	Fine motor delay Risk ratio <sup>b</sup> (95% CI)	P-value	Gross motor delay Risk ratio <sup>b</sup> (95% CI)	P-value	Language delay Risk ratio <sup>b</sup> (95% CI)	P-value	PSED delay Risk ratio <sup>b</sup> (95% CI)	P-value
Incidence <sup>c</sup> of 'presumed' malaria	1.03 (0.96 to 1.09)	0.435	1.04 (1.00 to 1.09)	0.059	0.99 (0.94 to 1.06)	0.926	0.95 (0.88 to 1.02)	0.145
> 1 malaria episodes (vs no malaria episode)	0.96 (0.78 to 1.19)	0.723	1.06 (0.89 to 1.27)	0.508	1.00 (0.82 to 1.23)	0.971	0.82 (0.66 to 1.03)	0.094
Incidence <sup>c</sup> of diarrhea	1.01 (1.00 to 1.02)	0.011	1.01 (1.00 to 1.02)	<0.001	1.00 (0.99 to 1.01)	0.129	1.00 (0.99 to 1.01)	0.953
Incidence <sup>c</sup> of ARI	0.99 (0.97 to 1.01)	0.367	0.99 (0.98 to 1.01)	0.445	0.99 (0.97 to 1.01)	0.338	1.02 (1.00 to 1.04)	0.025
Other predictors <sup>d</sup>								
Female sex (vs. male)	1.13 (0.91 to 1.41)	0.253	1.50 (1.22 to 1.85)	<0.001	0.92 (0.74 to 1.14)	0.438	0.77 (0.61 to 0.96)	0.023
LAZ at age 6 mo	0.90 (0.81 to 1.00)	0.059	0.82 (0.73 to 0.91)	<0.001	0.87 (0.78 to 0.97)	0.014	0.97 (0.86 to 1.08)	0.533
WLZ at age 6 mo	0.84 (0.76 to 0.93)	0.001	0.97 (0.88 to 1.07)	0.517	0.97 (0.88 to 1.07)	0.542	0.92 (0.83 to 1.03)	0.145
HFA score	0.98 (0.96 to 0.99)	0.034	0.99 (0.98 to 1.01)	0.702	1.01 (0.99 to 1.03)	0.091	1.02 (0.99 to 1.04)	0.075
Child's mood	0.38 (0.29 to 0.49)	<0.001	0.67 (0.55 to 0.83)	<0.001	1.08 (0.89 to 1.33)	0.435	0.78 (0.63 to 0.97)	0.023
Activity level	0.92 (0.67 to 1.25)	0.587	0.67 (0.51 to 0.87)	0.003	0.91 (0.65 to 1.29)	0.610	1.57 (1.12 to 2.21)	0.009

ARI acute respiratory infection, CI confidence interval, HFA household food insecurity access, LAZ length for age z-score, PSED Profile of Social and Emotional Development

<sup>a</sup>Defined as the bottom 25% of our sample

<sup>b</sup>Obtained by modified poisson regression (with a robust variance estimator)

<sup>c</sup>Total episodes/child years at risk

<sup>d</sup>Only predictors that showed statistical significance in any of the multivariate models are presented. Other variables entered in the regression, but not significant in any model, were: hemoglobin concentration; iron status; maternal education; interaction with the assessor during the Kilifi Developmental Inventory (KDI) assessment; and whether the child received an intervention (lipid-based nutrient supplements) during the study period

## Discussion

We tested the hypothesis that the linear growth, hemoglobin, iron status, and developmental outcomes at age 18 mo would be poorer in children with higher incidence of 'presumed' malaria. In a sample of 2016 Malawian children aged 6–18 mo, we found that malaria was not associated with change in LAZ, fine motor scores, gross motor scores, language development, iron status or hemoglobin concentration. Higher incidence of 'presumed' malaria was associated with higher risk of stunting and anemia (i.e. one additional episode of 'presumed' malaria per year was associated with 4 and 2% higher risk of stunting and anemia, respectively). Higher incidence of 'presumed' malaria was also associated with lower socio-emotional scores (i.e. one additional episode of 'presumed' malaria per year was associated with a reduction in PSED scores by 0.21), suggesting that children with higher malaria incidence tended to have fewer socio-emotional problems, possibly because malaria causes lethargy and inactivity which may manifest as fewer behavioral problems.

Our study had several strengths: the weekly home morbidity data collection for 1 year provided comprehensive data covering periods of both high and low malaria transmission; the longitudinal design made it possible to correlate the malaria exposure with the outcomes and interpret the directionality of association; and pooling of data from two studies helped us draw conclusions from a large sample. Although we did not calculate post-hoc power for this analysis, with a large sample size of 2016 children and narrow confidence intervals obtained for most of the morbidity outcomes, we believe the study was powered to detect clinically meaningful associations.

Our results should be interpreted with caution because we excluded children who did not have the outcomes measured at age 18 mo (26% of the sample), resulting in possible survival bias. However, this attrition rate is similar to that of other studies with a long follow up period [9, 10]. Moreover, we expect that the children lost to follow up may have had worse outcomes, which probably would have increased the strength of association in our findings.

Another possible cause of bias is the presumptive diagnosis of malaria, which could lead to misclassification. There is overlap in the symptoms of malaria, diarrhea and ARI [33] which may affect the sensitivity and specificity of presumptive malaria diagnosis depending on the intensity of malaria transmission. For example, presumptive malaria diagnosis usually has higher sensitivity and lower specificity in areas of high malaria transmission compared to areas of low transmission [34, 35]. With the global decline in malaria incidence and availability of malaria Rapid Diagnostic Tests (mRDTs), the WHO in 2010 recommended antimalarial treatment be provided when there is evidence of a positive malaria test result [36]. However, at

the time of conducting our study, mRDTs had not been rolled out nationwide, hence presumptive malaria diagnosis was used not only in this study but also in national prevalence surveys [18, 37], according to the practice of Integrated Management of Childhood Illness (IMCI) [38, 39]. Furthermore, in exploratory analysis using hospital diagnosed malaria (confirmed by mRDT, albeit with a lot of missing data), the direction of the associations was similar, suggesting that our findings are still valid (data not shown).

Our research assistants referred the children suspected of malaria to a clinic for treatment; the active surveillance and early treatment may have helped improve the study outcomes, which may have resulted in underestimation of the associations.

It is also possible that the association of malaria with stunting, anemia, and PSED scores was significant by chance due to multiple testing [40]. However, we believe the chance finding was less likely for the significant associations of diarrhoea and ARI with the outcomes because these associations were relatively strong based on *p*-values.

The available evidence on the association of malaria with growth and other outcomes is inconclusive. Some studies have reported significant associations of malaria with stunting, hemoglobin concentration, iron status and child development [11, 12, 41–44]. Children living in settings where infectious diseases are frequent and complementary food is of poor quality often fail to achieve catch up growth after illness episodes [45], hence frequent malaria could be associated with growth faltering. Earlier evidence suggested that anorexia, vomiting, and a catabolic state are responsible for the poor growth associated with febrile illnesses in children [46]. However, other studies have reported no association of malaria with growth outcomes [47–50]. Similar to our study, most of these studies provided active malaria diagnosis and treatment, which may have attenuated the strength of the association.

