**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Version 28.0 (18.05.2018)

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# 1 Version history

| Version number | Version date | Prepared by | Description of the completed editions |
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| 01.0 | 04.06.2013 | Lotta Alho<br>Yin Bun Cheung<br>Jan Peerson | Original document (no appendixes included) |
| 02.0 | 26.08.2013 | Alho, Cheung, Peerson | Added Appendix 01: Secondary growth outcomes (prepared by Lotta Alho).<br><br>Added Appendix 02: The impact of intervention on maternal fever (prepared by Lotta Alho).<br><br>Added a hypothesis on the incidence of small for gestational age and placental weight. Edited methods on pairwise comparisons and confidence intervals. Added site and maternal malaria at enrollment to effect modifier and covariate list. |
| 03.0 | 24.10.2013 | Alho, Cheung, Peerson | Added Appendix 03: Impact of supplementing maternal and infant diet with micronutrient fortified lipid-based nutrient supplements (LNS) upon postpartum symptoms of common mental disorder (prepared by Rob Stewart).<br><br>Added Appendix 04: Malaria immunity analyses (prepared by Upeksha Chandrasiri & Stephen Rogerson) |
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| 14.0 | 25.11.2014 | Alho, Cheung, Peerson | Revised Appendix 04: Secondary growth outcomes (prepared by Upeksha Chandrasiri). |
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| 25.0 | 19.06.2016 | Alho, Cheung, Peerson | Edited Appendix 15: Comparison of the main effect of treatment group on change in maternal vitamin B12 and folate status during pregnancy, in maternal and infant B12 and folate status at 6 months postpartum, in infant B12 and folate status at 18 months, and in vitamin B12 in breast milk at 6 months postpartum (version 2.0, prepared by Juliana Haber and Lindsay Allen) |
| 26.0 | 16.11.2016 | Alho, Cheung, Peerson | Added Appendix 27: The impact of the interventions on human milk oligosaccharides (HMOs) and proteins (version 1.0, prepared by Josh Jorgensen).<br><br>Added Appendix 28: The effect of the Dyad interventions on Malawian infant microbiota at 1, 6 12, 18 and 30 months (version 1.0, prepared by Arox Kamng'ona) |
| 27.0 | 12.12.2016 | Alho, Cheung, Peerson | Edited Appendix 04: Malaria immunity analyses (prepared by Upeksha Chandrasiri & Stephen Rogerson) (version 3.0, prepared by J Stephen Rogerson). |
| 28.0 | 18.05.2018 | Alho, Cheung, Peerson | Added Appendix 29: Assessment of whether the infant microbiota at 6 months modifies effects of the DYAD interventions on growth or inflammation (version 1.0, prepared by Riley Hughes). |

## 2 Introduction

Poor growth and severe childhood stunting are very common in rural Malawi and elsewhere in Sub-Sahara Africa, with known negative consequences for child development and long-term individual and household welfare. To date, few interventions have proven successful in preventing linear growth faltering in early childhood. Our previous results from trials in Ghana and Malawi suggest that a 6-12 month-long daily complementary feeding of infants with 20-50 g of an energy-dense and highly micronutrient fortified Lipid-based Nutrient Supplement (LNS) may markedly reduce the incidence of severe stunting before the age of 18 months. However, results from this and many other studies have indicated that linear growth retardation in low income countries typically starts before six months of age, often already in the foetal period. Hence, interventions targeting only complementary feeding are likely to have a rather limited impact on growth faltering.

The iLiNS-DYAD-M trial was designed to study the impact of an intervention that provides dietary LNS supplementation both to the mother during pregnancy and lactation and to her newly born child from 6 to 18 months of age. For this purpose 1391 pregnant mothers were enrolled in a rural area in Mangochi district, Malawi, and randomized to receive iron and folic acid supplementation (IFA group), multiple micronutrient supplementation (MMN group) or lipid-based nutrient supplements (LNS group). For a subgroup of 869 participants ("complete follow-up"), the intervention and a detailed follow-up will continue for 18 months after delivery. For the remaining participants (n=522, "simplified follow-up"), there will be no further interventions, but the children will be clinically examined at 6 and 18 months of age to assess their growth. Key details of the trial have been recorded at the clinical trial registry at the National Institute of Health (USA) (http://www.clinicaltrials.gov/), under the registration number NCT01239693. A full trial protocol is available from the contact person for this document.

This document (called "the statistical analysis plan" or SAP) describes the study group's plan for data analysis, management, and storage. The SAP is designed to be evolving over time. Version 1.0 documents the details of the hypothesis testing and other analyses on primary and selected secondary pregnancy outcomes. Subsequent versions of the SAP will give further details on the analyses and hypothesis testing of primary childhood outcomes and additional secondary outcome variables and exploratory analyses from the data.

## 3 Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the mother during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and

child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The objective of the first add-on component of the trial is to determine the prevalence of reproductive tract infections, periodontal disease, and symptomatic and asymptomatic malaria among the pregnant women, to study their association with the duration of pregnancy and birth size and to determine if LNS supplementation of pregnant women modifies the association between maternal reproductive tract infections and the duration of pregnancy or birth size. Further exploratory analyses will be done to study the association between the dietary intervention and the prevalence of defined infections or malaria immunity.

The objective of the second add-on component of the trial is to study the development of the infants' intestinal microbiome, its predictors, and its association to child nutrition and growth.

The above objectives have been broken down into the following first six aims that were predefined in the trial protocol. The safety aim was not explicitly stated among the predefined objectives in the trial protocol, but was listed under the safety outcomes for analysis.

1. To evaluate the effect of a novel lipid-based nutrient supplement (LNS-P&L) on pregnancy outcomes and the nutritional status of Malawian pregnant and lactating women.
2. To assess the effect on child growth, development, morbidity and micronutrient status of supplementing the maternal diet with LNS-P&L during pregnancy and lactation and the infant diet with another type of lipid-based nutrient supplement (LNS-20gM) from 6 to 18 mo of age.
3. To assess the extent to which household food insecurity and other individual, household, and village-level characteristics modify the effects of LNS on maternal or child outcomes.
4. To determine the prevalence of reproductive tract infections, periodontitis and symptomatic and asymptomatic malaria among the pregnant women, to study their association with the duration of pregnancy and birth size and to determine if the association is modified by maternal supplementation during pregnancy with LNS.
5. To collect information to facilitate future demand creation for LNS interventions, such as end-user knowledge, attitudes and practices related to LNS and other feeding and parental care-giving practices.
6. To study the development of the infants' intestinal microbiome, its predictors, and its association to child nutrition and growth.

## 4 General approach to data analysis

There will be four categories of data analysis.

1. For the main pregnancy outcomes (birth weight, placental weight, newborn length, other newborn size measurements, duration of pregnancy), the analyses will be driven by predefined study hypotheses (see chapter 4 below). Conclusions on this part of the study will be based on formal hypothesis testing.
2. For the main infant outcomes (length-for-age z-score and other child size measurements by 18 months of age, incidence of undernutrition during the intervention), the analyses will be driven by similar predefined study hypotheses. Conclusions on this part of the study will also be based on formal testing of predefined hypotheses. These analyses will not be described in version 1.0 of the SAP, but will appear in its subsequent versions.
3. For the secondary aims (other pregnancy and childhood outcomes), the analyses will be driven by similar hypotheses to those used for the pregnancy outcomes. These hypotheses have not been predefined in the trial protocol and hence they, too, do not appear in version 1.0 of this SAP. They will, however, be defined as appendixes in subsequent versions of the SAP. For each hypothesis-driven analysis, the SAP will be updated prior to starting the analysis.
4. In addition to the hypothesis-driven questions, there will be a large number of exploratory analyses. In the absence of predefined study hypotheses, these analyses will be considered hypothesis-generating, rather than confirmatory.

## 5 Hypotheses to be tested (pregnancy outcomes)

As indicated above, version 1.0 of the SAP describes predefined hypotheses only for the primary pregnancy outcomes (specific objective 1). Further hypotheses will be formulated and documented in subsequent SAP versions before the respective analyses are started.

Objective 1 / hypothesis 1: The mean birth weight among infants whose mothers were provided with LNS during pregnancy is higher than among infants whose mothers received either iron-folate or multiple micronutrient supplementation.

- As a secondary analysis (for this and to all other items below), we will also test hypotheses about differences between the MMN and IFA groups.

Objective 1 / hypothesis 2: The mean placental weight among women who were provided with LNS during pregnancy is higher than among women who received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 3: The proportion of low birth weight babies is lower among women who are provided with LNS during pregnancy than among women who receive either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 4: The mean newborn length-for-age Z-score (LAZ) is higher among babies whose mothers were provided with LNS during pregnancy than among babies whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 5: The prevalence of stunting (LAZ score <-2) is lower among newborns whose mothers were provided with LNS during pregnancy than among newborns whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 6: The mean duration of pregnancy among women who are provided with LNS during pregnancy is longer than among women who receive either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 7: The incidence of preterm delivery is lower among pregnant women who are provided with LNS during pregnancy than among pregnant women who receive either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 8: The incidence of being small for gestational age baby is lower among babies whose mothers are provided with LNS during pregnancy than among babies whose mothers receive either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 9: The mean newborn weight-for-age Z-score (WAZ) is higher among babies whose mothers were provided with LNS during pregnancy than among babies whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 10: The mean newborn mid upper arm circumference (MUAC) is higher among babies whose mothers were provided with LNS during pregnancy than among babies whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 11: The mean head circumference is higher among babies whose mothers were provided with LNS during pregnancy than among babies whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 1 / hypothesis 12: The prevalence of various forms of malnutrition (underweight, acute malnutrition, small head circumference) is lower among newborns whose mothers were provided with LNS during pregnancy than among newborns whose mothers received either iron-folate or multiple micronutrient supplementation.

## 6 Data cleaning and procedures on breaking the intervention code

The study group will adopt the following procedures for data cleaning and breaking the intervention code

1. In the first phase, a number of investigators will do preliminary cleaning of the data required for the main analyses (safety and pregnancy outcomes). At this point, all investigators are totally blinded to the intervention each participant has been receiving.

2. A study statistician (L.A) makes a preliminary database that contains semi-clean data required for the main analyses. The database and summary statistics for each variable are distributed to the principal investigators, the members of the board governing trial implementation and the principal biostatistician for the trial. Once these individuals agree that the data are sufficiently comprehensive and clean, the study statisticians (L.A, J.P, and Y.B.C) are provided with the database and a code that can be used to group the participants who received the same intervention together – i.e. that gives group codes 1, 2 and 3 without indicating the actual intervention each group number relates to.

3. The study statisticians review the data and complete preliminary analyses for group comparisons (without knowing the actual interventions). Based on these analyses, the study statisticians make suggestions for the amendment of the SAP (e.g. on the treatment of missing values). The investigators listed under 2) above then agree on a revised version of the SAP, after which the intervention code is broken and the main analyses are completed.

4. For secondary outcomes, the analyses will be mostly completed by investigators who are not study statisticians. For each of these analyses, data cleaning will be completed as above. Once the analyst has completed the first round of data cleaning without any knowledge about the group information, s/he will request masked group information from the statisticians. This information will again group the participants who received the same intervention together without indicating the actual intervention each group number relates to. For each analyst, the study statisticians provide a new / different set of scrambled group codes – so that two analysts cannot combine their results during the analysis.

5. Before the intervention code is fully broken, mistakes found in the data can be corrected in the database, as long as there is an audit trail that indicates the date of correction, the old and new value, justification for the correction and the identity of the person authorizing the change (this is not necessary for the correction of entry errors). After the code is broken, the data on main outcomes will be “frozen” and data can no longer be corrected in the database. Instead, all corrections (also entry errors) will be reviewed and need to be approved by the responsible investigator and documented before programmed into cumulative syntax-files (do-files, one for each data collection form) that will contain the same information as the

audit trail described above. These do-files need to be run to clean the data before any subsequent analyses.

6. Data cleaning for other data not used for the main analyses will continue even after breaking the intervention code. For each additional data collection form, the data will be similarly frozen by the time first real analyses will be completed from them (the time can vary form by form). Also for these forms, mistakes found before data freezing will be corrected straight into the database whereas those found after the data freezing will be corrected in separate data-cleaning do files. Both correction methods will contain the audit trail that can be used to track all completed changes.

7. Any investigator may raise a suspicion for a correctable mistake in the data. If such a suspicion arises, the investigator who has the responsibility over those particular data (each data collection form has a defined responsible investigator) should be informed and s/he should investigate if a correction is needed. If yes, the data managers in Finland and Malawi will be informed and the change will be made and documented either to the database (before data freezing, this will be done in Malawi), or to a correction do-file (after data freezing, this will be done in Finland).

## 7 Definition of the primary outcomes

Mean birth weight

Birth weight will be defined as a weight measured within 48 hours from delivery, expressed in grams, rounded to the nearest 10 g and with no decimals. *The data will be extracted from Form 23: Q2.1, Form 24: Q1.2, Q2.4.*

Proportion of low birth weight babies

Low birth weight will be defined as birth weight being less than 2500 g. The proportion of low birth weight babies will be calculated as the number of babies with a birth weight < 2500 g divided by the number of all babies with the valid birth weight data (measured within 48 hours of birth). The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 23: Q2.1, Form 24: Q1.2, Q2.4.*

Mean placental weight

Placental weight will be defined as a weight measured after delivery, expressed in grams, rounded to the nearest 1 g and with no decimals. *The data will be extracted from Form 23: Q4.6.*

Mean newborn length-for-age Z-score (LAZ)

Length-for-age will be calculated from age, sex, and length information from the first measurement taken at the study clinic within 6 weeks (42 days) from delivery, using the STATA macro developed by WHO using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.3.*

The prevalence of newborn stunting

Stunting will be defined as a LAZ-score < -2.0. The prevalence of stunting will be calculated by dividing the number of babies with LAZ < -2 Z-score units by the number of all babies with valid data on this outcome. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.3.*

Mean duration of pregnancy at delivery

The duration of pregnancy will be calculated from gestational age at enrollment, date of enrolment and date of delivery, using the following formula: The duration of pregnancy at birth = the duration of pregnancy at enrolment + (date of delivery – date of enrolment)/7. Women with twin pregnancy will be considered not having valid data on this outcome (because ultrasound dating of pregnancy is unreliable for twin pregnancies) and hence they will be excluded from this analysis. The values will be expressed as gestation weeks, with two decimals. *The data will be extracted from Form06a: Q1.2, Q7.6.1, Q7.6.2, Q7.7; Form 23: Q2.1.*

Incidence of preterm delivery

Preterm delivery will be defined as one occurring before 37.0 completed gestation weeks. The incidence of preterm delivery will be calculated by dividing the number of women with a preterm delivery by the number of all participating women with valid data on the duration of pregnancy. Women with twin pregnancy will be considered not having valid data on this outcome (because ultrasound dating of pregnancy is unreliable for twin pregnancies) and hence they will be excluded from this analysis. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form06a: Q1.2, Q7.6.1, Q7.6.2, Q7.7; Form 23: Q2.1.*

Incidence of small for gestational age

Small for gestational age will be defined by fetal growth curve developed by Alexander et. al. (1996). The incidence of small for gestational age babies will be calculated by dividing the number of small for gestational age babies by the number of all babies with valid data on duration of pregnancy and birth weight. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 06a: Q1.2, Q7.6.1, Q7.6; Form 23: Q2.1; Form 24: Q2.2, Q2.4.*

Mean weight-for-age Z-score (WAZ)

Weight-for-age will be calculated from age, sex, and weight information from the first measurement taken at the study clinic within 6 weeks (42 days) from delivery, using the STATA macro developed by WHO using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.2.*

The prevalence of newborn underweight

Underweight will be defined as a WAZ-score < -2.0. The prevalence of underweight will be calculated by dividing the number of babies with WAZ < -2 Z-score units by the number of all babies with valid data on this outcome. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.2.*

Mean MUAC-for-age Z-score

MUAC-for-age will be calculated from age, sex, and MUAC information from the first measurement taken at the study clinic within 6 weeks (42 days) from delivery, using the STATA macro developed by WHO using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.4.*

Prevalence of acute undernutrition

Acute undernutrition will be defined as a MUAC Z-score < -2.0. The prevalence of acute undernutrition will be calculated by dividing the number of babies with MUAC Z-score < -2 Z-score units by the number of all babies with valid data on this outcome. The proportion will be expressed with one decimal point. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.4.*

Mean head circumference-for-age Z-score

Head circumference-for-age will be calculated from age, sex, and head circumference information from the first measurement taken at the study clinic within 6 weeks (42 days) from delivery, using the STATA macro developed by WHO using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.5.*

Prevalence of small head circumference

Small head circumference will be defined as a head circumference Z-score < -2.0. The prevalence of small head circumference will be calculated by dividing the number of babies with head circumference Z-score < -2 Z-score units by the number of all babies with valid data on this outcome. The proportion will be expressed with one decimal point. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2; Form 29: Q1.2, Q2.5.*

## 8 Safety outcomes

Maternal serious adverse events

The occurrence of maternal SAEs will be expressed as the proportion of women with at least one SAE during the follow-up period (from enrolment to six weeks after delivery). The proportion will be calculated by dividing the number of women with at least one recorded SAE by the total number of enrolled participants. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). If any participant has experienced more than one type of SAE, the participant will be recorded in each category. *The data will be extracted from Form 23: Q2.1; Form 45: Q2.1, Q2.5.1, Q3.2.*

Infant serious adverse events

The occurrence of infant SAEs will be expressed as the proportion of babies with at least one SAE during the follow-up period (from enrolment to six weeks after delivery). The proportion will be calculated by dividing the number of babies with at least one recorded SAE by the total number of recorded newborns. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). The deaths will include abortions, stillbirths, and death after birth. If any participant has experienced more than one type of SAE, the participant will be recorded in each category. *The data will be extracted from Form 23: Q2.1; Form 45: Q2.1, Q2.5.1, Q3.2.*

Perinatal mortality rate

Perinatal mortality rate will be calculated using the following formula: the number of stillbirths or deaths occurring within 7 days from delivery divided by the total number of births, multiplied by 1000. A baby is considered having experienced a still birth if s/he was born dead from a pregnancy that lasted a minimum of 22.0 gestation weeks. If the pregnancy ended earlier than this, the termination will be considered “an abortion” and the individual will not be included in the calculation formula. The rate will be expressed as a plain figure, with no decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.1, Form 45: Q2.1, Q2.5.1, Q3.2.*

Neonatal mortality rate

Neonatal mortality rate will be calculated using the following formula: The number of deaths occurring within 28 days from delivery divided by the total number of live births, multiplied by 1000. The rate will be expressed as a plain figure, with no decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.1, Form 45: Q2.1, Q2.5.1, Q3.2.*

## 9 Basis for the analysis: Intention to treat and per protocol

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

All randomized participants will be eligible to be included in the analyses, with the exception that subjects with missing data on an outcome variable will be excluded for the analysis of that outcome. For outcome variables that reflect the duration of pregnancy, all twins will be considered not having valid date (because ultrasound assessment of the duration of pregnancy is less reliable in twin pregnancies). For variables targeted to be measured within 48 hours of delivery, the data are considered missing if the actual measurement time is over 48 hours. For variables targeted to be measured within 6 weeks of delivery, the data will be considered missing if the actual measurement time is over 6 weeks.