In the studies cited above, different exposure and outcome measures were used, which may also explain the inconsistency in the findings. For example, we defined linear growth as change in LAZ and stunting. Change in LAZ indicates the growth rate between two time-points and therefore provides more information about linear growth faltering than stunting status assessed at one time-point [51, 52]. Therefore, it is possible that there is no association between malaria and linear growth (based on change in LAZ), hemoglobin or iron status, or the association is very weak. It is also possible that the association between malaria and these outcomes is only seen in children in the left end of the curve (i.e. LAZ < -2 or hemoglobin < 110 g/L), hence the significant association of malaria with stunting and anemia but not change in LAZ or hemoglobin concentration. In contrast, diarrhea incidence was associated with an entire leftward shift in LAZ and gross motor scores



in this population. A leftward shift in mean LAZ for a population is associated with increased risk of mortality [53].

The association of diarrhea with growth has been reported in previous studies [7–9]. Frequent diarrhea episodes result in persistent loss of nutrients necessary for growth through malabsorption, changes in gut microbiota, continuous immune system activation, increased metabolism and anorexia resulting in growth suppression. In our study, the magnitude of the significant associations of malaria and diarrhea with the outcomes were small, consistent with other studies [7, 42, 44, 54–56]. This could be partly attributable to the active surveillance and treatment provided to all children, or unmeasured confounding [44], or that perhaps other conditions such as chronic inflammation and environmental enteric dysfunction may be more important determinants of child growth in developing countries [57, 58]. Nevertheless, in a study combining nutrition intervention with treatment of malaria and diarrhea there was greater growth velocity, a 25% reduction in prevalence of stunting and improved developmental outcomes at age 18 months [4], suggesting that interventions that combine improved nutrition with control of infections may have significant impact.

## Conclusions

We conclude that in this population of children aged 6–18 mo living in a malaria-endemic setting, with active surveillance and early treatment, ‘presumed’ malaria is not associated with change in LAZ, hemoglobin or iron status, but could be associated with stunting and anemia. In this population, diarrhoea was more consistently associated with growth than was malaria or ARI. These findings may be different in contexts where there is no active case finding and treatment for malaria is not promptly administered.

## Abbreviations

ARI: Acute respiratory infection; CI: Confidence interval; Hb: Hemoglobin; HFIA: Household food insecurity access; IFA: Iron and folic acid; IMCI: Integrated Management of Childhood Illness; KDI: Kilifi Developmental Inventory; LAZ: Length-for-age z-scores; LNS: Lipid-based nutrient supplements; MMN: Multiple micronutrients; mRDT: Malaria Rapid Diagnostic Test; PSED: Profile of Social and Emotional Development; RR: Risk ratio; SD: Standard deviation; WAZ: Weight-for-age z-scores; WHO: World Health Organization; WLZ: Weight-for-length z-scores; ZPP: Zinc protoporphyrin

## Acknowledgements

The authors thank the study participants, the local communities of Mangochi, the health service staff and their research personnel at the study sites as well as members of the trial’s data safety and monitoring board, the iLINS extended research team and the iLINS-Project Steering Committee for their contributions in all stages of the study.

## Funding

This publication is funded by a grant to the University of California, Davis from the Bill & Melinda Gates Foundation. The findings and conclusions contained within the article are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of this manuscript.

## Availability of data and materials

The datasets used and analysed during this study are available from the corresponding author on reasonable request.

## Authors’ contributions

The authors’ responsibilities were as follows: KM, JP, KGD, YBC, UA, and PA designed the study; JB, NP, JM, CM, LH, JP, YBC, UA, ELP, KGD, PA, and KM conducted the study; JB analysed the data and wrote the paper, with critical input and comments from all other authors; JB and KM had primary responsibility for final content. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was performed according to International Conference of Harmonization–Good Clinical Practice (ICH-GCP) guidelines and the ethical standards of the Helsinki Declaration. The protocol was reviewed and approved by the Institutional Review Boards of the University of Malawi, College of Medicine (IRB reference number P.01/09/722) and the Pirkanmaa Hospital District, Finland (IRB reference number R09130). At least one guardian signed or thumb-printed an informed consent form before enrolment of each participant. An independent data safety and monitoring board monitored the incidence of suspected SAE during the trial.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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Received: 5 July 2018 Accepted: 20 December 2018

Published online: 28 December 2018

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# PUBLICATION III

**The effect of providing Lipid-based Nutrient Supplements on morbidity in rural Malawian infants and young children: a randomized controlled trial.**

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Public Health Nutrition volume 19, 1893-1903 (2016)

<https://doi.org/10.1017/S1368980016000331>

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# The effect of providing lipid-based nutrient supplements on morbidity in rural Malawian infants and young children: a randomized controlled trial

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Submitted 28 September 2015: Final revision received 5 February 2016: Accepted 9 February 2016

## Abstract

**Objective:** Safety of home fortificants in children is uncertain in areas where infections are common. We tested the hypothesis that provision of lipid-based nutrient supplements (LNS) containing Fe does not increase infectious morbidity in children.

**Design:** Randomized controlled trial. Infants were randomised to receive 10, 20 or 40 g LNS/d; or no supplement until age 18 months. All LNS contained 6 mg Fe/d. Morbidity outcomes (serious adverse events, non-scheduled visits and guardian-reported morbidity episodes) were compared between control and intervention groups using a non-inferiority margin of 20%.

**Setting:** Namwera and Mangochi catchment areas in rural Malawi.

**Subjects:** Infants aged 6 months ( $n$  1932).

**Results:** The enrolled 1932 infants contributed 1306 child-years of follow-up. Baseline characteristics were similar across groups. Compared with the control group, the relative risk (95% CI) of serious adverse events was 0.71 (0.48, 1.07), 0.67 (0.48, 0.95) and 0.91 (0.66, 1.25) in 10, 20 and 40 g LNS/d groups, respectively. The incidence rate ratio (95% CI) of non-scheduled visits due to malaria was 1.10 (0.88, 1.37), 1.08 (0.89, 1.31) and 1.21 (1.00, 1.46), and of guardian-reported morbidity episodes was 1.04 (0.96, 1.11), 1.03 (0.97, 1.10) and 1.04 (0.97, 1.10), in the respective LNS groups.

**Conclusions:** Provision of 10 and 20 g LNS/d containing 6 mg Fe/d did not increase morbidity in the children. Provision of 40 g LNS/d did not affect guardian-reported illness episodes but may have increased malaria-related non-scheduled visits.