Number of participants with non-missing values analyzed for each end point will be presented by treatment groups.

## 10 Time points for the analyses

For the main pregnancy outcomes the time point for the analyses will cover the period from delivery to six weeks after delivery. This marks the end of puerperal period.

## 11 Presentation of the study findings and hypothesis testing

### 11.1 Success of enrolment and follow-up

All registered participants and the success of their follow-up will be described in a flow chart (Figure 1). For additional information the drop-out rate between groups will be tested with Fisher's exact test and baseline characteristics of drop-outs compared to those who completed the study will be tested with t-test or chi square. P-values for these tests will be shown in the text.

### 11.2 Baseline information

Participant characteristics at enrollment will be tabulated by treatment arms as indicated in table 1. Hypothesis testing will be performed for baseline information to give additional information but p-values will not be presented in Table 1 of the eventual manuscript. Methods used for hypothesis testing are indicated in Table 1.

### 11.3 Comparison of the continuous birth outcomes between the three intervention groups

The group means and standard deviations for birth weight, placental weight, duration of the pregnancy, and child anthropometrics in the newborn period will be tabulated by intervention group as shown in Table 2. The table will also indicate the differences in means and their 95 % confidence intervals between the intervention groups. Figure 2 will present the cumulative frequency plot for timing of deliveries in each group and Figure 3 will show the distribution of birth weight by intervention group.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be done (Stata command *pwcompare*). For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

### 11.4 Comparison of the dichotomous birth outcomes between the three intervention groups

The proportions of babies with low birth weight, preterm birth, or various forms of undernutrition in the newborn period will be tabulated by intervention group as shown in Table 3. Global null hypothesis of no differences between groups will be tested with Fisher's exact test. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with $P<0.05$. Pairwise comparisons will be done with log-binomial regression. Risk ratios between intervention groups are also presented in Table 3.

For the incidence of preterm birth, 12 sets of twin pregnancies will be excluded from the main analysis. As sensitivity analyses for incidence of preterm birth, adjustment for twin pregnancies will be done. Results of the sensitivity analysis will be presented in the text.

### 11.5 Safety profile: Analysis of serious adverse events

The total number of women or infants experiencing at least one SAE will tabulated by the intervention group and the SAE category and shown as described in Tables 4 (maternal SAEs) and 5(infant SAEs). Fisher'r exact test will be used to test the global null hypothesis of no differences between groups and the null hypothesis will be rejected if $P<0.05$. If the global null hypothesis is rejected, comparison between each pair of intervention groups will be conducted using log-binomial regression model.

Perinatal and neonatal mortality rates will be presented in the text.

## 12 General notes on statistical methods

### 12.1 Software

All analyses will be done in Stata version 12. The WHO 2006 Child Growth Standard will be used for age-and-sex standardization of weight and length and other anthropometrics.

### 12.2 Preparing anthropometric data for analysis

All the anthropometric measurements were completed in triplicate during each study visit. For the analysis, the team will use the mean of the first two readings if they do not differ by more than a pre-specified tolerance limit. If they do, the third measurement will be compared with the first and second measurements and the pair of measurements that has the smaller difference will be used to calculate the mean which will be used in analyses. If there are only one or two repeated measurements, the mean of those two will be used for the analyses.

The agreed tolerance limits between the first two measurements are:

1. length/height $\leq$ 0.5 cm
2. circumferences (head, MUAC) $\leq$ 0.5 cm
3. infant/child weight $\leq$ 0.1 kg
4. adult weight $\leq$ 0.1 kg
5. skinfold thickness $\leq$ 2.0 mm

The length, circumference and skinfold thickness measurements were recorded to the last complete unit (mm). To account for the bias of always rounding the values a bit downwards, half a unit will be added to all length, circumference and skinfold thickness measurements prior to the analysis. This procedure is not done for weight measurements, since they were recorded with precision scales to the nearest 10g.

Missing anthropometric values will be treated as missing, i.e. there will be no growth data imputation from the other data.

### 12.3 Multiple comparisons

The study involves multiple objectives and therefore multiple sets of hypothesis. Statistically, the different sets of hypotheses are considered independent families of hypotheses. Statistical adjustment for multiple comparisons in one family of hypotheses does not need to consider the other families.

For efficacy analysis, each family consists of 3 hypotheses, two comparing an intervention group versus the control group and one comparing the two intervention groups to each other. To account for the 3 comparisons, we will begin the analysis by testing the global null hypothesis of no difference between groups. If the global null hypothesis is rejected, raw P-values are used in the comparisons between intervention and control groups.

### 12.4 Confidence intervals

Regardless of results in hypothesis testing, the calculated ratios and differences in between-group comparisons will be complemented with confidence intervals (at 95% level), for descriptive purposes. For quantitative outcomes, confidence intervals will be based on ANOVA and for binary outcomes CI's will be based on log-binomial regression.

### 12.5 Interaction and effect modification

There will be two sets of tests for interaction between the intervention group and selected other variables on their association with the primary pregnancy and birth outcomes. All tests will be done using the likelihood ratio test.

The first set of analyses will be hypothesis-driven and will include unambiguous predefined variables that could logically modify the effect of the nutritional intervention on pregnancy and infancy. Variables included (as continuous variables where possible) in this analysis include:

1. Maternal height
2. Maternal BMI at enrolment
3. Gestational age at enrollment
4. Maternal age
5. Child sex
6. Maternal education
7. Proxy for SES
8. Number of previous pregnancies
9. Season at enrollment
10. Maternal anemia at enrollment
11. Maternal malaria at enrollment
12. Study site

The second set of analyses will be exploratory in nature and will include variables that can be constructed in several ways or that cannot *a priori* be logically linked to an effect modification. Themes or variables included in this analysis include:

1. Maternal knowledge, attitudes, and practices around child nutrition
2. Household wealth

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as stratified by the respective predictor variable. Variables that show no interaction with the intervention group can be used as covariates in the main analysis.

### 12.6 Covariate adjustment

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the four main outcome variables (mean birth weight, proportion of babies with low birth weight, mean newborn LAZ, and proportion of babies with newborn stunting. The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association with any of the four outcomes (a $p<0.1$ level), will be included in all the four models – i.e. all the models will be adjusted for the same set of covariates.

1. Maternal height
2. Maternal BMI
3. Gestational age at enrollment
4. Maternal age
5. Child sex
6. Maternal education
7. Proxy for SES
8. Number of previous pregnancies
9. Season at enrollment
10. Maternal anemia at enrollment
11. Maternal malaria at enrollment
12. Study site

If any of the above listed variables is found to be an effect modifier (see chapter 11.10), it will primarily not be included in the four adjusted models shown in the tables. However, as a sensitivity analysis we will also build supplementary models which may include effect modifiers and the respective interaction terms.

As another set of sensitivity testing, we will repeat the main analyses, adjusting them for the number of foetuses carried by the pregnant participant. There were 12 sets of twins in the study sample and this sensitivity analysis will study the possible confounding effect of twinning on the point estimates for the intervention effect.

## 13 Storage and release of data

The data meta-data will be stored in a tailor-made hierarchical database, consisting of a MS Access front-end and MySQL tables in the back-end. The database and a log file that records all

cumulative data corrections for the respective data collection forms are stored at a computer server at the University of Malawi and regularly copied to a server at the University of Tampere. A data manager in Malawi acts as the manager for these data.

When an investigator wishes to perform certain analyses, s/he will request the respective data from the above-indicated data manager. The data manager will export all the data from the respective data collection form into an excel or Stata file, run the cumulative data correction do-file and then provide the corrected data, together with the syntax for the correction do file (that documents all the completed data editions) to the person requesting the data.

The databases and the do-files will be named with systematic naming format and stored at the central server at the University of Tampere. For each article, the following files will be stored:

1. The database from which the analyses were performed
2. The data dictionary
3. The data correction do file(s)
4. The data analysis do file(s)
5. The actual scientific article

The data collection forms and respective user guides will be stored at the central study repository, in the computer server at the University of Tampere

In the longer run, there is a plan to place the data publicly available in the internet.

### 13.1 Data and output handling

To ensure reproducibility and to keep an audit trail, all data management, analysis and outputting procedures will be kept as Stata do files. All transformation, categorisation, or creation of variables as well as keeping or dropping of subjects in specific analyses will be written in the do files. The do files are to be executed in order to obtain these new data features temporarily, as opposed to saving these new features into permanent data files. It is envisaged that a large number of commands are required, and they may need to be partitioned in more than one do file. Numeric values will be used to indicate the correct sequence for running these files, and version number of the do file is indicated at the file name, e.g. iLiNS-DYAD data cleaning01, form 18, v01.0, 2013-04-27.do should be executed before iLiNS-DYAD data analysis02, form 18, v01.0, 2013-04-27. If data from more than one form are used the form number is not indicated in the do-file name but forms are listed in the comments section in the beginning of the do-file. Variables on data version and version date are included in the data file and people using the data are asked not to share the files with other approved data users. All approved users obtain the data from the data manager so that the latest version is distributed. Outputs will be saved as log files.

A master do file, for example, may include, but is not limited to, the following commands to execute all the data modification, analyses and outputting procedures in one go:

```
**** Example of a master do file

**** DYAD main paper, master do file

clear

version 12.1

set more off

set mem 50m

cd c:\dyad\mainpaper

capture log close

log using mainpaper.log, text replace

do iLiNS-DYAD data cleaning01, form 18, v01.0, 2013-04-27.do

do iLiNS-DYAD data analysis02, form 18, v01.0, 2013-04-27.do

do iLiNS-DYAD data analysis03, form 18, v01.0, 2013-04-27.do

log close
```

## 14 Procedures and history on modifications to the analysis plan

All new versions of and additions to the statistical plan will be approved by a team of core investigators, consisting of the senior researchers who oversee the trial implementation (iLiNS-Malawi Board of Directors) and the study statisticians. Each version will be identified with a new version number and a date of approval and named with standardized file-name format (iLiNS-DYAD analysis plan, version 00.3, 2012-12-27.docx).

In the file name, the first two digits before the decimal indicate an approved change to the SAP (ie version 01.0 denotes the first approved version, 03.0 the third approved version etc). The last digit after the decimal indicates a yet unapproved revision number for a document under editions (eg. 02.1 points to a document that is based on the second approved version, but has undergone one round of yet unapproved editions to it).

The table “Version history” on page 5 lists the editions made to the different approved versions of the SAP:

## 15 List of appendixes

Statistical Analysis Plan, Appendix 01: The impact of the intervention on child size at 6 months (added on 26.08.2013, revised on 19.04.2014)

Statistical Analysis Plan, Appendix 02: The impact of intervention on maternal fever (added on 26.08.2013)

Statistical Analysis Plan, Appendix 03: Impact of supplementing maternal and infant diet with micronutrient fortified lipid-based nutrient supplements (LNS) upon postpartum symptoms of common mental disorder (added on 24.10.2013)

Statistical Analysis Plan, Appendix 04: Malaria immunity analyses (added on 24.10.2013, revised on 25.11.2014, revised on 12.02.2017)

Statistical Analysis Plan, Appendix 05: The impact of intervention on maternal periodontal infections (added on 03.11.2013)

Statistical Analysis Plan, Appendix 06: Willingness-to-pay for lipid-based nutrient supplements during pregnancy (added on 21.03.2014)

Statistical Analysis Plan, Appendix 07: The impact of the interventions on iron status and inflammation (added on 12.06.2014)

Statistical Analysis Plan, Appendix 08: Characterization of microbial communities in the placenta, chorion, amnion, vagina and oral cavity (added on 04.07.2014)

Statistical Analysis Plan, Appendix 09: The impact of LNS on maternal salivary cortisol concentration (added on 04.07.2014)

Statistical Analysis Plan, Appendix 10: The impact of LNS on maternal cholesterol and triglycerides in plasma and fatty acids in plasma and breast milk (added on 04.07.2014)

Statistical Analysis Plan, Appendix 11: Developmental outcomes at age 18 months (added on 25.07.2014)

Statistical Analysis Plan, Appendix 12: Maternal cognition and mother-infant interaction at 6 months post-partum (added on 25.07.2014)

Statistical Analysis Plan, Appendix 13: The impact of intervention on maternal anthropometry and placental weight (added on 19.08.2014)

Statistical Analysis Plan, Appendix 14: The impact of the interventions on maternal vitamin A status (added on 31.08.2014)

Statistical Analysis Plan, Appendix 15: Comparison of the main effect of treatment group on change in maternal vitamin B12 and folate status during pregnancy, in maternal and infant B12 and folate status at 6 months postpartum, in infant B12 and folate status at 18 months, and in vitamin B12 in breast milk at 6 months postpartum (added on 11.09.2014, edited into version 02.0 on 19.06.2016)

Statistical Analysis Plan, Appendix 16: The impact of intervention on maternal reproductive tract infections and malaria (added on 13.09.2014)

Statistical Analysis Plan, Appendix 17: Effect on breastfeeding practices from birth to six months (added on 16.10.2014)

Statistical Analysis Plan, Appendix 18: Developmental milestones (added on 14.12.2014)

Statistical Analysis Plan, Appendix 19: Effect of lipid-based nutrient supplements on infant and young child feeding practices at age 18 months (added on 20.12.2014)

Statistical Analysis Plan, Appendix 20: Experience and Hypothetical Willingness-to-Pay for LNS-P&L and LNS-Child (added on 11.02.2015)

Statistical Analysis Plan, Appendix 21: Sleep patterns (added on 17.02.2015)

Statistical Analysis Plan, Appendix 22: Effect of lipid-based nutrient supplements on delivery complications (added on 25.05.2015)

Statistical Analysis Plan, Appendix 23: Impact of Complementary Feeding of Lipid-Based Nutrient Supplements (LNS) on Child Appetite (version 01.0 added on 22.06.2015, edited into version 02.0 on 19.04.2016)

Statistical Analysis Plan, Appendix 24: The effect of LNS on physical activity (added on 30.09.2015)

Statistical Analysis Plan, Appendix 25: The impact of LNS or MMN on child salivary cortisol concentration (added on 24.01.2016)

Statistical Analysis Plan, Appendix 26: Associations between environmental exposures, infant morbidity, and gut microbiota in iLiNS-DYAD-M (added on 05.05.2016)

Statistical Analysis Plan, Appendix 27: The impact of the interventions on human milk oligosaccharides (HMOs) and proteins (added on 27.07.2016)

Statistical Analysis Plan, Appendix 28: The effect of the Dyad interventions on Malawian infant microbiota at 1, 6 12, 18 and 30 months (added on 30.09.2016)

Statistical Analysis Plan, Appendix 29: Assessment of whether the infant microbiota at 6 months modifies effects of the DYAD interventions on growth or inflammation (added on 18.05.2018)

## 16 References

Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States National Reference for Fetal Growth. *Obstetrics & Gynecology* 1996; 87(2): 163-168.

## 17 Legends to the figures

Figure 1. Participant flow in CONSORT recommended format (Lancet 2001: 357: 1193)

Figure 2. Cumulative frequency plot showing timing (gestational weeks) of deliveries by intervention group.

Figure 3. Distribution of birth weight by intervention group

## 18 Tables

Table 1. Baseline characteristics of the participating women at enrolment, by study group

| **Characteristic** | **LNS** | **MMN** | **IFA** | **Test** |
|---|---|---|---|---|
| Number of participants | xxx | xxx | xxx | |
| Mean (SD) maternal age, years | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) maternal education, competed years at school | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) proxy for socioeconomic status | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) gestational age at enrolment, weeks | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) number of previous pregnancies | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of primiparous women | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Mean (SD) height, cm | xxx.x (xx.x) | xxx.x (xx.x) | xxx.x (xx.x) | ANOVA |
| Mean (SD) weight, kg | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) MUAC, cm | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) BMI, kg/m² | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of women with a low BMI (< 18.5 kg/m²) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Mean (SD) blood hemoglobin concentration, g/l | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of anaemic women (Hb < 110 g/l) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive HIV test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive malaria test (RDT) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |

Table 2. Continuous birth outcomes by intervention group

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) birth weight, g[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) birth weight, g, adjusted model[b] | | | | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) placental weight, g[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) newborn length-for-age (LAZ) z-score[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) newborn length-for-age (LAZ) z-score, adjusted model[b] | | | | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean (SD) duration of the pregnancy, weeks | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) newborn weight-for-age (WAZ) z-score[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) newborn MUAC for age z-score[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) newborn head circumference-for-age z-score[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

[a] Model without covariates

[b] Adjusted model, covariates based on model selection in 12.6

Table 3. Dichotomous birth outcomes by intervention group

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** |
| Incidence of low birth weight[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of low birth weight, adjusted model[b] | | | | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of newborn stunting[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of newborn stunting, adjusted model[b] | | | | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of preterm birth[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of small for gestational age [a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Prevalence of newborn underweight[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of acute newborn undernutrition[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of small newborn head circumference[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

[a] Model without covariates

[b] Adjusted model, covariates based on model selection in 12.6

Table 4. The incidence of maternal SAEs by study group

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Number of participants | xxx | xxx | xxx | | | | | | | |
| Number (%) of women who experienced any SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who died | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who were hospitalized (%) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who experienced other SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 5. The incidence of infant SAEs by study group

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Number of participants | xxx | xxx | xxx | | | | | | | |
| Number (%) of babies who experienced any SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of babies who died (abortion, stillbirth, neonatal death) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of babies who were hospitalized (%) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of babies who experienced other SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 01: The impact of the interventions on child size at 18 months (version 02.0, revised 10.04.2014)

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## 1 Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 26.08.2013 | Alho | Original document Appendix 01 added |
| 02.0 | 10.04.2014 | Alho | Updated Appendix 01 to cover growth analysis at 18 months, 6 and 12 months as complementary analysis. Modified methods, tables and figures to reflect this. Also added analysis of SAEs. |

## 2 Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The overall objective of the iLiNS-DYAD trial is to determine whether LNS consumed by the mother during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves maternal and child health during pregnancy and 18 months thereafter, as compared to consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The aim of the analyses described in appendix 1 is to compare child growth and mortality in the three intervention groups by 18 months of age. The following outcomes will be used to indicate child growth and mortality.

1. Mean length-for-age (LAZ), weight-for-age (WAZ), weight-for-length (WLZ), mid-upper arm circumference (MUAC)-for-age and head circumference-for-age Z-scores at 18 months of age (additionally at 6 and 12 months).
2. Mean change in length-for-age (LAZ), weight-for-age (WAZ), weight-for-length (WLZ) and head circumference-for-age Z-scores and mid-upper-arm circumference (MUAC) as an absolute value (cm) between birth and 18 months of age.
3. The prevalence of undernutrition (stunting, underweight, wasting, small mid-upper arm circumference and small head circumference) at 18 months of age (additionally at 6 and 12 months).
4. The incidence of undernutrition (stunting, underweight, wasting, small mid-upper arm circumference and small head circumference) between birth and 18 months of age.
5. Cumulative incidence of maternal serious adverse events by 6 months after birth and infant serious adverse events by 18 months after birth.

These analyses will primarily be completed with participants in the complete follow-up only. Secondarily, similar analyses, tables and figures will be made for participants in the simplified follow-up.

Complete follow-up means that women in the iron and folic acid group received IFA tablets during pregnancy and placebo tablets during first six months postpartum and participants in multiple micronutrient and LNS groups got either MMN tablet or LNS supplementation during pregnancy and first six months postpartum. The participating infants in IFA and MMN groups did not receive any supplements from 6 to 18 months of age. Infants in LNS group received the supplement from 6 to 18 months of age. Participants in complete follow-up group also underwent comprehensive follow-up and outcome assessment during infancy and early childhood.

Participants in simplified follow-up received similar interventions and follow-up as those in complete follow-up during pregnancy. After pregnancy, they received no intervention and a very limited number of follow-up visits (only clinical visits at 1, 6 and 18 months after delivery, no home visits).

The safety aim was not explicitly stated among the predefined objectives in the trial protocol, but was listed under the safety outcomes for analysis.

## 3 Hypotheses to be tested

1. At 18 months of age, the mean length-for-age (LAZ) Z-score will be greater among children born to mothers provided with LNS during pregnancy and for 6 months after delivery and who themselves received LNS from 6 to 18 months of age than among children who received no supplementation and whose mothers received either iron-folate or micronutrient supplementation.
    a. As secondary outcomes, we will use weight-for-age (WAZ), weight-for-length (WLZ), mid-upper arm circumference (MUAC)-for-age and head circumference-for-age Z-scores
        i. of these, we expect an inter-group difference in WAZ and head circumference, but not in WLZ or MUAC
    b. Besides the z-scores, we will describe the groups and group comparisons by absolute mean (SD) values in length (cm), weight (kg), MUAC (cm) and head circumference (cm)
    c. As a secondary analysis, we will also test hypotheses about differences between the MMN and IFA groups.
    d. As a supplementary analysis, we will complete similar analyses from data when the children were 6 and 12 months old

2. The mean change in length-for-age (LAZ) Z-score between 1 month of age and 18 months of age will be greater among children born to mothers provided with LNS during pregnancy and for 6 months after delivery and who themselves received LNS from 6 to 18 months of age than among children who received no supplementation and whose mothers received either iron-folate or micronutrient supplementation.

    a. As secondary outcomes, we will use change in weight-for-age (WAZ), weight-for-length (WLZ) and head circumference-for-age Z-scores.
    b. Besides the z-scores, we will describe the groups and group comparisons by absolute mean (SD) change in values in length (cm), weight (kg), MUAC (cm) and head circumference (cm)
    c. As a secondary analysis, we will also test hypotheses about differences between the MMN and IFA groups.

3. At 18 months of age the prevalence of severe stunting (LAZ<-3) will be lower among children born to mothers provided with LNS during pregnancy and for 6 months after delivery and who themselves received LNS from 6 to 18 months of age than among children who received no supplementation and whose mothers received either iron-folate or micronutrient supplementation.

    a. As secondary outcomes, we will use prevalence of stunting (LAZ<-2), underweight (WAZ<-2), severe underweight (WAZ<-3), wasting (WLZ<-2), severe wasting (WLZ<-3), small MUAC (MUAC Z-score <-2), very small MUAC (MUAC Z-score <-3), small head circumference (head circumference Z-score <-2) and very small head circumference (head circumference Z-score <-3)
    b. As a secondary analysis, we will also test hypotheses about differences between the MMN and IFA groups.
    c. As a supplementary analysis, we will complete similar analyses from data when the children were 6 and 12 months old

4. By 18 months of age the cumulative incidence of severe stunting (LAZ<-3) will be lower among children born to mothers provided with LNS during pregnancy and for 6 months after delivery and who themselves received LNS from 6 to 18 months of age than among children who received no supplementation and whose mothers received either iron-folate or micronutrient supplementation.

    a. As secondary outcomes, we will use incidence of stunting (LAZ<-2), underweight (WAZ<-2), severe underweight (WAZ<-3), wasting (WLZ<-2), severe wasting (WLZ<-3), small MUAC (MUAC Z-score <-2), very small MUAC (MUAC Z-score <-3), small head circumference (head circumference Z-score <-2) and very small head circumference (head circumference Z-score <-3)

b. As a secondary analysis, we will also test hypotheses about differences between the MMN and IFA groups.

## 4 Data cleaning and procedures on breaking the intervention code

The study group will adopt the following procedures for data cleaning and breaking the intervention code

1. In the first phase, the data will be cleaned by people blinded to the intervention.
2. Second, study statistician (L.A) makes a preliminary database that contains semi-cleaned data required for the analysis. Summary statistics for each variable are distributed to the principal investigators and people doing the data cleaning and additional data cleaning will be done if needed.
3. The study statistician reviews the data and completes preliminary analyses for group comparisons (without using the actual interventions). Based on these analyses, the study statistician makes suggestions for the amendment of the SAP (e.g. on the treatment of missing values). The investigators then agree on a revised version of the SAP, after which the intervention code is broken and the analyses are completed.
4. The data collection for complete follow-up participants ends in April 2014 and simplified follow-up participants in August 2014. Because the primary interest is in complete follow-up outcomes the code will be broken after the data collection and cleaning for the complete-follow up is finished. Additional analysis will be done for simplified follow-up after the data collection and cleaning has finished in August 2014.

## 5 Definition of the growth outcomes

Mean anthropometric Z-scores

Mean anthropometric Z-scores (LAZ, WAZ, WLZ, MUAC Z-score, head circumference Z-score) will be calculated from age, sex, and anthropometric information from the measurement taken at the study clinic at 1 (except for MUAC, for which the Z-scores are not available for children less than 1 mo), 6, 12 (complete follow-up only) and 18 months of age, using the STATA macro developed by WHO using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2, Q2.9; Form 29: Q1.2, Q2.2, Q2.3, Q2.4, Q2.5*

The prevalence of various forms of undernutrition

Moderate to severe undernutrition (stunting, underweight, wasting, small mid-upper arm circumference and small head circumference) will be defined as a Z-score < -2.0 and severe

undernutrition as Z-score < -3.0 for each variable (LAZ, WAZ, WLZ, MUAC Z-score, head circumference Z-score) separately. The prevalence of undernutrition or severe undernutrition will be calculated by dividing the number of children with Z-score < -2 or Z-score < -3 Z-score units by the number of all children with valid data on this outcome. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2, Q2.9; Form 29: Q1.2, Q2.2, Q2.3 , Q2.4, Q2.5*

The incidence of various forms of undernutrition by 18 mo

The incidence of each form of undernutrition (stunting, underweight, wasting, small mid-upper arm circumference and small head circumference) and severe undernutrition will be calculated by dividing the number of children who ever developed the form of undernutrition in question (Z-score < -2 or Z-score < -3 Z-score units at any visit at the age of 1, 6, 12 (complete follow-up only) or 18) by the number of children with valid data on at least one data collection point (1, 6, 12 (complete follow-up only), or 18 months of age). For the Kaplan-Meier analysis an event of undernutrition will be deemed to have happened at the midpoint between the last age when child was observed as being non-undernourished and the first age, when s/he was observed being undernourished. *The data will be extracted from Form 23: Q2.1; Form 24: Q2.2, Q2.9; Form 29: Q1.2, Q2.2, Q2.3 , Q2.4, Q2.5*

## 6 Safety outcomes

Maternal serious adverse events

Maternal SAE analysis will cover participants only in complete follow-up group. The occurrence of maternal SAEs will be expressed as the proportion of women with at least one SAE during the follow-up period. The proportion will be calculated by dividing the number of women with at least one recorded SAE by the total number of enrolled participants. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). If any participant has experienced more than one type of SAE, the participant will be recorded in each category. *The data will be extracted from Form 23: Q2.1; Form 43: Q2.2, Q3.1; Form 45: Q2.1, Q2.5.1, Q3.2.*

Infant serious adverse events

Infant SAE analysis will cover participants only in complete follow-up group. The occurrence of SAEs will be expressed as the proportion of infants with at least one SAE during the follow-up period. The proportion will be calculated by dividing the number of children with at least one recorded SAE by the total number of recorded newborns. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). The deaths will include abortions, stillbirths, and death after birth. If any

participant has experienced more than one type of SAE, the participant will be recorded in each category. *The data will be extracted from Form 23: Q2.1; Form 43: Q2.2, Q3.1; Form 45: Q2.1, Q2.5.1, Q3.2.*

## 7 Basis for the analysis: Intention to treat and per protocol

Primarily, the analysis will be based on the principle of modified intention-to-treat. The modification concerns two participants who were accidentally allocated to another group than the one to which they were actually randomized. The modified ITT analysis will include these two participants under the intervention they received.

Secondarily, we will run the analyses per protocol. We will run the analyses with 60%, 70% and 80% adherence to the supplement but the final level to be used will be decided after the data for adherence are available.

All randomized participants will be eligible to be included in the analyses, with the exception that subjects with missing data on an outcome variable will be excluded for the analysis of that outcome. For variables targeted to be measured within 4 weeks from the target age, the data will be considered missing if the actual measurement time is over 4 weeks.

Number of participants with non-missing values analyzed for each end point will be presented by treatment groups.

## 8 Time points for the analyses

All the above analyses will primarily be done when the child is 18 months old. Secondarily, we will complete similar analyses from data when the children were 6 and 12 months old.

For variables targeted to be measured at 6, 12 or 18 months of age, the data are considered missing if the actual measurement date is off by +/- 4 weeks from target. For variables targeted to be measured at 1 month of age the time point will be within 6 weeks of delivery (the same as that for the main birth outcome analysis). The data for 1 month measurements will be considered missing if the actual measurement time is over 6 weeks.

Maternal SAEs will be analyzed up to 7 months after birth and child SAEs up to 19 months after birth for participants in complete follow-up. Intervention for mothers stops at 6 months after birth and intervention for children stops at 18 months of age but we will analyze all SAEs that occurred within 4 weeks from the target end date. SAEs that occurred after these time points will be excluded from the analyses.

## 9 Presentation of the study findings and hypothesis testing

### 9.1 Baseline information

All enrolled participants and the success of their follow-up will be described in a flow chart (Figure 1). Participant characteristics at enrollment will be tabulated by treatment arms as indicated in Table 1. Hypothesis testing will be performed for baseline information to give additional information but P-values will not be presented in Table 1 of the eventual manuscript. Methods used for hypothesis testing are indicated in Table 1.

We will create more outcome tables and figures than are expected to be published. For tables and figures planned to be published in the first publication, see Appendix.

### 9.2 Comparison of the anthropometric measurements at 18 months of age between the three intervention groups

Table 2 will present the group means and standard deviations at 18 months of age and Table 3 will present the mean change between 1 and 18 months of age for LAZ, WAZ, WLZ, MUAC-Z score (change in MUAC Z-score between 1 and 18 months not presented in Table 3 because MUAC Z-scores are not available for children less than 1 mo) and head circumference Z-score and absolute values in length (cm), weight (kg), MUAC (cm) and head circumference (cm). Tables 2 and 3 will also indicate differences in means and their 95% confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. For pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected only if the global null-hypothesis is also rejected.

Figures 2-3 will show the Kernel plots and cumulative percentages of LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-scores at 18 months of age. Mean change in LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-scores by group between 1, 6, 12 and 18 months of age will be presented in Figures 4-8.

### 9.3 Comparison of the dichotomous growth outcomes at 18 months of age between the three intervention groups

The prevalence of various forms of undernutrition at 18 months by intervention group will be presented in Table 4 and the incidence of various forms of undernutrition by 18 months by intervention group in Table 5. Outcomes describing prevalence and incidence of undernutrition will be stunting, severe stunting, underweight, severe underweight, wasting, severe wasting, small MUAC, very small MUAC, small head circumference and very small head circumference.

Global null hypothesis of no differences between groups will be tested with Fisher's exact test and the global null-hypothesis is rejected with $P<0.05$. Pairwise comparisons will be done with

log-binomial regression. For pairwise comparisons with P<0.05 the hypothesis of no differences between groups will be rejected only if the global null-hypothesis is also rejected. Risk ratios between intervention groups are also presented in Tables 4 and 5.

Cumulative incidence of stunting, severe stunting, underweight, severe underweight, wasting, severe wasting, small MUAC, very small MUAC, small head circumference and very small head circumference will be presented in Figures 9-13. The log rank test will be used for testing global hypothesis and pair-wise comparisons. Hypothesis of equality of survivor functions is rejected if P<0.05.

### 9.4 Safety profile: Analysis of serious adverse events

The total number of women or children experiencing at least one SAE will be tabulated by the intervention group and the SAE category and shown as described in Tables 6 (maternal SAEs) and 7 (child SAEs). Fisher's exact test will be used to test the global null hypothesis of no differences between groups and the null hypothesis will be rejected if P<0.05. Comparison between each pair of intervention groups will be conducted using log-binomial regression model. For pairwise comparisons with P<0.05 the hypothesis of no differences between groups will be rejected only if the global null-hypothesis is also rejected

Kaplan-Meier survival curve for child mortality will be presented graphically by intervention group as illustrated in Figure 14. The log-rank test will be used for testing global hypothesis and pair-wise comparisons. Hypothesis of equality of survivor functions is rejected if P<0.05. Hazard ratios and their confidence intervals will be estimated by the Cox regression model. The sts and stcox commands will be used.

## 10 General notes on statistical methods

### 10.1 Software

All analyses will be done in Stata version 12. The WHO 2006 Child Growth Standard will be used for age-and-sex standardization of weight and length and other anthropometrics.

### 10.2 Preparing anthropometric data for analysis

All the anthropometric measurements were completed in triplicate during each study visit. For the analysis, the team will use the mean of the first two readings if they do not differ by more than a pre-specified tolerance limit. If they do, the third measurement will be compared with the first and second measurements and the pair of measurements that has the smaller difference will be used to calculate the mean which will be used in analyses. If there are only one or two repeated measurements, the mean of those two will be used for the analyses.