## Keywords

Lipid-based nutrient supplements  
Infectious disease morbidity  
Iron  
Children  
Low-income countries

The WHO recommends the use of Fe supplements or home fortificants to improve Fe status and reduce anaemia prevalence among infants and children aged 6–23 months in low-income countries<sup>(1)</sup>. Although home fortificants (such as multiple micronutrient powders (MNP) and small-quantity lipid-based nutrient supplements (LNS)) may have positive effects on children's micronutrient status, their safety is less well documented, especially in areas where infections are common<sup>(2)</sup>. While some studies suggest that home fortificants are safe<sup>(3,4)</sup> and may reduce morbidity<sup>(5)</sup>, others have reported harmful effects. In Pakistan, provision of MNP with or without Zn as home fortificants was associated with increased risk of diarrhoea

and reported chest in-drawing in children<sup>(6)</sup>. Findings from a study in Kenya suggest that Fe fortification modifies the gut microbiome, increasing pathogenic bacteria and causing intestinal inflammation<sup>(7)</sup>. An increased risk of malaria infection and deaths has also been reported in a large trial using Fe and folic acid supplements in Zanzibar, also raising concerns about Fe supplementation in Fe-replete children living in malaria-endemic regions. Excess free Fe circulating in blood may also aid growth of pathogens such as malaria parasites<sup>(8)</sup>.

Currently, there is interest in testing the efficacy and effectiveness of providing LNS as home fortificants to prevent undernutrition and micronutrient deficiencies for

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programmatic use in vulnerable populations. LNS products are similar to MNP because they contain a range of vitamins and minerals, including Fe. There are concerns about Fe provision to children in areas where malaria is endemic<sup>(9)</sup>. However, there is little evidence regarding the safety of Fe-containing LNS. Safety studies have mainly been reported about MNP, although with conflicting results<sup>(3,4,6,10)</sup>. A few reports on LNS provision for prevention of child undernutrition suggest that it is safe<sup>(11–13)</sup>, but the evidence is not conclusive because those studies had either a relatively short duration (i.e. 6 months or less) or insufficient power because of small sample sizes<sup>(14)</sup>.

The iLiNS-DOSE study was a large randomized controlled trial with a primary objective of testing the efficacy of different doses of LNS in promoting linear growth in children in a rural Malawian population. In the main outcome paper, we reported that supplementation with LNS for 1 year did not promote length gain or prevent stunting<sup>(15)</sup>. However, other studies in Ghana<sup>(11)</sup>, Burkina Faso<sup>(16)</sup> and Malawi<sup>(17)</sup> reported significant length gain and reduction in stunting associated with LNS. Considering the evidence from these studies, we aimed to assess, as a secondary outcome, the safety of LNS when provided to young children in a malaria-endemic area. In the current analysis, we tested the hypothesis that provision of Fe-containing LNS does not increase infectious disease morbidity when provided to infants and young children for 12 months.

## Methods

### **Study sites and participants**

We conducted the study in communities within the catchment areas of Mangochi district hospital and Namwera health centre in the south-eastern part of Malawi. Mangochi district hospital outpatient department serves an estimated population of 100 000 people, whereas Namwera health centre serves a rural population of ~22 000. The hospital catchment area is partly semi-urban, while Namwera is predominantly rural. In this area, the major causes of death among children aged <5 years are malaria (17%), pneumonia (13%) and diarrhoeal diseases (11%)<sup>(18,19)</sup>. These diseases are prevalent in the catchment area throughout the year with seasonal fluctuations. The prevalence of HIV is 10.3% (ages 15–49 years) and an estimated 170 000 children aged 0–14 years are living with HIV/AIDS in Malawi<sup>(20)</sup>.

For a period of 18 months (November 2009 to May 2011), we identified potential participants in the catchment areas through community surveys and invited them to the study clinic for further eligibility assessment. Children who were 5.5 to 6.49 months old, whose guardians had signed informed consent and planned to be available during the whole study period were considered eligible. Exclusion criteria were any severe illness warranting hospital referral, bipedal oedema, Hb < 50 g/l, family history of

peanut allergy, concurrent participation in another clinical trial and weight-for-height Z-score < -2. We excluded children with weight-for-height Z-score < -2 because these children are at risk of developing severe acute malnutrition. Children with severe acute malnutrition are treated through the national nutritional rehabilitation programmes, therefore were more likely to deviate from our trial protocol. To identify the lowest growth-promoting daily dose and formulation of LNS and to test the hypothesis that milk-free LNS would promote growth equally well as milk-containing LNS, we randomly assigned the children to six groups as follows: milk-containing LNS 10 g/d, 20 g/d and 40 g/d; milk-free LNS 20 g/d and 40 g/d; and a control group which did not receive LNS during the 12-month study period. The nutrient compositions of the five different doses of LNS are reported in Table 1. We used a reduced dose of Fe (6 mg) in the LNS because of safety concerns based on recommendations from the WHO<sup>(9)</sup>. The Zn content (8 mg) was based on the WHO/FAO Recommended Nutrient Intakes for diets with low bioavailability<sup>(21)</sup>. The rationale for selecting specific nutrient levels for LNS is described in detail elsewhere<sup>(22)</sup>.

### **Randomization and masking**

The details of the randomization process are described in the main outcome paper<sup>(15)</sup>. Briefly, when the guardian consented to let her infant participate and the infant met all the enrolment criteria, the guardian was asked to choose and open one randomization envelope from a block of six unused envelopes. The envelope contained the participant identification code and supplement code. Randomization into the trial and group allocation was done by a randomizer not participating in the analysis. The investigators involved in data cleaning and analysis were blinded to the group allocation.

### **Data collection and participant follow-up**

Research assistants visited the participants' homes every week to deliver supplements and collect morbidity data. The data were collected by interviewing the guardians about the child's health in the previous 7 d using a structured questionnaire. The information was complemented by a picture calendar filled out by the guardians on a daily basis to aid memory of their child's morbidity status. These were done to minimize problems of recall associated with community morbidity assessments<sup>(23)</sup>. The research assistants collecting morbidity data knew which children were receiving LNS.

We trained health workers to collect data on non-scheduled visits made to health centres when the child was sick, including data for hospitalizations and hospital deaths. For deaths occurring at home, the information was collected by a verbal autopsy method, previously validated in the study area<sup>(24)</sup>. Records of all hospitalizations

**Table 1** Nutrient and energy contents of the food supplements used in the present study

Nutrient	10 g milk LNS	20 g milk LNS	20 g milk-free LNS	40 g milk LNS	40 g milk-free LNS
Daily ration (g)	10	20	20	40	40
Total energy (kJ)	230	490	490	1008	1008
Total energy (kcal)	55	117	117	241	241
Protein (g)	1.3	2.5	1.0	5.0	2.0
Fat (g)	4.7	9.5	9.4	18.9	18.8
Linoleic acid (g)	2.22	4.44	4.44	8.88	8.88
$\alpha$ -Linolenic acid (g)	0.29	0.58	0.58	1.16	1.16
Vitamin A ( $\mu$ g RE)	400	400	400	400	400
Vitamin C (mg)	30	30	30	30	30
Thiamin (mg)	0.3	0.3	0.3	0.3	0.3
Riboflavin (mg)	0.4	0.4	0.4	0.4	0.4
Niacin (mg)	4	4	4	4	4
Folic acid ( $\mu$ g)	80	80	80	80	80
Pantothenic acid (mg)	1.8	1.8	1.8	1.8	1.8
Vitamin B <sub>6</sub> (mg)	0.3	0.3	0.3	0.3	0.3
Vitamin B <sub>12</sub> ( $\mu$ g)	0.5	0.5	0.5	0.5	0.5
Vitamin D ( $\mu$ g)	5	5	5	5	5
Vitamin E (mg)	6	6	6	6	6
Vitamin K ( $\mu$ g)	30	30	30	30	30
Fe (mg)	6	6	6	6	6
Zn (mg)	8	8	8	8	8
Cu (mg)	0.34	0.34	0.34	0.34	0.34
Ca (mg)	240	240	240	240	240
P (mg)	208	208	208	208	208
K (mg)	265	265	265	265	265
Mg (mg)	50	50	50	50	50
Se ( $\mu$ g)	20	20	20	20	20
Iodine ( $\mu$ g)	90	90	90	90	90
Mn (mg)	1.2	1.2	1.2	1.2	1.2
Phytate (mg)	28	56	56	112	112