The agreed tolerance limits between the first two measurements are:

1. length/height ≤ 0.5 cm
2. circumferences (head, MUAC) ≤ 0.5 cm
3. infant/child weight ≤ 0.1 kg
4. adult weight ≤ 0.1 kg
5. skinfold thickness ≤ 2.0 mm

The length, circumference and skinfold thickness measurements were recorded to the last complete unit (mm). To account for the bias of always rounding the values a bit downwards, half a unit will be added to all length, circumference and skinfold thickness measurements prior to the analysis. This procedure is not done for weight measurements, since they were recorded with precision scales to the nearest 10g.

Missing anthropometric values will be treated as missing, i.e. there will be no growth data imputation from the other data.

### 10.3 Multiple comparisons

The study involves multiple objectives and therefore multiple sets of hypothesis. Statistically, the different sets of hypotheses are considered independent families of hypotheses. Statistical adjustment for multiple comparisons in one family of hypotheses does not need to consider the other families.

For efficacy analysis, each family consists of 3 hypotheses, two comparing an intervention group versus the control group and one comparing the two intervention groups to each other. To account for the 3 comparisons, we will begin the analysis by testing the global null hypothesis of no difference between groups. If the global null hypothesis is rejected, raw P-values are used in the comparisons between intervention and control groups.

### 10.4 Confidence intervals

Regardless of results in hypothesis testing, the calculated ratios and differences in between-group comparisons will be complemented with confidence intervals (at 95% level), for descriptive purposes. For quantitative outcomes, confidence intervals will be based on ANOVA and for binary outcomes CI's will be based on log-binomial regression.

### 10.5 Interaction and effect modification

There will be tests for interaction between the intervention group and selected other variables on their association with the primary growth outcomes (LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score).

Analyses will be hypothesis-driven and will include unambiguous predefined variables that could plausibly modify the effect of the nutritional intervention on pregnancy and infancy. Variables included (as continuous variables where possible) in this analysis include:

1. Maternal height
2. Maternal BMI at enrolment
3. Gestational age at enrollment
4. Maternal age
5. Child sex
6. Maternal education
7. Proxy for SES
8. Number of previous pregnancies
9. Season at enrollment
10. Maternal anemia at enrollment
11. Maternal malaria at enrollment
12. Study site
13. Food security

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as separate analyses for each stratum by the respective predictor variable. Variables that show no interaction with the intervention group can be used as covariates in the main analysis.

### 10.6 Covariate adjustment

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the main growth outcome variables (LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score). The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association with any of the five outcomes (a $p<0.1$ level), will be included in all the four models – i.e. all the models will be adjusted for the same set of covariates.

1. Maternal height
2. Maternal BMI
3. Gestational age at enrollment
4. Maternal age
5. Child sex
6. Maternal education
7. Proxy for SES
8. Number of previous pregnancies
9. Season at enrollment
10. Maternal anemia at enrollment
11. Maternal malaria at enrollment
12. Study site
13. Food security

If any of the above listed variables is found to be an effect modifier (see chapter 10.5), it will primarily not be included in the four adjusted models shown in the tables. However, as a

sensitivity analysis we will also build supplementary models which may include effect modifiers and the respective interaction terms.

## 11 Legends to the figures

Figure 1. Participant flow in CONSORT recommended format (Lancet 2001: 357: 1193)

Figure 2. Kernel density plots of LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score at 18 mo by intervention group

Figure 3. Cumulative frequency plots of LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score at 18 mo by intervention group

Figure 4. Mean change in length-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 5. Mean change in weight-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 6. Mean change in weight-for-length Z-score between 1, 6, 12 and 18 months by intervention group

Figure 7. Mean change in MUAC (cm) between 1, 6, 12 and 18 months by intervention group

Figure 8. Mean change in head-circumference-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 9. Cumulative incidence of stunting and severe stunting by intervention group

Figure 10. Cumulative incidence of underweight and severe underweight by intervention group

Figure 11. Cumulative incidence of wasting and severe wasting by intervention group

Figure 12. Cumulative incidence of small MUAC and very small MUAC by intervention group

Figure 13. Cumulative incidence of small head circumference and very small head circumference by intervention group

Figure 14. Cumulative survival curve for infant mortality by intervention group

## 12 Figures

Figure 1. Participant flow in CONSORT recommended format (Lancet 2001: 357: 1193)

Figure 2. Kernel density plots of LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score at 18 mo by intervention group

Figure 3. Cumulative frequency plots of LAZ, WAZ, WLZ, MUAC Z-score and head circumference Z-score at 18 mo by intervention group

Figure 4. Mean change in length-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 5. Mean change in weight-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 6. Mean change in weight-for-length Z-score between 1, 6, 12 and 18 months by intervention group

Figure 7. Mean change in MUAC (cm) between 1, 6, 12 and 18 months by intervention group

Figure 8. Mean change in head-circumference-for-age Z-score between 1, 6, 12 and 18 months by intervention group

Figure 9. Cumulative incidence of stunting and severe stunting by intervention group

Figure 10. Cumulative incidence of underweight and severe underweight by intervention group

Figure 11. Cumulative incidence of wasting and severe wasting by intervention group

Figure 12. Cumulative incidence of small MUAC and very small MUAC by intervention group

Figure 13. Cumulative incidence of small head circumference and very small head circumference by intervention group

Figure 14. Cumulative survival curve for infant mortality by intervention group

## 13 Tables

Table 1. Baseline characteristics of the participating women at enrolment, by study group

| **Characteristic** | **LNS** | **MMN** | **IFA** | **Test** |
|---|---|---|---|---|
| Number of participants | xxx | xxx | xxx | |
| Mean (SD) maternal age, years | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) maternal education, competed years at school | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) proxy for socioeconomic status | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) gestational age at enrolment, weeks | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) number of previous pregnancies | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of primiparous women | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Mean (SD) height, cm | xxx.x (xx.x) | xxx.x (xx.x) | xxx.x (xx.x) | ANOVA |
| Mean (SD) weight, kg | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) MUAC, cm | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) BMI, kg/m² | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of women with a low BMI (< 18.5 kg/m²) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Mean (SD) blood hemoglobin concentration, g/l | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of anemic women (Hb < 100 g/l) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive HIV test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive malaria test (RDT) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |

Table 2. Continuous growth outcomes by intervention group at 18 mo

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) length-for-age z-score (LAZ) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) length, cm | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) weight-for-age z-score (WAZ) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) weight, kg | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) weight-for-length z-score (WLZ) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) MUAC-for-age z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) MUAC, cm | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean (SD) head circumference-for-age z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) head circumference, cm | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 3. Change in continuous growth outcomes by intervention group at 18 mo

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean change (SD) in length-for-age z-score (LAZ) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in length (cm) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in weight-for-age z-score (WAZ) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in weight (kg) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in weight-for-length z-score (WLZ) between 1 and 18 | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| mo | | | | | | | | | | |
| Mean change (SD) in MUAC (cm) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in head circumference-for-age z-score between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean change (SD) in head circumference (cm) between 1 and 18 mo | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 4. Prevalence of dichotomous growth outcomes by intervention group at 18mo

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Prevalence of moderate to severe stunting (LAZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of severe stunting (LAZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of moderate to severe underweight (WAZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of severe underweight (WAZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of moderate to severe wasting (WLZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of severe wasting (WLZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of small MUAC (Z-score<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of very small MUAC (Z-score<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Prevalence of small head circumference (head circumference Z-score<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of very small head circumference (head circumference Z-score<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

Table 5. Incidence of dichotomous growth outcomes by intervention group at 18mo

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Incidence of moderate to severe stunting (LAZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of severe stunting (LAZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of moderate to severe underweight (WAZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of severe underweight (WAZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of moderate to severe wasting (WLZ<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of severe wasting (WLZ<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of small MUAC (Z-score<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of very small MUAC (Z-score<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Incidence of small head circumference (head circumference Z-score<-2) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Incidence of very small head circumference (head circumference Z-score<-3) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

Table 6. The incidence of maternal SAEs by study group up to 6 months after delivery

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Number of participants | xxx | xxx | xxx | | | | | | | |
| Number (%) of women who experienced any SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who died | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who were hospitalized (%) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of women who experienced other SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 7. The incidence of infant SAEs by study group up to 18 months of age

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Number of participants | xxx | xxx | xxx | | | | | | | |
| Number (%) of infants who experienced any SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of infants who died (abortion, stillbirth, neonatal death) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of infants who were hospitalized (%) | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) of infants who experienced other SAEs | xxx (x.x %) | xxx (x.x %) | xxx (x.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

## 14 Appendix: Tables and figures planned for 1st publication

1. Figure 1. Trial profile (Participant flow in CONSORT recommended format)
2. Table 1. Baseline characteristics of the participating women at enrolment by study group
3. Table 2. Anthropometrics at 18 months by study group, continuous outcomes
4. Figure 2. Kernel density plots or cumulative frequency plots of anthropometric Z-scores at 18 mo by study group
5. Table 3. Mean change in anthropometrics between birth and 18 months by study group
6. Figure 3. Mean change in anthropometric Z-scores between 1, 6, 12 and 18 months by study group
7. Figures 4-5. Incidence of severe and moderate to severe stunting by study group
8. Table 4. Tables for stratified analyses (based on interaction tests, with whatever seems statistically significant)
9. Tables 5-6. SAE tables for mothers and children
10. Figure 6. Cumulative survival curve for infant mortality by intervention group

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 02: The impact of intervention on maternal fever (version 01.0, added on 26.08.2013)

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## 1. Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The aim of the secondary analyses described in appendix 2 is to compare maternal fever in three different intervention groups between enrollment and delivery and from delivery to six months postpartum. The following outcomes will be used to indicate maternal fever.

1. Proportion of visits when the woman reported having fever yesterday
2. Proportion of visits when the woman reported having fever on one or more days in the past week
3. Proportion of days when the woman reported having fever

## 2. Hypotheses to be tested

The same hypothesis will be tested separately in pregnancy and in lactation.

Proportion of visits when the woman reported having fever yesterday is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Proportion of visits when the woman reported having fever on one or more days in the past week is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Proportion of days when the woman reported having fever is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

- As a secondary analysis (for this and to other items above), we will also test hypotheses about differences between the MMN and IFA groups.

## 3. Definition of the maternal fever outcome variables

Percentage of visits when woman reported having fever yesterday

Percentage of visits when woman reported having fever yesterday will be calculated by dividing the number of visits when the woman reports having had fever yesterday by the number of visits when the question was asked. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 18: Q3.7.*

Percentage of visits when woman reported having fever one or more days in the past week

Percentage of visits when woman reported having fever on one or more days in the past week will be calculated by dividing the number of visits when the woman reports having had fever on one or more days by the number of visits when the question was asked. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 18: Q3.7.1.*

Percentage of days when the woman reported having fever

Number of fever days will be calculated by adding up the number of days when the woman reported having had fever during the past week. Number of days when the woman could have had fever will be calculated by adding up number of forms when the question was asked and multiplying it by 7. Percentage of days when the woman reported having fever will be calculated by dividing the number of fever days by the number of days when the woman could have had fever. The values will be expressed as a percentage, with one decimal. *The data will be extracted from Form 18: Q3.7.1.*

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes.

## 5. Time points for the analyses

All the above analyses will cover time period from enrollment to delivery and from delivery to six months postpartum.

## 6. Presentation of the study findings and hypothesis testing

### 6.1 Comparison of the maternal fever outcomes from enrollment to delivery and from delivery to six months after delivery between the three intervention groups

The group means and standard deviations for percentage of visits when the woman reported having fever yesterday, percentage of visits when the woman reported having fever on one or more days in the past week and percentage of days when the woman reported having fever will be tabulated by intervention group as shown in Tables 1 and 2. Table 1 presents the results in pregnancy and Table 2 in lactation. The tables will also indicate the differences in means and their 95 % confidence intervals between the intervention groups.

Distributions of all three outcome variables are skewed and thus log transformation will be done before the analysis. The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be done (Stata command *pwcompare*). For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

## 7. General notes on statistical methods

### 7.1 Software

The same as that for the primary outcome analyses

### 7.2 Preparing anthropometric data for analysis

The same as that for the primary outcome analyses

### 7.3 Multiple comparisons

The same as that for the primary outcome analyses.

### 7.4 Confidence intervals

The same as that for the primary outcome analyses.

### 7.5 Interaction and effect modification

The same as that for the primary outcome analyses.

### 7.6 Covariate adjustment

The same adjustments will be done as for the main analyses.

## 8. Legends to the figures

None

## 9. Figures

None

## 10. Tables

Table 1. Maternal fever outcomes by intervention group in pregnancy

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) % of visits when the woman reported having fever yesterday | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) % of visits when the woman reported having fever 1 or more days in the past week | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) % of days when the woman reported | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| having fever | | | | | | | | | | |

Table 2. Maternal fever outcomes by intervention group in lactation

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) % of visits when the woman reported having fever yesterday | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) % of visits when the woman reported having fever 1 or more days in the past week | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) % of days when the woman reported having fever | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 03: Impact of supplementing maternal and infant diet with micronutrient fortified lipid-based nutrient supplements (LNS) upon postpartum symptoms of common mental disorder (version 01.0, added on 24.10.2013)

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## 1 Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 24.10.2013 | RC Stewart | Original document |
| | | | |
| | | | |

## 2 Introduction

Poor maternal nutritional status during the perinatal period has been linked to increased risk of common mental disorders (CMDs) (Leung & Kaplan 2009). CMDs include depression, anxiety and clinical states of mixed depressive, anxious and somatic symptoms, occurring during pregnancy and the first year postpartum.

Although the link between nutrition and mental disorders outside of the perinatal period has also been investigated, there has been particular focus on the perinatal period because of the nutritional stresses associated with pregnancy and lactation, and the implications of perinatal CMDs for infant development. In low- and middle-income countries (LMIC), perinatal CMDs (and higher counts on screening measures of CMD symptoms) are associated with poor infant outcomes including impaired intrauterine and postnatal growth, and increased diarrheal episodes (Stewart 2007).

Deficiencies in iron, zinc, B-vitamins and essential fatty acids (EFAs) have been associated with perinatal depression in observational studies (Leung & Kaplan 2009). There have been a limited number of trials investigating the impact of nutritional supplementation upon maternal mental health during the perinatal period, and only 4 RCT's conducted in LMIC.

Beard et al (2005) conducted an RCT of iron supplementation for women in the first postnatal year in Khayelitsha, Cape Town, South Africa. Women with mild anaemia were recruited at 6-8 weeks postpartum and randomized to receive 125mcg iron daily or placebo. Edinburgh Postnatal Depression Scale (EPDS) scores at 9 months postpartum were significantly lower in the treatment vs the control group.

In an RCT in HIV-infected women recruited in second trimester in Tanzania, multivitamin supplementation (B-complex, C and E) demonstrated a protective effect on incidence of CMD symptoms equivalent to major depressive disorder (measured using an validated adapted version of the Hopkins Checklist 25) during the follow up period (Smith Fawzi et al. 2007).

Maternal distress (measured using the Self Reporting Questionnaire (SRQ) was reported as an outcome measure in a trial comparing multi-micronutrient (MMN) vs iron and folic acid (IFA) supplementation amongst women in pregnancy and the early postpartum period in Bangladesh (Frith et al. 2009). The MMN group had a lower mean SRQ score at 3.4-4 months postpartum than those receiving 30mg of iron supplementation but not 60-mg.

In a double-blind cluster-randomized trial in Indonesia comparing MMN to IFA supplementation during pregnancy and until three months postpartum, Prado et al (2012) investigated the effect on maternal cognition and mood (measured using an adaption of the Centre for Epidemiologic Studies-Depression (CES-D) scale). Women were recruited in pregnancy and outcomes measured after a mean of 25 weeks of supplementation. There was no effect of MMN

supplementation on mood despite improvement in overall cognitive function equivalent to 1 year of schooling.

To date there have been no trials investigating essential fatty acid supplementation on maternal CPMDs in a low-income setting. A number of trials conducted in high income countries found a beneficial effect of omega 3 fatty acid supplementation on depression, but a meta-analysis concluded that most of the apparent effect could be explained by publication bias (Bloch MH, Hannestad J. 2011).

A nutritional intervention might reduce symptoms of perinatal CMD through (1) a direct effect upon physiological processes including neurotransmitter synthesis, membrane function and inflammatory processes, (2) a psychological response to having increased energy, fewer infections, reduced obstetric complications etc, or (3) by reducing maternal stress through improving infant health. Caring for a sick infant may be a risk factor for CMD; in particular, there is an association between infant diarrhoeal episodes and CMD symptoms that may be bidirectional (Rahman et al. 2007).

The iLiNS-DYAD-M trial was designed to study the impact of an intervention that provides dietary LNS supplementation both to the mother during pregnancy and lactation and to her newly born child from 6 to 18 months of age. For this purpose 1391 pregnant mothers were enrolled in a rural area in Mangochi district, Malawi, and randomized to receive iron and folic acid supplementation (IFA group), multiple micronutrient supplementation (MMN group) or lipid-based nutrient supplements (LNS group). For a subgroup of 869 participants ("complete follow-up"), the intervention and a detailed follow-up will continue for 18 months after delivery. For the remaining participants (n=522, "simplified follow-up"), there will be no further interventions, but the children will be clinically examined at 6 and 18 months of age to assess their growth. Key details of the trial have been recorded at the clinical trial registry at the National Institute of Health (USA) (http://www.clinicaltrials.gov/), under the registration number NCT01239693.

In this sub-study we investigated the impact of the intervention upon maternal symptoms of CMD at 4-6 weeks and 6 months postpartum.