LNS, lipid-based nutrient supplement; RE, retinol equivalents.

and deaths were reviewed as serious adverse events (SAE) by a study physician and reported to members of the data and safety monitoring board within 48 h of occurrence.

Anthropometric measurements were taken by research assistants who underwent training and standardization every 6 months. Research assistants who took anthropometric measurements were not aware of group allocation. The details of anthropometric, biochemical and socio-economic measurements and cleaning of anthropometry data are explained in the main outcome paper<sup>(15)</sup> and in the statistical analysis plans published on the iLiNS website<sup>(25)</sup>.

### Ethics

The study was performed according to International Conference of Harmonization–Good Clinical Practice (ICH-GCP) guidelines and the ethical standards of the Helsinki Declaration. The protocol was reviewed and approved by the Institutional Review Boards of the University of Malawi, College of Medicine (IRB reference number P.01/09/722) and the Pirkanmaa Hospital District, Finland (IRB reference number R09130). At least one guardian signed or thumb-printed an informed consent form before enrolment of each participant. The trial was registered at the clinical trials registry ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) with the registration ID of NCT00945698.

An independent data safety and monitoring board monitored the incidence of suspected SAE during the trial.

### Outcomes

We assessed the following morbidity outcomes at the end of the follow-up: SAE, non-scheduled visits, diagnoses made at non-scheduled visits and guardian-reported morbidity symptoms and disease episodes.

SAE were comprised of hospitalizations and deaths, defined according to the US Department of Health and Human Services Office for Human Research Protections<sup>(26)</sup>. Non-scheduled visits were defined as visits made by the participants to any health facility because of illness. At each non-scheduled visit, a diagnosis of malaria, gastroenteritis, acute respiratory infection or other illnesses was made by health workers.

For home visits, diagnoses of gastroenteritis, acute respiratory infection and 'undefined fever' were derived from a combination of guardian-reported morbidity symptoms recorded on one or more days.

To ensure that the diagnoses derived from symptoms at home visits were mutually exclusive a diagnosis algorithm was created, whereby any diarrhoea episode (three or more loose stools in 24 h) was categorized as gastroenteritis with or without other symptoms. If diarrhoea was



absent but there was presence of any respiratory symptoms (cough, rapid or difficult breathing and nasal discharge) with or without fever, a diagnosis of acute respiratory infection was made. Fever episodes in the absence of diarrhoea and respiratory symptoms, with or without other symptoms, were categorized as 'undefined fever'. The rest of the symptoms in the absence of diarrhoea, respiratory symptoms and fever were categorized as other illnesses. For all diseases, an episode was defined as the period starting from the day the child had symptoms if preceded by at least two days of either no symptoms or no data. The episode ended on the last day the child had symptoms which was then followed by at least two symptom-free days.

We defined anaemia as  $Hb < 105$  g/l based on suggested reference values for infants<sup>(27)</sup>. We defined Fe deficiency as  $Zn$  protoporphyrin  $> 70$   $\mu$ mol/mol haem, measured from washed red blood cells<sup>(28,29)</sup>.

### Sample size calculation

The sample size was calculated based on the primary objective of the main study: to test the hypothesis of non-inferiority of LNS without milk on change in length-for-age Z-score as compared with LNS containing milk. Assuming an sd for the change in length-for-age Z-score of 1.0, a predetermined non-inferiority margin of 0.25 Z-score units and an estimated 15% attrition rate, a sample size of 320 per group was estimated to provide the trial with 90% power and 95% confidence (one-sided test) to discard an inferiority null hypothesis. Morbidity (the outcome for the present paper) was a secondary outcome of the study. We did not calculate a separate sample size or *post hoc* power for the morbidity outcome. We relied on the confidence interval to determine if the sample size was adequate for each of the morbidity outcomes, as recommended by several scholars<sup>(30,31)</sup>.

### Data entry and management

The data were double-entered using Microsoft® Access, REDCap™ and TELEform®. Typographical errors, extreme observations and discrepancies were resolved prior to breaking the randomization code. Analyses were done using the statistical software package Stata version 12.1.

### Statistical analysis

Our hypothesis was that the risk of morbidity among children would not be significantly higher in the intervention groups compared with the control group. We used a non-inferiority approach to compare the risk of morbidity between the intervention groups and the control group. We chose a predefined non-inferiority margin of no greater than 20% increase in morbidity in the intervention groups compared with the control group to conclude that there was no difference in morbidity. We assumed that an

increase in morbidity of 20% or more in the LNS groups relative to the control would be clinically significant, with negative impact on the overall health of the children. There is no agreed definition of non-inferiority in this context, but an increase between 15% and 20% in morbidity has previously been considered clinically significant<sup>(3,4,6,8,13)</sup>. The non-inferiority approach and the margin of 20% were predefined in the statistical analysis plan published at the iLiNS website<sup>(25)</sup> before starting the analysis.

We intended to confirm non-inferiority if the entire 95% CI (two-sided) for the risk ratio (RR)/incidence rate ratio (IRR) was below the value of 1.20. For each morbidity outcome, we anticipated the following four possible conclusions: (i) if the upper bound of the 95% CI for the RR/IRR was  $< 1.20$ , a conclusion of non-inferiority would be made; (ii) if the lower bound of the 95% CI for the RR/IRR was  $> 1.20$ , a conclusion of inferiority (suggesting a harmful effect) would be made; (iii) if the upper bound of the 95% CI for the RR/IRR was  $< 1.00$ , a conclusion of superiority (suggesting a beneficial effect) would be made; and (iv) if the upper bound of the 95% CI for the RR/IRR was  $> 1.20$  and lower bound was  $< 1.20$ , the findings would be considered inconclusive for target group inference.