## 3 Study objectives

Objective 1: To investigate the impact of the intervention upon maternal symptoms of CMD at 4-6 weeks postpartum

Objective 2: To investigate the impact of the intervention upon maternal symptoms of CMD at 6 months postpartum

Objective 3: To identify covariates, effect modifiers and mediators of any effect of intervention upon maternal symptoms of CMD at 4-6 weeks and 6 months postpartum

## 4 Hypotheses to be tested

Objective 1 / hypothesis 1: The mean Self Reporting Questionnaire (SRQ) score at 4-6 weeks postpartum amongst mothers provided with LNS during pregnancy is lower than among infants whose mothers received either iron-folate or multiple micronutrient supplementation.

- As a secondary analysis (for this and to all other items below), we will also test hypotheses about differences between the MMN and IFA groups.

Objective 1 / hypothesis 2: The proportion of women scoring ≥5 and ≥8 on the SRQ at 4-6 weeks postpartum is lower among women who are provided with LNS during pregnancy than among women who receive either iron-folate or multiple micronutrient supplementation.

Objective 2 / hypothesis 1: The mean SRQ score at 6 months postpartum amongst mothers provided with LNS during pregnancy is lower than among infants whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 2 / hypothesis 2: The proportion of women scoring ≥5 and ≥8 on the SRQ at 6 months postpartum is lower among women who are provided with LNS during pregnancy than among women who receive either iron-folate or multiple micronutrient supplementation.

Objective 2 / hypothesis 3: The mean EPDS score at 6 months postpartum amongst mothers provided with LNS during pregnancy is lower than among infants whose mothers received either iron-folate or multiple micronutrient supplementation.

Objective 2 / hypothesis 4: The proportion of women scoring ≥9 and ≥13 on the EPDS at 6 months postpartum is lower among women who are provided with LNS during pregnancy than among women who receive either iron-folate or multiple micronutrient supplementation.

## 5 Data cleaning and procedures on breaking the intervention code

As per main study

## 6 Definition of the primary outcomes

**SRQ total** is a continuous outcome describing the total score on the Self Reporting Questionnaire (SRQ).

**EPDS total** is a continuous outcome describing the total score on the Edinburgh Postnatal Depression Scale (EPDS)

CMD screening tools are best analysed as continuous measures. However, dichotomising scores into high and low scorers can have more clinical utility. As scores are unimodally distributed, the choice of cut-off score is usually made based on the desired balance of sensitivity and specificity for detection of a gold standard diagnosis, usually major depressive episode.

We validated Chichewa and Yao versions of the SRQ and EPDS amongst women attending the antenatal clinic at Mangochi District Hospital (Stewart et al. 2013).

**SRQ**

**SRQ≥8:** This is the cut off most commonly used. In our validation study 16% scored above a cut off score of ≥8. At this cutoff, the test characteristics (Chichewa version) for detection of DSM-IV major depressive episode were sensitivity 50.4%, specificity 88.4%, and PPV 41.2%.

**SRQ≥5:** 34% scored above a cut off score of ≥5. At this cutoff, the test characteristics (Chichewa version) for detection of DSM-IV major depressive episode were sensitivity 73.3% specificity 70.9%, and PPV 31.6%. This is the cutoff that best balances sensitivity and specificity.

**EPDS**

**EPDS≥13**: This is the most commonly used cutoff. 8.2% scored above a cut off score of ≥13. At this cutoff, the test characteristics (Chichewa version) for detection of DSM-IV major depressive episode were sensitivity 33.7%, specificity 94.9% and PPV 50.0%.

**EPDS≥9**: This is the most commonly used cutoff. 16.0% scored above a cut off score of ≥9. At this cutoff, the test characteristics (Chichewa version) for detection of DSM-IV major depressive episode were sensitivity 44.4%, specificity 85.4% and PPV 35.3%.

## 7 Safety outcomes

As per main study

## 8 Basis for the analysis: Intention to treat and per protocol

As per main study

## 9 Time points for the analyses

For the main outcomes the time point for the analyses between 4 -6 weeks postpartum, and between 26 weeks postpartum.

## 10 Presentation of the study findings and hypothesis testing

### 10.1 Success of enrolment and follow-up

All registered participants and the success of their follow-up will be described in a flow chart (as per main study). For additional information the drop-out rate between groups will be tested with Fisher's exact test and baseline characteristics of drop-outs compared to those who completed the study will be tested with t-test or chi square. P-values for these tests will be shown in the text.

### 10.2 Baseline information

Participant characteristics at enrollment will be tabulated by treatment arms as indicated in table 1. Hypothesis testing will be performed for baseline information to give additional information but p-values will not be presented in Table 1 of the eventual manuscript. Methods used for hypothesis testing are indicated in Table 1.

### 10.3 Comparison of the continuous CPMD outcomes between the three intervention groups

The group means and standard deviations for SRQ total at 4-6 weeks and 6 month postpartum and EPDS total at 4-6 weeks and 6 month postpartum will be tabulated by intervention group as shown in Table 2. The table will also indicate the differences in means and their 95 % confidence intervals between the intervention groups. Figure x will show the distribution of SRQ and EPDS total by intervention group.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the

three intervention groups will be done using Tukey's method (Stata command *pwcompare*). For all pairwise comparisons with P<0.05, the null-hypothesis of no difference in means between groups will be rejected.

10.4 Comparison of the dichotomous birth outcomes between the three intervention groups

The proportions of women scoring SRQ≥8 and ≥5 at 4-6 weeks and 6 month postpartum and EPDS≥9 and ≥13 at 4-6 weeks and 6 month postpartum and will be tabulated by intervention group as shown in Table x. Global null hypothesis of no differences between groups will be tested with logistic regression. Pairwise comparisons will be tested by Tukey's method. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with P<0.05. Odds ratios between intervention groups are also presented in Table 3.

10.5 Safety profile: Analysis of serious adverse events

As per main study

## 11 General notes on statistical methods

11.1 Software

As per main study

11.2 Preparing anthropometric data for analysis

As per main study

11.3 Multiple comparisons

As per main study

11.4 Confidence intervals

As per main study

11.5 Interaction and effect modification

We will test for interaction between the intervention group and selected other variables on their association with 4-6 week and 6-month SRQ score, and 6-month EPDS score. All tests will be done using the likelihood ratio test.

We will analyse variables that could logically modify the effect of the nutritional intervention on 6-month SRQ score. Variables included (as continuous variables where possible) in this analysis include:

1. Antenatal SRQ score

2. Proxy for SES
3. Social support
4. Maternal height
5. Maternal BMI at enrolment
6. Gestational age at enrollment
7. Maternal anemia at enrollment
8. Maternal age
9. Maternal education
10. Number of previous pregnancies
11. Season at enrollment
12. Child sex

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as stratified by the respective predictor variable. Variables that show no interaction with the intervention group can be used as covariates in the main analysis.

11.6 Covariate adjustment

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show adjusted regression models for 4-6 week and 6-month SRQ score, and 6 month EPDS score. The covariates to be included in the models will be derived from the list below (for 4-6 week outcomes, variables from after 6 weeks postpartum will be excluded). All variables which show a statistically significant association (at $p<0.1$ level), will be included in all the model

1. Antenatal SRQ score
2. Proxy for SES
3. Social support
4. Maternal height
5. Maternal BMI at enrolment
6. Gestational age at enrollment
7. Maternal anemia at enrollment
8. Maternal age
9. Maternal education
10. Number of previous pregnancies
11. Season at enrollment
12. Child sex
13. Maternal BMI at 6 months
14. Delivery complications
15. Infant growth at 6 months
16. No. of infant diarhoeal episodes
17. Number of maternal morbidity episodes
18. Anaemia and iron status (Hb, ZPP,), malarial antigen at ~ 36 wk gestation and 6 mo postpartum

19. Breast milk composition (essential fatty acids, vitamin A, B-vitamins) at 6 mo postpartum
20. Compliance with intervention
21. Serious adverse events (including child death)

If any of the above listed variables is found to be an effect modifier (see chapter 11.10), it will primarily not be included in the four adjusted models shown in the tables. However, as a sensitivity analysis we will also build supplementary models which may include effect modifiers and the respective interaction terms.

As another set of sensitivity testing, we will repeat the main analyses, adjusting them for the number of foetuses carried by the pregnant participant. There were 12 sets of twins in the study sample and this sensitivity analysis will study the possible confounding effect of twinning on the point estimates for the intervention effect.

**Analysis of potential mediators**

A nutritional intervention might reduce symptoms of common mental disorder at 6 months postpartum by a number of mechanisms including:

1. Improved maternal nutritional status. This might improve mood through maternal response to increased energy, fewer infections, reduced obstetric complications etc, or by a direct nutritional effect upon physiological processes including neurotransmitter synthesis, membrane function and inflammatory processes.

2. Reduced stress through improved infant health. Caring for a sick infant may be a risk factor for postnatal common mental disorder.

To investigate which, if any, of these pathways mediate an effect of the intervention upon symptoms of common mental disorder at 6 months postpartum, we will model the effect of including the following variables as mediators.

Maternal health and nutritional status:

1. Maternal morbidity (episodes of diarrhoea and malaria, delivery complications)
2. Red blood cell essential fatty acid status at ~ 36 wk gestation and Breast milk composition (essential fatty acids, vitamin A, B-vitamins) at 6 mo postpartum
3. Anaemia and iron status (Hb, ZPP, transferrin receptor) at 36 weeks and 6 months.
4. Micronutrient status (vitamin A, B-vitamins, zinc) at 36 weeks and 6 months.

Child health

5. Infant length for age z score at 6 months
6. No. of infant diarhoeal episodes from 0-6 months

7. Child sleep.

## 12 Storage and release of data

As per main study

### 12.1 Data and output handling

As per main study

## 13 Procedures and history on modifications to the analysis plan

As per main study

## 14 References

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## 15 Legends to the figures

Figure 1. Participant flow in CONSORT recommended format (Lancet 2001: 357: 1193)

## 16 Tables

Table 1. Baseline characteristics of the participating women at enrolment, by study group

As per main study plus:

| **Characteristic** | **LNS** | **MMN** | **IFA** | **Test** |
|---|---|---|---|---|
| Antenatal SRQ score | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Antenatal Multidimensional Scale of Perceived Social Support (MSPSS) score | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |

Table 2. SRQ and EPDS total scores (continuous measure) by intervention group

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) SRQ at 4-6 weeks [a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) SRQ at 4-6 weeks, adjusted model[b] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) SRQ score at 6 months [a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) SRQ score at 6 months, adjusted model[b] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) EPDS score at 6 months[a] | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) | x.xx | x.xx | x.xx | x.xxx | x.xx (xx to | x.xxx | x.xx (xx to | x.xxx | x.xx (xx to | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| EPDS score at 6 months , adjusted model[b] | (x.xx) | (x.xx) | (x.xx) | | xx) | | xx) | | xx) | |

[a] Model without covariates

[b] Adjusted model, covariates based on model selection in 11.11

Table 3. SRQ ≥5, SRQ≥8 and EPDS≥9 and ≥13 (dichotomous outcomes) by intervention group

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** |
| Prevalence of SRQ≥5 at 4-6 weeks[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥5 at 4-6 weeks, adjusted model[b] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥8 at 4-6 weeks[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥8 at 4-6 weeks, adjusted model[b] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥5 at 6 months[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Prevalence of SRQ≥5 at 6 months, adjusted model[b] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥8 at 6 months[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of SRQ≥8 at 6 months, adjusted model[b] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of EPDS≥9 at 6 months[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of EPDS≥9 at 6 months, adjusted model[b] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of EPDS≥13 at 6 months[a] | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of EPDS≥13 at 6 months, adjusted | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| model[b] | | | | | | | | | | |

[a] Model without covariates

[b] Adjusted model, covariates based on model selection in 11.11

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 04: Analyses on malaria immunity (version 03.0, modified on 12.02.2017)

Prepared by: Ms. Upeksha Chandrasiri (PhD student), Prof. Stephen Rogerson

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**Figure 4 B:** Antibodies to variant surface antigens at 18 months by supplementation group 31

# 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 24.10.2013 | Upeksha Chandrasiri<br>Prof. Stephen Rogerson | Original appendix 04 |
| 02.0 | 17.11.2014 | Upeksha Chandrasiri<br>Prof. Stephen Rogerson | Updated Appendix 04 to cover malaria immunity at 6 months. Additional objectives, methods, tables and figures highlighted. |
| 03.0 | 12/02/2017 | Prof. Stephen Rogerson | Updated Appendix 04 to cover malaria immunity at 18 months. Additional objectives, methods, tables and figures highlighted. |

# 2. Introduction

Malaria places nearly 125 million pregnancies at risk with almost 30 million of them occurring in malaria endemic African countries alone. Malaria in pregnancy leads to over 10,000 maternal and 200,000 infant deaths each year as a result of adverse pregnancy outcomes; severe maternal anaemia, low birth weight infants, small for gestational age, still births and preterm delivery. This significant susceptibility could be attributable to the lack of acquired immunity to malaria that provides protection against clinical disease.

In addition, pregnant women residing in malaria high prevalent regions often suffer from undernourishment adding extra burden on maternal and child morbidity and mortality. Certain nutrient deficiencies such as protein energy malnutrition (PEM), Vitamin A and Zinc are known to increase susceptibility to malaria infection, possibly via suppression of the immune system. Therefore an additional supply of essential nutrients during this critical time, particularly among women living in disadvantaged families with limited resources may benefit leading to improved pregnancy or birth outcomes, child growth outcomes and even malaria clinical outcomes.

Our study is a subproject of the iLiNS-DYAD Malawi clinical trial investigating the influence of a lipid based nutrient supplementation (LNS) on the acquisition of antibody immunity to malaria in pregnant women and their children. Total IgG and functional IgG to a range of malaria antigens expressed during the asexual blood stage of the parasite; merozoite antigens (expressed on the surface and rhoptry bodies of merozoites), variant surface antigens (VSA, expressed on the surface of infected red blood cells) and schizont extract antigens were measured in plasma samples collected at enrolment (≤ 20 gestation weeks) and at 36 gestation weeks (gw) for the mothers and at 6 months and 18 months for their children.

This appendix describes the approaches taken for the statistical analyses for determining the influence of maternal nutrient supplementation on malaria antibody immunity during pregnancy and

at 6 and 18 months in infants, prepared by the iLiNS sub-contract investigators at the University of Melbourne.

## 3. Hypotheses to be tested

The primary hypotheses of the study are detailed as following.

Hypothesis 1: Lipid based nutrient supplementation during pregnancy increases antibody responses to malaria at 36 gw compared to multiple micronutrient supplementation (MMN) and iron and folic acid supplements (IFA)

Hypothesis 2: Maternal LNS supplementation increases malaria antibody responses in children at 6 months

Hypothesis 3: LNS supplementation during pregnancy and lactation increases antibody acquisition in children at 6 months of age compared to children of the mothers who received MMN or IFA.

Hypothesis 4: LNS supplementation during pregnancy and lactation and LNS supplementation in 6-18 month old children increases antibody acquisition in children at 18 months of age compared to children of the mothers who received MMN or IFA.

## 4. Study objectives

The primary aim of the iLiNS-DYAD-M study is to determine the beneficial role of nutrient supplementation on child growth outcomes. The sub-contract for the current study will investigate the secondary outcomes, determining the influence of nutrient supplementation on malaria antibody immunity during pregnancy and antibody acquisition in early childhood. Further secondary outcomes include determining the association between antibody levels and pregnancy/birth outcomes, impact of maternal nutrient supplementation on malaria antibody acquisition in infants as described below.

1. Determine the effect of the type of nutrient supplementation on malaria antibody immunity at 36 weeks' gestation. (LNS, multiple micronutrient supplementation (MMN) and iron and folic acid supplements (IFA))

2. Investigate the association between malaria antibody immunity at 6 months and the influence of maternal nutrient supplementation

3. Determine the association between maternal nutrient supplementation type and malaria antibody acquisition at 6 months

We will compare seroprevalence, and relative concentrations, of antibodies to malaria antigens (variant surface antigens, merozoite antigens and schizont extract as previously described) between children in different supplementation groups; antibodies in LNS group compared to MMN, LNS compared to IFA and MMN compared to IFA

4. Determine the association between maternal nutrient supplementation type and malaria antibody acquisition at 18 months

We will compare seroprevalence, and relative concentrations, of antibodies to malaria antigens (variant surface antigens, merozoite antigens and schizont extract as previously described) between children in different supplementation groups; antibodies in LNS group compared to MMN, LNS compared to IFA and MMN compared to IFA.

## 5. Definition of primary outcomes

a. Malaria antibody measurements at enrolment and at 36gw

Malaria antibody levels were measured in the peripheral plasma samples collected from pregnant women at enrolment (≤20 gw) and at 36 gw. Antibodies were measured against pregnancy-specific variant surface antigens (VSA) (including VAR2CSA DBL5 antigen), non-pregnancy-specific VSA, merozoite antigens; MSP-1 19kD, MSP-2, MSP-3, Rh2A9 (PfRh2-2030), EBA-175 and schizont extract. The antibody levels were measured by fluorimetry and will be reported as fluorescence intensity (FI), or by flow cytometry and will be reported as geometric mean fluorescence intensity (MFI) as a percentage relative to the positive control.

5.2. Changes in antibody levels and magnitude of change in antibody levels from enrolment to 36gw

Changes in antibody levels measured against all malaria antigens will be compared between enrolment and at 36gw to determine crude changes in antibody levels in the current pregnancy. For additional analyses antibody levels will be divided based on their tertiles for each antigen at each time point. Antibody levels will be first sorted from lowest to the highest. The first 1/3$^{rd}$ of the lowest antibody levels category will be defined as low responders

In order to calculate the magnitude of change in antibody levels following formula will be used.