Risks of experiencing an SAE (hospitalization or death) were calculated as numbers of participants experiencing an SAE divided by the total number of participants in each group. We used generalized linear modelling (log-binomial family) to estimate and compare the risks between the intervention groups and the control group, reported as RR (95% CI). Longitudinal prevalences of common morbidity symptoms were defined as proportions of days when the child had an illness among all days of observation for each child. We compared the longitudinal prevalence for each intervention group with the control group by first log-transforming the data, then performed ordinary least squares regression, and exponentiated the regression coefficients and their 95% CI. Incidences of non-scheduled visits and guardian-reported disease episodes in each group were calculated as the sum of visits or episodes across individuals divided by total child-years in each group. We used negative binomial regression to compare the incidences between the intervention groups and the control group, reported as IRR (95% CI).

The primary analysis was done on the intention-to-treat basis. As secondary analysis, the outcomes were fitted in models that included the following covariates at baseline: age, sex, weight-for-length Z-score, weight-for-age Z-score, length-for-age Z-score, Fe status, Hb status, seasonality, maternal education, marital status, household food insecurity (using the Household Food Insecurity Access Scale), household water source and sanitation.

We planned first to compare between the milk and non-milk groups of the same dose, then proceed to collapse the groups if there were no differences between the milk





and non-milk groups of the same LNS dose. We outlined this plan in the statistical analysis plan published at our website<sup>(25)</sup>. Although the Fe content was originally the same in all LNS doses, we observed differences in daily intake of LNS between the groups (those given the higher doses of LNS/d consumed a lower proportion)<sup>(32)</sup>, suggesting that the actual daily dose of Fe and other micronutrients also varied among the intervention groups. Therefore we analysed the groups according to the different daily doses of LNS provided.

### Results

We conducted the iLiNS-DOSE study between November 2009 and May 2012. Out of 2136 infants who came to the study clinic for assessment, 1932 were enrolled and randomized into the six study groups. The group allocation, reasons for exclusion and loss to follow-up are shown in Fig. 1. At baseline, the mean age of the participants was 5.9 (SD 0.3) months and their mean length-for-age Z-score and weight-for-height Z-score were -1.4 (SD 1.1) and +0.3 (SD 1.1), respectively. The proportions of children with stunting, a positive malaria test, severe anaemia

(Hb < 80 g/l), moderate to severe anaemia (Hb < 105 g/l), Fe deficiency (Zn protoporphyrin > 70 μmol/mol haem) or Fe-deficiency anaemia were 29.3%, 16.3%, 7.9%, 50.8%, 66.0% and 42.0%, respectively. The reported bed-net utilization by participants was 81.0%. There were no differences between the groups in the baseline characteristics (Table 2). No episodes of suspected allergy or intolerance of the supplement were recorded during the trial.

The enrolled children contributed a total of 1306 child-years of follow-up, i.e. the mean length of follow-up was 251 (SD 94) d/child. A total of 1534 (79.4%) children remained in follow-up at 18 months of age and we managed to see 1407 (72.8%) children on the final home visit. There were no intergroup differences either in the mean length of follow-up ( $P=0.974$ ) or the proportion of children who remained in the study until its end ( $P=0.334$ ). We did not see differences in morbidity outcomes between the milk and non-milk groups of the same dose (each  $P>0.05$ ); therefore we proceeded to collapse the groups. We present all the results by daily ration of LNS (0, 10, 20 or 40 g/d), irrespective of the milk content of the supplement.

We recorded 271 SAE among the study participants. Of these, seventy-eight were deaths (4.0% of the enrolled

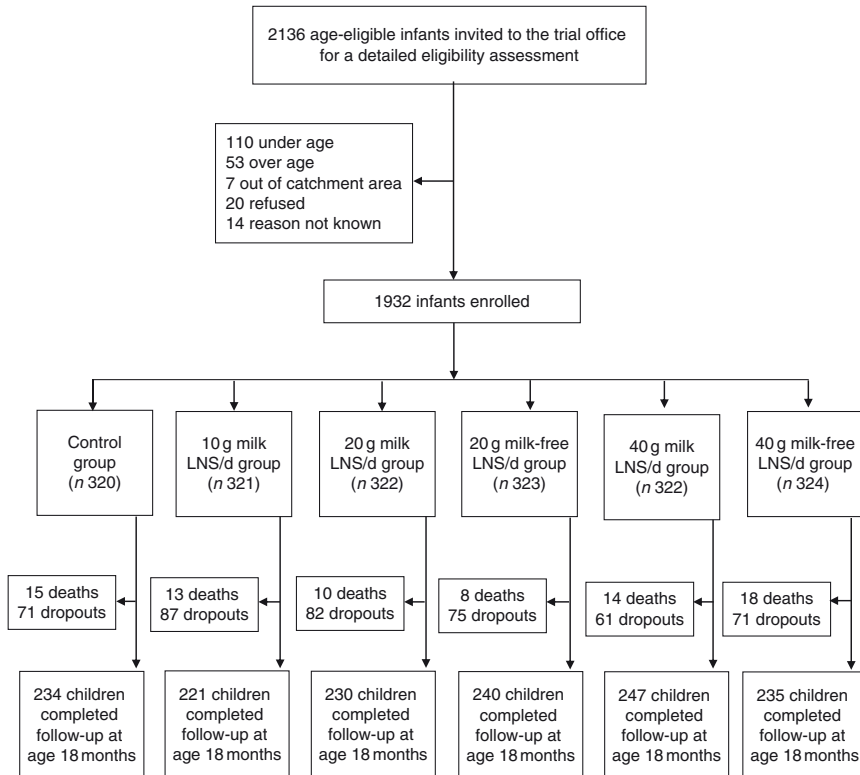


Fig. 1 Participant flow in the present study (LNS, lipid-based nutrient supplement)

**Table 2** Baseline characteristics of the participating rural Malawian infants and young children, iLINS-DOSE study, November 2009–May 2012

Variable	Control (n 320)		10 g LNS/d (n 321)		20 g LNS/d (n 645)		40 g LNS/d (n 646)	
	n or Mean	% or sd	n or Mean	% or sd	n or Mean	% or sd	n or Mean	% or sd
Participants (n, % boys)	168	52.5	156	48.6	331	51.3	314	48.6
Age in months (mean, sd)	5.9	0.3	5.9	0.3	5.9	0.3	5.9	0.3
WAZ (mean, sd)	-0.7	1.2	-0.7	1.2	-0.7	1.2	-0.8	1.1
LAZ (mean, sd)	-1.4	1.1	-1.4	1.1	-1.4	1.0	-1.4	1.1
WLZ (mean, sd)	0.3	1.2	0.3	1.1	0.3	1.1	0.2	1.1
Hb < 105 g/l (n, %)	145	45.6	165	51.6	321	50.2	343	53.8
ZPP > 70 µmol/mol haem (n, %)	208	67.5	195	62.9	389	63.7	423	69.1
Malaria RDT-positive (n, %)	54	18.2	51	17.2	92	15.5	94	15.8
Maternal education, completed years (mean, sd)	4.7	3.7	4.7	3.6	4.7	3.5	4.6	3.5
Persons in the household (mean, sd)	5.5	2.3	5.4	2.4	5.4	2.4	5.5	2.2

LNS, lipid-based nutrient supplement; WAZ, weight-for-age Z-score; LAZ, length-for-age Z-score; WLZ, weight-for-length Z-score; ZPP, Zn protoporphyrin; RDT, rapid diagnostic test.

**Table 3** Risk of serious adverse events (SAE) by intervention group among rural Malawian infants and young children, iLINS-DOSE study, November 2009–May 2012

Variable	Results by study group								Comparison between the groups†					
	Control (n 320)		10 g LNS/d (n 321)		20 g LNS/d (n 645)		40 g LNS/d (n 646)		10 g LNS/d v. control		20 g LNS/d v. control		40 g LNS/d v. control	
	n	%	n	%	n	%	n	%	RR	95% CI	RR	95% CI	RR	95% CI
Total SAE	57	–	39	–	77	–	98	–	0.71	0.48, 1.07	0.67	0.48, 0.95*	0.91	0.66, 1.25
Children who reported any SAE	50	15.6	36	11.2	68	10.5	92	14.2	0.66	0.41, 1.06	0.69	0.47, 1.03	0.83	0.57, 1.22
Children who were hospitalized	38	11.9	25	7.8	53	8.2	64	9.9	0.86	0.42, 1.79	0.60	0.30, 1.17	1.05	0.58, 1.92
Children who died	15	4.7	13	4.1	18	2.8	32	5.0						

LNS, lipid-based nutrient supplement; RR, risk ratio.

\*Statistically significant result.

†Groups were compared using generalized linear modelling (log-binomial family).

participants) and 193 were hospitalizations. Compared with the control group, the 95% CI for the RR of experiencing an SAE was entirely below 1.20 (suggestive of non-inferiority) in the 10 g LNS/d group, entirely below 1.00 (suggestive of a protective effect) in the 20 g LNS/d group and ranged from 0.66 to 1.25 (inconclusive) in the 40 g LNS/d group. The 95% CI for the RR of hospitalizations was entirely below 1.20 (suggestive of non-inferiority) in the 10 and 20 g LNS/d groups, and ranged from 0.57 to 1.22 (inconclusive) in the 40 g LNS/d group (Table 3). The 95% CI for the risk of death was entirely below 1.20 (suggestive of non-inferiority) in the 20 g LNS/d group, and ranged from 0.42 to 1.79 and 0.58 to 1.92 (inconclusive) in the 10 and 40 g LNS/d groups, respectively (Table 3).

In total, we recorded 9034 non-scheduled visits to health facilities due to illnesses (5.2 non-scheduled visits/child per year of follow-up). Compared with the control group, the 95% CI for the IRR of non-scheduled visits was entirely below 1.20 (suggestive of non-inferiority) in the 10 and 20 g LNS/d groups. The incidence was 13% higher in the 40 g LNS/d group but the lower bound of the 95% CI for the IRR was <1.20 (inconclusive; Table 4). For non-scheduled visits due to malaria (confirmed by rapid

diagnostic test), acute respiratory infection and other illnesses, the incidences were, respectively, 21%, 14% and 13% higher in the 40 g LNS/d group than in the control group, but the comparisons were also inconclusive (Table 4).

The mean longitudinal prevalence of all guardian-reported illness symptoms was 29.1 (sd 19.6)%. Compared with the control group, the 95% CI for the geometric means ratio of the longitudinal prevalence of all symptoms was entirely below 1.20 (suggestive of non-inferiority) in the 10 g LNS/d and 40 g LNS/d groups and ranged from 0.97 to 1.22 (inconclusive) in the 20 g LNS/d group (Table 5). For most of the individual symptoms, comparisons of the longitudinal prevalences between the LNS groups and the control group were inconclusive (Table 5).

From the guardians' symptom recalls, we identified 19690 separate illness episodes among the study participants. We excluded thirty-four children from this analysis because of missing data. The mean incidence of illness episodes was 15.6 (sd 8.8)/child per follow-up year. Compared with the control group, the 95% CI for the IRR of all illnesses, 'undefined fever' and acute respiratory infection episodes were entirely below 1.20 (suggestive of non-inferiority) in all the intervention groups (Table 6).



**Table 4** Incidence† of non-scheduled visits and clinical diagnoses among rural Malawian infants and young children, iLiNS-DOSE study, November 2009–May 2012

Variable	Results by study group								Comparison between the groups‡					
	Control (follow-up years: 296.7)		10 g LNS/d (follow-up years: 294.9)		20 g LNS/d (follow-up years: 595.8)		40 g LNS/d (follow-up years: 601.9)		10 g LNS/d v. control		20 g LNS/d v. control		40 g LNS/d v. control	
	Total visits	Incidence	Total visits	Incidence	Total visits	Incidence	Total visits	Incidence	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI
All non-scheduled visits	1412	4.8	1417	4.8	2988	5.0	3217	5.3	1.02	0.89, 1.18	1.05	0.93, 1.19	1.13	1.00, 1.27*
Gastroenteritis	163	0.5	159	0.5	298	0.5	324	0.5	0.98	0.76, 1.27	0.91	0.73, 1.14	0.98	0.79, 1.22
ARI	454	1.5	446	1.5	938	1.6	1041	1.7	1.02	0.84, 1.22	1.04	0.88, 1.22	1.14	0.98, 1.34
Clinical malaria	185	0.6	174	0.6	418	0.7	409	0.7	0.94	0.74, 1.20	1.12	0.92, 1.37	1.08	0.89, 1.33
Confirmed malaria§	385	1.3	418	1.4	834	1.4	941	1.6	1.10	0.88, 1.37	1.08	0.89, 1.31	1.21	1.00, 1.46*
Total malaria	570	1.9	592	2.0	1252	2.1	1350	2.2	1.04	0.87, 1.25	1.09	0.93, 1.28	1.16	1.00, 1.36*
Other diseases	274	0.9	244	0.8	581	1.0	627	1.0	0.89	0.71, 1.12	1.05	0.87, 1.27	1.13	0.93, 1.36

LNS, lipid-based nutrient supplement; IRR, incidence rate ratio; ARI, acute respiratory infection.

\*Statistically significant results.

†Incidence = number of cases/child per year of follow-up.

‡Groups were compared using negative binomial regression.

§Measured by rapid diagnostic test (RDT) for malaria parasites based on histidine-rich protein (HRP-II).

||Other diseases = sepsis, measles, anaemia, etc.