Magnitude of antibody level change = Antibody levels at 36gw – Antibody levels at enrolment

5.3. Rate of change in antibody levels by 36gw

The rate of change in antibody levels will be defined as following

$$\text{Rate of change in antibody levels} = \frac{\text{Magnitude of antibody level change}}{\text{Number of weeks from enrolment to 36gw}}$$

The number of weeks from enrolment to 36gw varies among women due to different gestational weeks at enrolment

5.4. Seropositivity to malaria antigens at enrolment and at 36gw

The seropositivity of each participant for each malaria antigen at a particular time point, enrolment or 36gw; will be defined as following

For merozoite antibodies and anti-VAR2CSA DBL5 antibodies measured by fluorimetry,

A plasma sample is considered seropositive if the MFI of the sample > average MFI of the negative controls + (3X standard deviation of the MFI of negative control)

For VSA antibodies measured via flow cytometry,

A plasma sample is considered seropositive if the Geometric MFI of sample > average geometric MFI of the negative controls + (2X standard deviation of the geometric MFI of negative control).

If seropositive to a particular malaria antigen at a particular time point “1” or if seronegative “0” will be reported. This information is included as a variable next to the respective antibody level variables in the database

5.5. Seroprevalence at enrolment and at 36gw

The seroprevalence will be defined as the proportion of women seropositive to a particular antigen at a particular time point. .

5.6. Seroconversion to malaria antigens by 36gw

Seroconversion to each malaria antigen will be determined and will be categorized as either positive or negative. A positive seroconversion is defined when the plasma collected from one pregnant woman at enrolment is seronegative (enrolment = 0) to a particular antigen when her plasma collected at 36gw becomes seropositive (36gw = 1) to the same antigen and vice versa for the negative seroconversion (enrolment = 1, 36gw = 0). The number of women who gained seropositivity and who lost seroconversion by 36gw will be calculated as a percentage of the total number of women.

5.7. Categorising pregnant women based on malaria infection status (effect modifiers and covariate adjustments)

Pregnant women will be categorised into infected and uninfected malaria based on the presence of parasitaemia by light microscopy (LM+ and LM-). This categorisation will be used in the analyses of adjustment for covariates and effect modifiers.

*Light microscopy data will be obtained from Form 07, Q3*

5.8. Antibody levels at 6 months (continuous outcome),

Antibodies to malaria were measured in plasma samples collected at 6 months. Antibodies to the same malaria schizont extract and merozoite antigens that were used in the maternal study were used; merozoite surface protein 1 (MSP-1), MSP-2, MSP-3, reticulocyte binding homologue 2A9 (Rh2A9) and erythrocyte binding antigen 175 (EBA-175). In addition we measured antibodies to 3 different parasite lines expressing different *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) protein (PfEMP-1 is the major variant surface antigen [VSA] involved in malaria pathogenesis). Measured antibody levels (in optical density [OD] for schizont and merozoite antigens and geometric mean fluorescence intensity [MFI] for VSA) were presented as a percentage of the positive control.

5.9. Antibody seropositivity at 6 months (dichotomous outcome)

A participant is considered seropositive for an antigen if the percentage of the sample's OD or MFI was greater than the sum of the average and 3 standard deviations (SD) of the percentage OD or MFI of the negative controls. A child who is seropositive for a particular antigen at 6 months will be assigned 1 and a child who is not seropositive will be assigned 0.

5.10. Antibody levels at 18 months (continuous outcome),

Antibodies to malaria will be measured in plasma samples collected at 18 months. Antibodies to the same malaria schizont extract and merozoite antigens that were used in the maternal study will be used; merozoite surface protein 1 (MSP-1), MSP-2, reticulocyte binding homologue 2A9 (Rh2A9) and erythrocyte binding antigen 175 (EBA-175). In addition, we will measure antibodies to 3 different parasite lines expressing different *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) protein (PfEMP-1 is the major variant surface antigen [VSA] involved in malaria pathogenesis). Measured antibody levels (in optical density [OD] for schizont and merozoite antigens and geometric mean fluorescence intensity [MFI] for VSA) will be presented as a percentage of the positive control.

5.11. Antibody seropositivity at 18 months (dichotomous outcome)

A participant will be considered seropositive for an antigen if the percentage of the sample's OD or MFI is greater than the sum of the average and 3 standard deviations (SD) of the percentage OD or MFI of the negative controls. A child who is seropositive for a particular antigen at 18 months will be assigned 1 and a child who is not seropositive will be assigned 0.

## 6. Basis of the analysis for objective 1: Intention to treat and per protocol

The basis for the analysis is the same as for the main trial.

## 7. Time points for analyses

All the analyses will be performed using antibody measures at enrolment and 36 gw. Measurements of covariates and effect modifiers collected during this time period will be used in the following analyses.

## 8. Presentation of study findings and hypothesis testing

8.1. Baseline information

Participant characteristics including demographic and basic clinical characteristics will be categorised by intervention groups as shown in table 1. The median and interquartile range for each characteristic will be tabulated unless otherwise stated. Differences in characteristics across the groups will be determined by Kruskal Wallis (non-parametric continuous variables), $Chi^2$ or Fisher Exact test (for categorical variables) where applicable.

Statistical differences between the groups will be reported as $p<0.05$ and 95% confidence intervals will be reported for the analyses.

8.2. Comparison of antibody levels and seroprevalence at enrolment and at 36gw among pregnant women in different intervention groups

Differences in antibody levels at enrolment and at 36gw across the 3 intervention groups will be compared by performing Kruskal Wallis test. If a significant difference was found in the antibody levels at enrolment between the intervention groups, enrolment malaria antibodies will be considered as a covariate in further analyses. To determine differences between MMN, LNS groups with IFA, Mann Whitney test will be performed (malaria antibody levels are not normally distributed). If the null-hypothesis (no change in antibody levels between intervention groups) was rejected for the above comparisons, Bonferroni correction will be performed adjusted for covariates and confounders described in sections 9.5 and 9.6 during the period from enrolment to 36gw. Scatter plot (similar to the presentation in figure 1) or box-whiskers plot will be constructed to display antibody levels in each supplementation group.

The seroprevalence for each antigen at 36gw will be compared between the 3 intervention groups by performing logistic regression (Table 2). Any statistically significant differences will be reported as $p<0.05$.

### 8.3. Magnitude and rate of change in antibody levels among pregnant women in different intervention groups

The magnitude and rate of antibody level change will be compared between the intervention groups by performing Kruskal Wallis test or if the above data is normally distributed one-way ANOVA will be performed. If null-hypothesis will be rejected, $p<0.05$, Bonferroni correction or Holm-Šídák method will be performed adjusted for the confounders and covariates. Magnitude and rate of antibody level change will be reported in table format (Table 3) or bar graphs with mean and standard error of the mean (SEM) (Figure 2 legend). Statistical differences will be reported as $p<0.05$ accompanied by 95% confidence interval.

### 8.4. Changes in seroconversion to malaria across the supplementation groups

The positive seroconversions at 36gw for each antigen across the intervention groups will be compared across the supplementation groups. Logistic regression will be performed to determine any differences between supplementation groups and data will be presented in table format similar to table 2. Statistical differences will be reported as $p<0.05$.

### 8.5. Comparison of malaria antibody levels and seroprevalence at 6 months between the three maternal supplementation arms

Figure 3A and 3B present antibody levels at 6 months reported as a percentage of the positive control stratified by the supplementation groups. Kruskal Wallis test will be performed to determine the differences in antibody levels between the supplementation arms.

Table 4 will present the median percentage antibody levels with interquartile range (IQR) at 6 months of age. Kruskal Wallis test will be performed to compare antibody levels across supplementation groups. Linear regression univariate analysis will be performed between LNS versus IFA, LNS versus MMN and MMN versus IFA to determine the antibody level differences between supplementation groups. Multivariate regression will be performed adjusting for confounders and effect modifiers. Coefficients and 95% confidence intervals (CI) will be reported.

Table 5 will present the number and the percentage of children with seropositivity to each malaria antigen by supplementation groups. $Chi^2$ test will be performed to determine the differences across the supplementation groups. A multivariate logistic regression will be performed adjusting for confounders, reporting relative risk ratios (RR) and 95% CI.

### 8.6. Testing the effect modification of factors interacting with the association between malaria immunity at 6 months and supplementation type

Table 6 will present the influence of effect modifiers on the association between antibody levels at 6 months and the supplementation type. Likelihood-ratio test will be performed to determine potential interactions. If the p-value for the ratio is $<0.1$, we will stratify antibody levels by the effect modifier and examine the effect of supplementation within each group.

### 8.7. Comparison of malaria antibody levels and seroprevalence at 18 months between the three maternal supplementation arms

Figure 4A and 4B will present antibody levels at 18 months reported as a percentage of the positive control stratified by the supplementation groups. Kruskal Wallis test will be performed to determine the differences in antibody levels between the supplementation arms.

Table 7 will present the median percentage antibody levels with interquartile range (IQR) at 18 months of age. Kruskal Wallis test will be performed to compare antibody levels across supplementation groups. Linear regression univariate analysis will be performed between LNS versus IFA, LNS versus MMN and MMN versus IFA to determine the antibody level differences between supplementation groups. Multivariate regression will be performed adjusting for confounders and effect modifiers. Coefficients and 95% confidence intervals (CI) will be reported.

Table 8 will present the number and the percentage of children with seropositivity to each malaria antigen by supplementation groups. $Chi^2$ test will be performed to determine the differences across the supplementation groups. A multivariate logistic regression will be performed adjusting for confounders, reporting relative risk ratios (RR) and 95% CI.

### 8.8. Testing the effect modification of factors interacting with the association between malaria immunity at 18 months and supplementation type

Table 9 will present the influence of effect modifiers on the association between antibody levels at 18 months and the supplementation type. Likelihood-ratio test will be performed to determine potential interactions. If the p-value for the ratio is <0.1, we will stratify antibody levels by the effect modifier and examine the effect of supplementation within each group.

## 9. General notes on statistical methods

### 9.1. Software

STATA 13 will be used to perform all the statistical analyses. Additionally, Graphpad Prism 5 will be used for constructing graphs. Database including all the variables for the analyses was constructed in Microsoft Excel and converted to dat* format to be used on STATA

### 9.2. Preparing malaria antibody data for analyses

Maternal peripheral plasma samples were heat inactivated and diluted to a working concentration prior to analysis. Both enrolment and 36gw samples for the same participant were assayed in the same plate on the same day. Every sample including the negative (malaria unexposed and non-immune Melbournian plasma) and positive (pool of sera collected from malaria immune individuals) controls were performed in triplicates

The MFI of antibodies were determined by taking the average of the triplicates (fluorimetry for determining antibodies to merozoite antigens) or duplicates (flow cytometry assays for VSA). The MFI for each sample is adjusted for intra and inter-plate variability. Intra-plate variability was determined by calculating the percentage variance of the MFI of the replicates. The samples were re-run if the variance between the triplicates/duplicates was greater than 20%, with their respective enrolment/ 36gw samples in the same assay.

Inter-plate variability was determined by calculating the percentage coefficient of variation (CV%) for each assay as following. Standard deviation of the MFIs of the positive control standard (highest dilution of the positive, see below) will be averaged for all the assays for each antigen and will be divided by the average MFI of the same positive standard for these assays to determine the CV as shown below.

$$CV\% = \frac{\text{Standard deviation}}{\text{Average}} \times 100\%$$

If the CV% for an assay was >30% this assay was repeated.

The positive pool sera was serially diluted to create a standard curve which was then used to determine the antibody levels as a percentage of the positive control with the lowest dilution set to 0% and the highest dilution set to 100%. Seropositivity for each antigen for each participant was determined using the formulas in section 5.4 and included in the database alongside the adjusted antibody levels.

### 9.3. Multiple comparisons

Bonferroni or Holm-Šídák method will be used for multiple comparisons.

Statistical adjustment for multiple comparisons for malaria immunity at 6 months will be performed for all the analyses as mentioned above; sections 8.5 – 8.6.

Similar adjustment for multiple comparisons for malaria immunity at 18 months will be performed for all the analyses as mentioned above; sections 8.7 – 8.8.

### 9.4. Confidence intervals

All the statistical analyses will be complemented with 95% confidence interval (CI) calculated based on t-test

### 9.5. Interactions and effect modifiers

9.5.1. We will test for interactions between the intervention groups and selected effect modifiers (list below) on their association with malaria antibody levels at enrolment and 36gw, magnitude and rate of antibody level change. All tests will be done using the likelihood ratio test.

1. Maternal age
2. Gravidity
3. HIV status
4. Bed net use
5. Season at enrolment
6. Malaria infection at enrolment(based on LM+ and LM-)
7. Neighborhood of residence (categorized based on the closest health centre)

*Data will be obtained from Form 02, Q2.3; Q2.5; Form 06, Q3.3, Q6.2, Form 18, Q2.3, Form 03, Q2*

9.5.2. Potential effect modifiers will be tested for any interactions between the intervention group and antibody levels and seropositivity at 6 and 18 months using likelihood-ratio test.

These variables include (as continuous variables where possible):

1. Maternal BMI at enrolment
2. Duration of gestation (from enrolment to delivery)
3. Number of pregnancies
4. Sex of the child
5. Maternal education
6. Proxy for SES
7. Study site
8. Maternal anaemic status at enrolment
9. Maternal HIV status
10. Bed net use by children

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as separate analyses for each stratum by the respective predictor variable.

## 9.6. Adjustment for covariates

Following covariates will be used to construct adjusted regression models for the outcome variables (antibody levels at 36gw, seroprevalence, magnitude of change and rate of change in antibody levels). If a statistically significant association was found (a $p<0.05$ level), these covariates will be included in all the four models – i.e. all the models will be adjusted for the same set of covariates.

1. No covariate adjustment
2. Maternal malaria antibody levels at enrolment
3. Malaria infection at enrolment
4. Maternal age
5. Gravidity

6. HIV status
7. Bed net use
8. Season at enrolment

9.6.1. Covariate adjustment for malaria immunity at 6 months.

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the outcome variables; antibody levels at 6 months and seropositivity. The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association (at $p<0.1$ level), will be included in the regression model

1. No covariate adjustment
2. Maternal BMI at enrolment
3. Duration of gestation (from enrolment to delivery)
4. Number of pregnancies
5. Sex of the child
6. Maternal education
7. Proxy for SES
8. Study site
9. Maternal anaemic status at enrolment
10. Maternal HIV status
11. Bed net use by children

9.6.2. Covariate adjustment for malaria immunity at 18 months.

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the outcome variables; antibody levels at 18 months and seropositivity. The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association (at $p<0.1$ level), will be included in the regression model

1. No covariate adjustment
2. Maternal BMI at enrolment
3. Duration of gestation (from enrolment to delivery)
4. Number of pregnancies
5. Sex of the child
6. Maternal education
7. Proxy for SES
8. Study site
9. Maternal anaemic status at enrolment
10. Maternal HIV status
11. Bed net use by children

# 1. Tables

**Table 1:** Patient demographic and clinical characteristics

| **Characteristics** | IFA | MMN | LNS | All women | P (95% CI) |
|---|---|---|---|---|---|
| **No. pregnant women** (maternal samples received at Melbourne) | XXX | XXX | XXX | 1008 | X.XX (XX to XX) |
| **Gestation weeks (gw) at enrolment: median (IQR)** | XX | XX | XX | 17 (15-18.4) | X.XX (XX to XX) |
| **Maternal age : median (IQR)**<br><20 years No. (%)<br>20-25<br>26-30<br>>30 | XX | XX | XX | 24 (20-28)<br>310 (30.83%)<br>298 (29.62%)<br>238 (23.66%)<br>160 (15.90%) | X.XX (XX to XX) |
| **Gravidity: Number (%)**<br>Primigravidae<br>Secundigravidae<br>Multigravidae (3-5 pregnancies)<br>Grand multigravidae (>5 pregnancies) | XX | XX | XX | 199 (19.76%)<br>202 (20.06%)<br>381 (37.84%)<br>225 (22.34%) | X.XX (XX to XX) |
| **Malaria prevalence: Number (%) at enrolment,**<br>PCR<br>Blood film | XX | XX | XX | XX (XX) | X.XX (XX to XX) |
| **HIV prevalence**: No: (%) | XX | XX | XX | 130 (13%) | X.XX (XX to XX) |
| **Anaemia: total (%)**<br>Haemoglobin levels<br>Iron deficiency anaemia | XX | XX | XX | XX (XX) | X.XX (XX,XX) |
| **Bednet use** | XX | XX | XX | XX | X.XX (XX,XX) |

**Table 2:** Seroprevalence to malaria among pregnant mothers across the supplementation groups at 36gw

| | **Pregnant women seropositive at 36gw/ total pregnant women in each group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** |
| Total IgG to pregnancy- specific VSA | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Opsonising antibodies to pregnancy-specific VSA | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| VAR2CSA-DBL5 | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Schizont extract | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Total IgG to non-pregnancy-specific VSA | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Opsonising antibodies to pregnancy-specific VSA | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| MSP-1 19kD | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MSP-2 | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| MSP-3 | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| EBA-175 | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Rh2A9 | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

Data presented as the proportion of women seropositive at 36 gw (seroprevalence) for the 3 supplementation arms. Logistic regression will be performed adjusting for covariates and confounders

**Table 3:** Magnitude of antibody level change or rate of antibody level change categorized by supplementation groups

| | **Magnitude of antibody level change by 36gw or rate of antibody level change** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS** | **MMN** | **IFA** | **P-value KW** | **Median difference (95 % CI)** | **P-value MW** | **Median difference (95 % CI)** | **P-value MW** | **Median difference (95 % CI)** | **P-value MW** |
| Total IgG to pregnancy- specific VSA, median (IQR) | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Opsonising antibodies to pregnancy-specific VSA | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| VAR2CSA-DBL5 | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Schizont extract | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Total IgG to non-pregnancy-specific VSA | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Opsonising antibodies to pregnancy-specific VSA | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| MSP-1 19kD | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MSP-2 | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| MSP-3 | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| EBA-175 | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Rh2A9 | xx (xx, xx) | xx (xx, xx) | xx (xx, xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

Data presented as median magnitude of change in antibody levels or median rate of antibody level and interquartile range (IQR). Comparisons between the 3 groups will be performed by Kruskal Wallis test (KW) while comparisons between 2 groups will be performed by Mann Whitney test (MW). Adjusted for covariates and confounders.