**Table 5** Longitudinal prevalence† of guardian-reported common childhood morbidity symptoms among rural Malawian infants and young children, iLiNS-DOSE study, November 2009–May 2012

Variable	Results by study group								Comparison between the groups‡					
	Control (n 314)§		10 g LNS/d (n 315)§		20 g LNS/d (n 636)§		40 g LNS/d (n 633)§		10 g LNS/d v. control		20 g LNS/d v. control		40 g LNS/d v. control	
	Geometric mean (%)	SD	Geometric mean (%)	SD	Geometric mean (%)	SD	Geometric mean (%)	SD	GMR	95 % CI	GMR	95 % CI	GMR	95 % CI
All symptoms	21.9	2.3	22.6	2.3	23.8	2.4	22.3	2.4	1.03	0.90, 1.18	1.09	0.97, 1.22	1.02	0.91, 1.14
Fever	6.7	2.5	6.1	2.7	6.8	2.5	6.3	2.8	0.90	0.77, 1.06	1.00	0.88, 1.15	0.93	0.81, 1.07
Cough	9.2	2.7	9.4	2.7	10.0	2.6	9.3	2.7	1.02	0.86, 1.20	1.09	0.95, 1.25	1.01	0.88, 1.16
Diarrhoea	2.8	3.0	3.1	3.4	3.1	3.1	2.7	3.2	1.13	0.92, 1.39	1.09	0.92, 1.30	0.96	0.81, 1.14
Nasal discharge	10.4	2.9	11.4	2.7	11.8	2.7	11.3	2.7	1.09	0.92, 1.30	1.14	0.98, 1.32	1.09	0.94, 1.26
Difficult breathing	2.3	3.5	2.5	3.3	2.3	3.5	2.1	3.3	1.12	0.87, 1.45	1.00	0.80, 1.27	0.94	0.75, 1.18
Vomiting	2.5	2.8	2.2	3.3	2.6	2.9	2.6	2.9	0.88	0.72, 1.07	1.04	0.89, 1.23	1.06	0.90, 1.24

LNS, lipid-based nutrient supplement; GMR, geometric means ratio.

†Longitudinal prevalence = number of days with symptom/total number of days of follow-up.

‡Groups were compared using ordinary least squares regression.

§Excluded thirty-four participants from analysis due to missing data (six in the control group, six in the 10 g LNS/d group, nine in the 20 g LNS/d group and thirteen in the 40 g LNS/d group).

Finally, we combined the intervention groups into one group to compare all participants who received LNS with those who did not. In this comparison, the 95 % CI for all morbidity outcomes were entirely below 1.20 (suggestive of non-inferiority); the 95 % CI for the incidence of non-scheduled visits was just touching the non-inferiority margin of 1.20. The 95 % CI for the risk of death was too wide to draw any conclusions (Fig. 2). Adjusting for baseline anaemia and Fe status did not significantly alter any of the results (data not shown).

## Discussion

We tested the hypothesis that long-term supplementation with LNS containing 6 mg Fe would not increase morbidity in children. In a sample of 1932 Malawian infants and young children aged 6–18 months, we found that daily provision of LNS for 1 year was not associated with excess hospitalizations, episodes of common childhood diseases or symptoms when all intervention groups were combined. The incidence of non-scheduled visits was

**Table 6** Incidence of guardian-reported disease episodes among rural Malawian infants and young children, ILINS-DOSE study, November 2009–May 2012

Variable	Results by study group				Comparison between the groups†												
	Control (follow-up years: 220·0)		10 g LNS/d (follow-up years: 209·7)		20 g LNS/d (follow-up years: 433·6)		40 g LNS/d (follow-up years: 443·1)		10 g LNS/d v. control		20 g LNS/d v. control		40 g LNS/d v. control				
	Total episodes	Incidence	Total episodes	Incidence	Total episodes	Incidence	Total episodes	Incidence	IRR	95% CI	IRR	95% CI	IRR	95% CI			
All diseases	3215	14·7	3187	15·1	6572	15·2	6716	15·1	1·04	0·96	1·11	1·03	0·97	1·10	1·04	0·97	1·10
Gastroenteritis	408	1·8	405	1·9	837	1·9	761	1·7	1·07	0·89	1·30	1·05	0·89	1·24	0·94	0·80	1·12
ARI	1866	8·5	1829	8·7	3794	8·8	3876	8·7	1·02	0·92	1·12	1·02	0·94	1·11	1·02	0·94	1·11
Undefined fever	345	1·6	322	1·5	657	1·5	687	1·6	0·97	0·81	1·16	0·96	0·83	1·12	0·99	0·85	1·16
Other diseases	596	2·7	631	3·0	1284	3·0	1392	3·1	1·13	0·97	1·31	1·09	0·96	1·24	1·16	1·02	1·32

LNS, lipid-based nutrient supplement; IRR, incidence rate ratio; ARI, acute respiratory infection.

†Incidence = number of episodes/child per year of follow up.

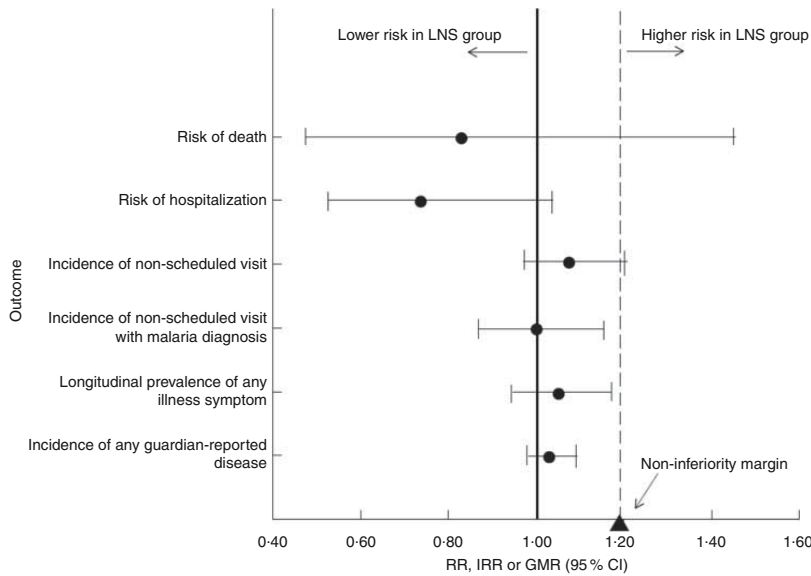
‡Groups were compared using negative binomial regression.

inconclusive. For group comparisons, the 10 and 20 g LNS/d groups had similar outcomes to the control group, except the risk of SAE which was significantly lower in the 20 g LNS/d group. For the 40 g LNS/d group, the comparisons were inconclusive, with increased incidence of non-scheduled visits due to malaria in this LNS group. Our study was not powered to assess mortality outcome.

Our study had several strengths: random allocation of participants; blinding of the investigators involved in data analysis; frequent morbidity data collection; and the year-long follow-up period. Our findings may need to be interpreted with caution since the primary outcome results showed no effect of LNS on growth or stunting<sup>(15)</sup>. However, studies prior to this reported significant length gain and reduction in stunting associated with LNS<sup>(11,16,17)</sup>. Therefore we believe our results showing that LNS is safe are relevant on a wider scale.