**Table 4.** Malaria antibody levels at 6 months stratified by supplementation groups

| | **Result by study group** | | | | **Comparison between LNS and IFA group** | | **Comparison between LNS and MMN group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **IFA** | **MMN** | **LNS** | **P-value[a]** | **Coefficient (95 % CI)** | **P-value[b]** | **Coefficient (95 % CI)** | **P-value[b]** | **Coefficient (95 % CI)** | **P-value[b]** |
| Number of participants | N=XXX | N=XXX | N=XXX | | | | | | | |
| MSP-1 19kD | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-2 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-3 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| EBA-175 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Rh2A9 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Schizont extract | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antibodies to VSA of E8B parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of R29 parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of 3D7 parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

a. P-value calculated using Kruskal Wallis test
b. Linear regression of antibody levels between supplementation groups
c. Multivariate regression adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

**Table 5.** Antibody seropositivity at 6 months by supplementation groups

| | **Number of children seropositive/ total number of children** | | | | **Comparison between LNS and IFA group** | | **Comparison between LNS and MMN group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **IFA** | **MMN** | **LNS** | **P-value[a]** | **RR (95 % CI)** | **P-value[b]** | **RR (95 % CI)** | **P-value[b]** | **RR (95 % CI)** | **P-value[b]** |
| MSP-1 19kD | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-2 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-3 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| EBA-175 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Rh2A9 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Schizont extract | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of E8B parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antibodies to VSA of R29 parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of 3D7 parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

a. P-value calculated using the Chi2 test
b. P-value calculated using logistic regression reporting Relative Risk Ratios (RR)
c. P-value calculated using multivariate logistic regression reporting odds ratios (OR) while adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

**Table 6:** Effect modification- dichotomous/continuous variables (by supplementation group)

| | | Result by study group | | | | Comparison between LNS and IFA group | | Comparison between LNS and MMN group | | Comparison between MMN and IFA group | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | Interaction test p-value[a] | IFA | MMN | LNS | P-value[b] | RR/difference in means (95 % CI)[c] | P-value[c] | RR/difference in means (95 % CI)[c] | P-value[c] | RR/difference in means (95 % CI)[c] | P-value[c] |
| Antibodies to MSP-1 19kD | | | | | | | | | | | |
| HIV=1 | 0.xx* | xxx/n (xx.x %)[1] | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y)[2] | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| HIV=0 | | xxx/n (xx.x %) | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Primi = 0 | 0.xx* | xxx/n (xx.x %)[1] | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y)[2] | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Multi = 1 | | xxx/n (xx.x %) | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Duration of gestation | 0.xx* | mean (SD) | mean (SD) | mean (SD) | 0.xxx | diff in means (x-y) | 0.xxx | diff in means (x-y) | 0.xxx | diff in means (x-y) | 0.xxx |

a. P-value calculated using likelihood ratio test
b. P-value calculated using $Chi^2$ test (dichotomous) or ANOVA (continuous, comparing mean differences)
c. Relative risk with 95 % confidence interval and the p-value

**Table 7.** Malaria antibody levels at 18 months stratified by supplementation groups

| | Result by study group | | | | Comparison between LNS and IFA group | | Comparison between LNS and MMN group | | Comparison between MMN and IFA group | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **IFA** | **MMN** | **LNS** | **P-value[a]** | **Coefficient (95 % CI)** | **P-value[b]** | **Coefficient (95 % CI)** | **P-value[b]** | **Coefficient (95 % CI)** | **P-value[b]** |
| Number of participants | N=XXX | N=XXX | N=XXX | | | | | | | |
| MSP-1 19kD | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-2 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| EBA-175 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Rh2A9 | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Schizont extract | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of E8B parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antibodies to VSA of R29 parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of 3D7 parasite line | Median (IQR) | Median (IQR) | Median (IQR) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

a. P-value calculated using Kruskal Wallis test
b. Linear regression of antibody levels between supplementation groups
c. Multivariate regression adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

**Table 8.** Antibody seropositivity at 18 months by supplementation groups

| | Number of children seropositive/ total number of children | | | | Comparison between LNS and IFA group | | Comparison between LNS and MMN group | | Comparison between MMN and IFA group | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **IFA** | **MMN** | **LNS** | **P-value[a]** | **RR (95 % CI)** | **P-value[b]** | **RR (95 % CI)** | **P-value[b]** | **RR (95 % CI)** | **P-value[b]** |
| MSP-1 19kD | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| MSP-2 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| EBA-175 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Rh2A9 | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Schizont extract | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of E8B parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Antibodies to VSA of R29 parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antibodies to VSA of 3D7 parasite line | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | xxx/xxx (xx.x%) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |
| Adjusted model[c] | | | | | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx | x.xx (xx.x, xx.x) | x.xx |

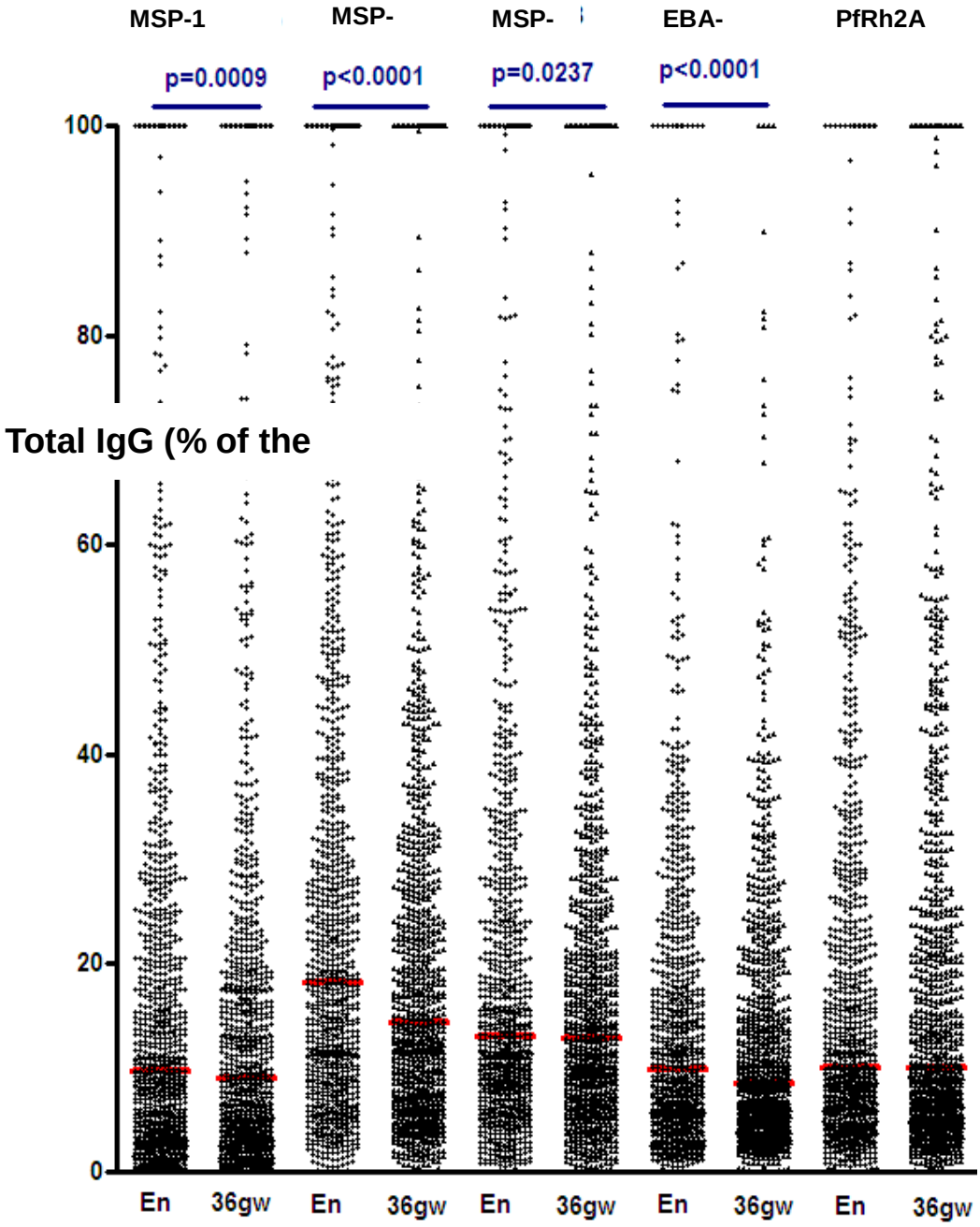
a. P-value calculated using the Chi2 test
b. P-value calculated using logistic regression reporting Relative Risk Ratios (RR)
c. P-value calculated using multivariate logistic regression reporting odds ratios (OR) while adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

**Table 9:** Effect modification at 18 months- dichotomous/continuous variables (by supplementation group)

| | | Result by study group | | | | Comparison between LNS and IFA group | | Comparison between LNS and MMN group | | Comparison between MMN and IFA group | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | Interaction test p-value[a] | IFA | MMN | LNS | P-value[b] | RR/difference in means (95 % CI)[c] | P-value[c] | RR/difference in means (95 % CI)[c] | P-value[c] | RR/difference in means (95 % CI)[c] | P-value[c] |
| Antibodies to MSP-1 19kD | | | | | | | | | | | |
| HIV=1 | 0.xx* | xxx/n (xx.x %)[1] | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y)[2] | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| HIV=0 | | xxx/n (xx.x %) | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Primi = 0 | 0.xx* | xxx/n (xx.x %)[1] | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y)[2] | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Multi = 1 | | xxx/n (xx.x %) | xxx/n (xx.x %) | xxx/n (xx.x %) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx | RR (x-y) | 0.xxx |
| Duration of gestation | 0.xx* | mean (SD) | mean (SD) | mean (SD) | 0.xxx | diff in means (x-y) | 0.xxx | diff in means (x-y) | 0.xxx | diff in means (x-y) | 0.xxx |

d. P-value calculated using likelihood ratio test
e. P-value calculated using $Chi^2$ test (dichotomous) or ANOVA (continuous, comparing mean differences)

## 2. Figures and legends




**Example figure 1:** Antibody levels to merozoite antigens reported as a percentage of the positive control at enrolment (En) and 36 gestation weeks (36gw). Each symbol represents an individual pregnant woman with %total IgG. Red horizontal bar indicates the median and the yellow dashed line denotes the average seronegative cut off. Sample size, n=1008.

**Figure 2:** Bar graph representing magnitude of antibody level change categorised by supplementation groups.

**Figure 3 A:** Antibodies to merozoite antigens and schizont extract at 6 months by supplementation group

**Figure 3 B:** Antibodies to variant surface antigens at 6 months by supplementation group

**Figure 4 A:** Antibodies to merozoite antigens and schizont extract at 18 months by supplementation group

**Figure 4 B:** Antibodies to variant surface antigens at 18 months by supplementation group

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 05: The impact of intervention on maternal periodontal infections (version 01.0, 03.11.2013, prepared by Ulla Harjunmaa)

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## 1. Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The aim of the secondary analyses described in appendix 5 is to compare maternal periodontal infection prevalence and caries prevalence in three different intervention groups one week after delivery (0 to 6 weeks). The following outcomes will be used to indicate maternal oral infection status.

1. Number of mouth sextants with bleeding on probing
2. Prevalence of gingivitis
3. Mean periodontal pocket depth (clinical)
4. Mean number of periodontal pockets >3mm
5. Prevalence of periodontitis
6. Mean number of caries lesions
7. Prevalence of deep caries lesions
8. Prevalence of periapical infections

## 2. Hypotheses to be tested

Number of mouth sextants with bleeding on probing is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Prevalence of gingivitis is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Mean periodontal pocket depth is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Mean number of periodontal pockets >3mm is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Prevalence of periodontitis is lower among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Mean number of caries lesions is higher among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Prevalence of deep caries lesions is higher among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

Prevalence of periapical infections is higher among women provided with LNS during pregnancy than among women who received either iron-folate or micronutrient supplementation.

As a secondary analysis, we will also test hypotheses about differences between the MMN and IFA groups.

## 3. Definition of the maternal periodontal infection and caries variables

**Prevalence of gingivitis**
Gingivitis is defined as at least one dental arch sextant (dd18-14, 13-23, 24-28, 38-34, 33-43, 44-48) with profound bleeding after probing

**Prevalence of periodontitis**
Periodontal pockets were examined clinically and radiologically. In the clinical examination, periodontal pockets were measured from six sites of each tooth, (wisdom teeth excluded) and the deepest measurement for each tooth was recorded in millimeters without decimals, rounded to the nearest millimeter. Radiologically periodontal pockets were measured from the dento-enamel junction to the deepest point of the bony pocket and expressed relative to the full length of the root (cervical, mid or apical third of root length).

Periodontitis will be defined as number of participants who have at least three periodontal pockets deeper than 3mm in clinical examination or /and at cervical root length in the x-ray and gingivitis present at least at one sextant (dichotomous, no/yes)

**Mean periodontal pocket probing depth**
Mean probing depth will be calculated using clinical pocket measurements and expressed in millimeters. Wisdom teeth will be excluded from the analysis.

**Prevalence of caries**
Caries lesions were assessed clinically and radiologically. Caries was defined as carious lesion penetrating the enamel of the tooth. Number of carious lesions will be calculated from clinical and radiographic data and expressed as number of teeth affected by caries (0 to 32).

**Prevalence of deep caries**

Caries lesions were assessed clinically and radiologically. Deep caries was defined as carious lesion reaching the pulp of the tooth. Participant will be defined as having the diseased if at least one deep caries lesion was seen in the radiograph.

**Prevalence of periapical infections**

Periapical infections were assessed radiologically and defined as osteolytic finding >1mm with diffuse margins surrounding the apex of the root. Participant will be defined as having the diseased if at least one infection finding was seen in the radiographs.

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes.

## 5. Time points for the analyses

All the above analyses will cover time period from delivery to six weeks postpartum. This marks the end of puerperal period.

## 6. Presentation of the study findings and hypothesis testing

### 6.1 Comparison of the continuous oral infection outcomes between the three intervention groups

The group means and standard deviations for number of sextants with bleeding of probing, number of periodontal pockets >3mm, periodontal pocket probing depth and number of caries lesions and will be tabulated by intervention group as shown in Table 1. The table will also indicate the differences in means and their 95 % confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be (Stata command *pwcompare*). For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

### 6.2 Comparison of the dichotomous birth outcomes between the three intervention groups

The proportions of mothers with periodontitis (clinical and clinical+radiographic diagnosis separately), gingivitis, deep caries lesions and periapical infections will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups will be tested with Fisher's exact test. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with $P<0.05$. Risk ratios between intervention groups are also presented in Table 2.

## 7. General notes on statistical methods

### 7.1 Software

The same as that for the primary outcome analyses

### 7.2 Preparing anthropometric data for analysis

The same as that for the primary outcome analyses

### 7.3 Multiple comparisons

The same as that for the primary outcome analyses.

### 7.4 Confidence intervals

The same as that for the primary outcome analyses.

### 7.5 Interaction and effect modification

The same as that for the primary outcome analyses.

### 7.6 Covariate adjustment

The same adjustments will be done as for the main analyses.