Potential causes of bias were: higher than anticipated attrition (20·4%); diagnostic malaria tests not done at home visits; and possible differential reporting of illness by guardians who were not blinded. Theoretically, it is also possible that the research assistants may have under-reported morbidity for children in the LNS groups since they were not blinded. However, we had other objective tools for morbidity data collection such as non-scheduled visits reported by clinicians not employed by the study and SAE assessed by the study physician who was blinded to group allocation. All these showed consistent results of no difference in morbidity, except at non-scheduled visits where malaria-related visits were inconclusive in the 40 g LNS/d group. There were no intergroup differences in proportion of children whose data were available and all participants were included in the analysis up to the time of their dropout or death, suggesting that attrition was balanced among the groups. Reimbursement of medical costs could also inflate the non-scheduled visits data. However, Malawi provides a free national health-care system in public health facilities where most of our participants were treated, and we did not reimburse transport for non-scheduled visits. A study in children aged 6–36 months in Chad<sup>(12)</sup> recorded an incidence of reported disease episodes of 1·17 per child-month (or 14·04 per child-year) which is close to our findings, suggesting that the high disease incidence at home visits was a true observation. Thus, we believe our results are still valid and representative of the target population, suggesting that 10 and 20 g LNS/d did not increase morbidity in our population.

Our findings of no excess morbidity in the 10 and 20 g LNS/d groups are similar to those of a previous study done in Malawi, but in a different area. In that study of 840 children, daily provision of 54 g LNS (containing the same amount of Fe as the LNS used in the present study, 6 mg) did not increase malaria or respiratory morbidity<sup>(15)</sup>. Similar findings have been reported in previous studies that used LNS<sup>(11,12)</sup> or MNP<sup>(3–5)</sup>. In our study, although we



**Fig. 2** Plot of selected morbidity outcomes among rural Malawian infants and young children, iLiNS-DOSE study, November 2009–May 2012. All intervention groups receiving lipid-based nutrient supplement (LNS) were pooled together and compared with the control group, with results (●) reported as incidence rate ratios (IRR), relative risks (RR) or geometric means ratios (GMR) with their 95% CI (represented by horizontal lines). The vertical dashed line represents the margin of non-inferiority. The solid line represents the null effect. None of the point estimates and their 95% CI fell entirely above the non-inferiority margin (1.20; ▲). We conclude that LNS does not increase morbidity

did not routinely test for malaria during home visits, cases of ‘undefined fever’ could be classified as suspected malaria cases according to the Integrated Management of Childhood Illnesses classification for high-malaria-risk areas<sup>(33)</sup>. In general, Fe-containing home fortificants are assumed to be safer than Fe supplements (such as liquid Fe drops) for children living in areas where infectious diseases are common because home fortificants provide a physiological dose of Fe distributed throughout the day, which potentially avoids the adverse effects associated with Fe given as a bolus dose<sup>(15)</sup>. However, another key mechanism by which Fe may mediate malaria morbidity is by increasing reticulocytosis<sup>(34)</sup>. In unadjusted and preliminary analyses of Hb and Zn protoporphyrin status in this study cohort, LNS provision was associated with improved Fe status and a reduction in the prevalence of Fe deficiency, but no improvement in blood Hb concentration. Thus, the Fe in the LNS improved Fe stores but may not have stimulated reticulocytosis, which might explain the lack of adverse effects on malaria morbidity.

Our findings are different from the reports of the studies in Pakistan<sup>(6)</sup>, Kenya<sup>(7)</sup>, Pemba<sup>(8)</sup>, Zambia<sup>(10)</sup> and Cote d’Ivoire<sup>(35)</sup>. These studies reported increases in malaria-related hospitalizations and deaths, respiratory infections, diarrhoea and intestinal inflammation associated with Fe supplements or Fe-containing MNP provision in children.

These differences could be due to: (i) the dose of Fe; (ii) prevalence of Fe deficiency in the study population; and (iii) intensive morbidity surveillance.

The dose of Fe used in the above studies was 12.5 mg/d, whereas we used 6 mg/d in our study. We advised that the LNS be eaten on two or more occasions during the day, so as to limit the amount of Fe ingested in a single meal<sup>(22)</sup>. This might have eliminated the detrimental effects of Fe. The high prevalence of Fe deficiency (66%) in our population may also offer protection to infections aggravated by provision of Fe as suggested by other studies in Africa<sup>(36,37)</sup>, although not all<sup>(38)</sup>. In the Ghana and Pemba studies, children with Fe deficiency at baseline were not adversely affected by the intervention<sup>(4,8)</sup>. We provided intensive morbidity surveillance and referral for treatment to the national health system which may have improved the overall health of the children, an observation also highlighted in the Ghana and Pemba studies<sup>(4,8)</sup>. Thus, it appears that although in some settings provision of Fe may increase the risk of morbidity, this was not the case in our population with a high prevalence of Fe deficiency, using a modest dose of Fe in LNS and providing morbidity surveillance.

The finding that the 40 g LNS/d dose may have been associated with excess malaria-related non-scheduled visits is puzzling, especially given that the 10 and 20 g LNS/d doses were conclusively not associated with increased

morbidity. We do not have a clear biological explanation for this because the dose of Fe was intended to be similar in all the LNS groups. Our dietary intake data suggest that children in the 40 g LNS/d group were actually getting less Fe, as the proportion of LNS eaten by children in this group was only about half that of the 10 g LNS/d group<sup>(32)</sup>. In addition, there were no differences in breast milk intake observed between the groups in intention-to-treat analysis<sup>(39)</sup>. Other factors such as modifications of gut microbiota<sup>(7,40)</sup> may be involved in the pathways that link home fortificants and infections, and these require further study.

## Conclusion

In conclusion, long-term provision of 10 and 20 g LNS/d containing 6 mg Fe/d did not increase morbidity in infants and young children in a population where Fe deficiency and infectious diseases are both common. Provision of 40 g LNS/d did not affect guardian-reported illness symptoms and episodes but may have increased non-scheduled visits because of malaria. A larger study would be needed to assess the effect of LNS on child mortality.

## Acknowledgements

**Acknowledgements:** The authors thank the study participants, the local communities, the health service staff and their research personnel at the study sites as well as members of the trial's data safety and monitoring board, the iLiNS extended research team and the iLiNS-Project Steering Committee (<http://www.ilins.org>) for their contributions in all stages of the study. **Financial support:** This publication is funded by a grant to the University of California, Davis from the Bill & Melinda Gates Foundation. The findings and conclusions contained within the article are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. **Conflict of interest:** The authors declare no conflict of interest. **Authorship:** The authors' responsibilities were as follows: K.M., J.P., K.G.D., S.A.V., Y.B.C., U.A. and P.A. designed the study; J.B., L.A., K.M., J.P., S.A.V., Y.B.C., U.A. and P.A. conducted the study; J.B. analysed data and wrote the paper, with critical input and comments from all other authors; J.B. and P.A. had primary responsibility for final content. All authors read and approved the final manuscript. **Ethics of human subject participation:** The study was performed according to International Conference of Harmonization–Good Clinical Practice (ICH-GCP) guidelines and the ethical standards of the Helsinki Declaration. The protocol was reviewed and approved by the Institutional Review Boards of the University of Malawi, College of Medicine (IRB reference

number P.01/09/722) and the Pirkanmaa Hospital District, Finland (IRB reference number R09130). At least one guardian signed or thumb-printed an informed consent form before enrolment of each participant. The trial was registered at the clinical trials registry ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) with the registration ID of NCT00945698. An independent data safety and monitoring board monitored the incidence of suspected SAE during the trial.

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