## 8. Tables

Table 1. Continuous oral infection outcomes by intervention groups

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) n:o of sextants with bleeding on probing | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) n:o of periodontal pockets >3mm | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) periodontal pocket depth | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) n:o of caries lesions | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 2. Dichotomous oral infection outcomes by intervention groups

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Prevalence of gingivitis | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of periodontits (clinical) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of periodontits (clinical+radiolog.) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of deep caries lesions | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Prevalence of periapical infections | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 06: Willingness-to-Pay for Lipid-based Nutrient Supplements During Pregnancy (LNS-P&L), added on 21.03.2014)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 21.03.2014 | Reimao | Original document Appendix 06 added |
| | | | |

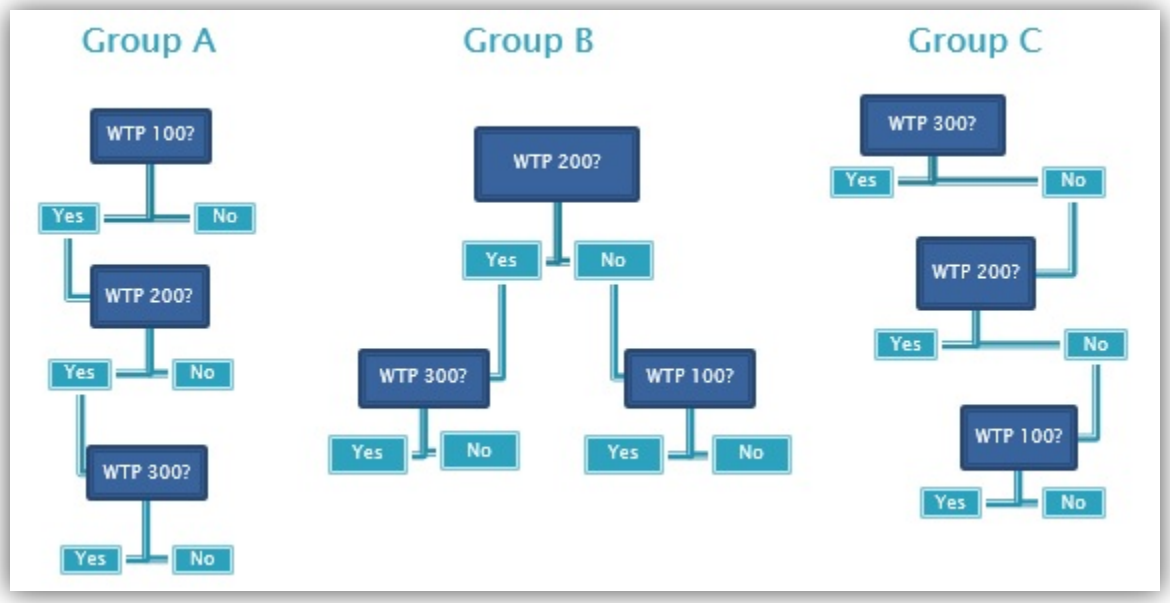
## 2. Introduction and Context

The proposed paper studies individual hypothetical willingness-to-pay (WTP) for lipid-based nutritient supplements for pregnant and lactating women (LNS-P&L) as distributed by the iLiNS study in Malawi. These supplements are designed for the prevention of malnutrition in pregnant mothers and their babies, and thus far have been provided for free to randomly selected pregnant and lactating women participating in the project.[1] The viability of a self-sustaining, non-experimental distribution of LNS, however, depends on the WTP for LNS in the target population. Specifically, the introduction of LNS into the market, where it can reach more people and be available well beyond an experimental timeframe, is contingent not only on identifying its potential health and nutritional benefits, but also on gauging the demand curve for LNS. Measuring individual hypothetical WTP and identifying its determinants is the first step in this direction.

We use the term "hypothetical", however, because the WTP measurement used in this analysis is based on individual statements only, as opposed to one elicited through an experiment or observed in a real market. Specifically, interviewees are first encouraged to think about their WTP through a contingent valuation tree, through which they are asked about their WTP for LNS at three different price points, as depicted in Figure 1. With this frame of reference, they are then asked the maximum price they would be willing to pay for a week's worth of LNS-P&L; this is recorded as their hypothetical WTP. Because respondents may have different timeframes when giving this value – some considering the purchase of only one week's worth of LNS while others considering the price they would be willing to pay for various months, for instance, – follow-up questions ask for the maximum they would be willing to pay every week for the duration of the pregnancy. This measurement is the long-term hypothetical WTP. In our analysis, we will use both measurements.

[1] Within this study, the treatment arm receiving LNS is given both LNS-P&L during pregnancy and lactation and child LNS once the focal child is six months of age. The current paper focuses on LNS-P&L; child LNS will be studied in future work.

Figure 1. Contingent Valuation Tree for Eliciting Hypothetical WTP




To benchmark the hypothetical WTP measurements, respondents are also asked about their WTP for 147 grams of *bonya*, a small dried local fish.[2] This information is elicited through the same contingent valuation tree, and is also collected for both short and long timeframes.

## 3. Objective of Analysis

The objective of this particular analysis is to estimate the distribution of individual willingness-to-pay for LNS-P&L and determine whether it is affected by exposure to LNS. In addition, it aims to identify other individual and household characteristics that may also influence WTP.

[2] A reasonable and comparable daily serving of *bonya* is estimated to be around 21 grams (market price about U$0.25).

## 4. Description of Variables

The planned analysis for this paper focuses on WTP for LNS-P&L during pregnancy only and will use data collected at baseline. The principal pre-requisite for participating in this iLiNS study was to be pregnant during the enrollment period and live within the catchment area encompassing Mangochi, Malindi, Lungwena, and Namwera. In enrolling, participants were each assigned into one of three study arms: a group receiving LNS-P&L during pregnancy and lactation and child LNS at 6-18 months; a second receiving micronutrient tablets only during pregnancy and lactation; and a control group.

The data to be used in this paper was collected around five weeks after enrollment, and collectively belongs to what we refer to as baseline or "round one" data. There may be some variation in the exact number of weeks elapsed between enrollment and subsequent interviews, however, with some baseline data collected as much as nine weeks after enrollment. The following subsections list the variables of interest, all from baseline.

### 41 Dependent Variables

The dependent variables in our analysis are the WTP measurements, converted into 4$^{th}$ quarter 2011 US dollars for ease of analysis and comparability with other studies. For the same reasons, while the questionnaire asks for WTP for a week's supply of the product, we divide the stated responses by seven to get individual WTP for a day's worth of LNS or *bonya*. Specifically, the dependent variables are the natural logarithms of:

- Hypothetical WTP for LNS: stated maximum price willing to pay for LNS
- Hypothetical WTP for *bonya* : stated maximum price willing to pay for *bony*a
- Long-term hypothetical WTP for LNS: stated maximum price willing to pay for LNS for the duration of the pregnancy
- Long-term hypothetical WTP for *bonya*: stated maximum price willing to pay for *bonya* for the duration of the pregnancy.

The logarithmic form of the variables above are used because of the skewness of the distribution, a standard adjustment made when studying prices. Additionally, we also consider:

- Difference in hypothetical WTP for LNS and *bonya*
- Difference in long-term hypothetical WTP for LNS and *bonya.*

### 4.2 Explanatory and Control Variables

We propose the following reduced-form model for estimating the determinants of WTP:

$$WTP_i =$$
$$\beta_0 + \beta_1 iLiNS\ treatment_i + \beta_2 iLiNS\ mother\ characteristics_i +$$
$$\beta_3 (iLiNS\ mother\ characteristics)_i * (i\ not\ iLiNS\ mother)_i +$$
$$\beta_4 i\ is\ not\ iLiNS\ mother_i + \beta_5 household\ characteristics_i + \beta_6 X_i + \epsilon_i \quad ,$$

where $WTP_i$ is each one of the six dependent variables listed in the previous subsection. Explanatory variables are indicators of the treatment arm, characteristics of the iLiNS mother, and household characteristics. Control variables are included in $X_i$.

*iLiNS treatment*

- Study arm: participants are assigned into one of three study arms, only one of which receives LNS-P&L. In this paper, we are interested in measuring whether even a short exposure to LNS-P&L affects individual WTP for LNS.

*iLiNS mother characteristics*

- Gestational weeks at enrollment: number of weeks of pregnancy at the time the mother enrolled in the iLiNS study
- Mother's age: age in years
- Mother's education: number of years of education
- Mother's BMI: calculated using the mother's height and weight at enrollment, and adjusted for weeks of gestation; may be normalized for the sample
- Mother's HIV status: HIV status as recorded at enrollment
- Baby is mother's first child: whether, at enrollment, the mother has other children or not
- Mother's tribe: the tribe of the iLiNS mother
- Risk aversion: final bet amount in the risk aversion experiment
- Discount rate: amount of rice added in the final round of the discount rate experiment

Note that the last two measurements are technically from experiments applied to the iLiNS mother or the father of the iLiNS baby and to the iLiNS mother or the male head of household, respectively, but over 97% of respondents to the risk aversion questions are iLiNS mothers. This share is closer to 90% for the discount rate questionnaire.

*i not iLiNS mother*

In about 10% of observations, the respondent for the WTP questionnaire at baseline was the household head rather than the pregnant women enrolled in the iLiNS study, and data collection limitations prevent us from identifying individual characteristics for respondents who are not the iLiNS mother. Given this restriction, we allow for possible systematic differences in WTP for household heads relative to iLiNS mothers, both with respect to its level ($\beta_4$) and to how the iLiNS mother's characteristics affect WTP ($\beta_3$).

*household characteristics*

- Household size: number of people that are part of the iLiNS mother's household at baseline
- Number of children under 5: number of children below the age of five who are part of the iLiNS mother's household at baseline
- Household Asset Index: principal components score based on baseline ownership of a set of assets and household quality. A higher score is associated with better living conditions.
- Household Food Insecurity Access (HFIA) Score: indicator of the household's food insecurity, generated by adding the value of responses to nine questions regarding food insecurity. The higher the score, the higher degree of food insecurity in the last four weeks.
- Household per capita expenditures: total daily per capita expenditures by the household, calculated as a sum of all food and non-food expenditures and converted into 4$^{th}$ quarter 2011 US dollars
- Share of food expenditures: the portion of total expenditures that were used for the purchase of food, calculated as the ratio of food expenditures to total expenditures for the household

Control Variables in $X$

- Month: month in which the baseline WTP questionnaire was administered, as there may be systematic variation across seasons
- Year: year in which the baseline WTP questionnaire was administered
- Enumerator: code of the enumerator who administered the baseline WTP questionnaire
- Contingent valuation group: a set of dummy variables indicating to which group the respondent to the WTP questionnaire was assigned and, consequently, the order in which the framing questions were posed, as detailed in Figure 1
- Weeks from enrollment to enumeration: the number of weeks elapsed between when the woman enrolled in the study and when the baseline WTP questionnaire was applied.

## 5. Statistical Methods

### 5.1 Data Cleaning

Cleaning of the SES variables, including the WTP data, will follow standard protocol and on-going practice, with Maira Reimao identifying issues, verifying discrepancies, and submitting corrections and/or verification requests through Form 99 (copying Steve Vosti and Per Ashorn). These are then processed by the data management team in Malawi or verified by Robert Mataya if further investigation is required.

Non-SES variables will be received from the nutrition team in Finland. They will be verified for cleanliness, but no changes will be made without authorization from the nutrition team.

## 5.2 Software

All of the analysis will be done using Stata 13 (student edition), and the final draft will be prepared using Latex.

## 5.3 Analysis

The statistical analysis in the proposed paper will be based on various regressions, using the reduced-form model described in the previous section of this document. Results will be presented in a series of tables. The interpretation of results related to non-SES indicators will be done in collaboration with the scientific teams responsible for those data.

### 5.3.1 Descriptive Statistics

Summary statistics for the explanatory variables will be shown on Table 1. The next two tables will focus on the short-term WTP for LNS, first giving summary statistics for the entire sample (Table 2) and then divided by each of the study arms (Table 3). Similarly, Table 4 will display the summary statistics for the entire sample while Table 5 will distinguish between study arms.

### 5.3.2 Regression Results

The regressions for the proposed paper will use ordinary least squares (OLS) with robust standard errors, following the model described in sub-section 2.2. In total, six regressions will be run, each with one of the variables described in sub-section 2.1 as the dependent variable. Table 6 will present the results for the initially stated hypothetical WTP and Table 7 will detail the results for long-term WTP. While the control variables will be included in the regressions used to generate the two tables, their respective estimates will not be reported as they are not of interest for the study at hand.

## 6. Tables

Table 1: Summary Statistics for Explanatory Variables
Baseline Mother and Household Characteristics, Full Sample

| | Variable | Mean/Count | Std Dev/ Percent | Min | Max |
|---|---|---|---|---|---|
| **Mother characteristics** | **Gestational weeks** | | | | |
| | **Age** | | | | |
| | **Education** | | | | |
| | **BMI** | | | | |
| | **HIV status** | | | | |
| | **Baby is first child** | | | | |
| | **Tribe** | | | | |
| | **Risk aversion** | | | | |
| | **Discount rate** | | | | |
| **Household characteristics** | **Household size** | | | | |
| | **# of children under 5** | | | | |
| | **Asset index** | | | | |
| | **HFIA score** | | | | |
| | **Per capita expenditures** | | | | |
| | **Share of food expenditures** | | | | |

Table 2. Summary Statistics on Short-Term WTP, Full Sample
Baseline Short-Term WTP

| Variable | N | Mean/Count | Std Dev/ Percent | Min | Max |
|---|---|---|---|---|---|
| **WTP for LNS** | | | | | |
| **WTP for *bonya*** | | | | | |
| **Difference between WTP for LNS and *bonya*** | | | | | |

Values pertain to WTP for a day's serving, converted into 4th quarter 2011 US dollars.

Table 3. Summary Statistics on Short-Term WTP, by Treatment Arm
Baseline Short-Term WTP

| | | Treatment Arm | | |
|---|---|---|---|---|
| | **Full Sample** | **Receiving LNS-P&L** | **Receiving Micronutrient Tablets** | **Control** |
| WTP for LNS (all) | Mean (std dev) | Mean (std dev) | Mean (std dev) | Mean (std dev) |
| WTP for LNS (non-zero) | | | | |
| WTP for *bonya* (all) | | | | |
| WTP for *bonya* (non-zero) | | | | |
| Difference in WTP for LNS and *bonya* (all) | | | | |

Values pertain to WTP for a day's serving, converted into 4th quarter 2011 US dollars.

Standard deviations are given in parenthesis.

In the last two columns, the following markers *** ($p<0.01$), **($p<0.05$), and *($p<0.10$) indicate a difference in means between the group receiving LNS-P&L and the group in the respective column, significant at the given level.

Table 4. Summary Statistics on Long-Term WTP, Full Sample
Baseline Short-Term WTP

| Variable | N | Mean/Count | Std Dev/ Percent | Min | Max |
|---|---|---|---|---|---|
| **WTP for LNS** | | | | | |
| **WTP for *bonya*** | | | | | |
| **Difference between WTP for LNS and *bonya*** | | | | | |

Values pertain to WTP for a day's serving, converted into 4th quarter 2011 US dollars.

Table 5. Summary Statistics on Long-Term WTP, by Treatment Arm
Baseline Short-Term WTP

| | | Treatment Arm | | |
|---|---|---|---|---|
| | **Full Sample** | **Receiving LNS-P&L** | **Receiving Micronutrient Tablets** | **Control** |
| WTP for LNS (all) | Mean (std dev) | Mean (std dev) | Mean (std dev) | Mean (std dev) |
| WTP for LNS (non-zero) | | | | |
| WTP for *bonya* (all) | | | | |
| WTP for *bonya* (non-zero) | | | | |
| Difference in WTP for LNS and *bonya* (all) | | | | |

Values pertain to WTP for a day's serving, converted into 4th quarter 2011 US dollars.

Standard deviations are given in parenthesis.

In the last two columns, the following markers *** ($p<0.01$), **($p<0.05$), and *($p<0.10$) indicate a difference in means between the group receiving LNS-P&L and the group in the respective column, significant at the given level.

Table 6. Regression Results for Hypothetical WTP for a Day's Supply
Baseline Short-Term WTP

| | | Dependent Variable | | |
|---|---|---|---|---|
| | | **WTP for LNS (ln)** | **WTP for *bonya* (ln)** | **Difference in WTP for LNS and *bonya*†** |
| | **Receiving LNS-P&L** | | | |
| | **Receiving micronutrient tablet** | | | |
| **Mother characteristics** | **Gestational weeks** | Coefficient (std error) | Coefficient (std error) | Coefficient (std error) |
| | **Age** | | | |
| | **Education** | | | |
| | **BMI** | | | |
| | **HIV status** | | | |
| | **Baby is first child** | | | |
| | **Tribe** | | | |
| | **Risk aversion** | | | |
| | **Discount rate** | | | |
| **Respondent is not iLiNS mother** | | | | |
| **Respondent is not iLiNS mother interacted with mother characteristics** | **Gestational weeks** | | | |
| | **Age** | | | |
| | **Education** | | | |
| | **BMI** | | | |
| | **HIV status** | | | |
| | **Baby is first child** | | | |
| | **Tribe** | | | |
| | **Risk aversion** | | | |
| | **Discount rate** | | | |
| **Household characteristics** | **Household size** | | | |
| | **# of children under 5** | | | |
| | **Asset index** | | | |
| | **HFIA score** | | | |
| | **Per capita expenditures** | | | |
| | **Sample size** | N | N | N |

| | |
|---|---|
| | **R-Squared** |

Statistical significance: *** (p<0.01), **(p<0.05), and *(p<0.10)

† For each respondent, the difference between their short-term WTP for LNS and their short-term WTP for *bonya*

The regressions above also included controls for month and year of enumeration, enumerator, contingent valuation group, and time between enrollment and enumeration (estimates not reported).

Table 7. Regression Results for Baseline Hypothetical WTP for a Day's Supply
Baseline Long-Term WTP

| | | **Dependent Variable** | | |
|---|---|---|---|---|
| | | **WTP for LNS (ln)** | **WTP for *bonya* (ln)** | **Difference in WTP for LNS and *bonya*†** |
| | **Receiving LNS-P&L** | | | |
| | **Receiving micronutrient tablet** | | | |
| **Mother characteristics** | **Gestational weeks** | Coefficient (std error) | Coefficient (std error) | Coefficient (std error) |
| | **Age** | | | |
| | **Education** | | | |
| | **BMI** | | | |
| | **HIV status** | | | |
| | **Baby is first child** | | | |
| | **Tribe** | | | |
| | **Risk aversion** | | | |
| | **Discount rate** | | | |
| **Respondent is not iLiNS mother** | | | | |
| **Respondent is not iLiNS mother interacted with mother characteristics** | **Gestational weeks** | | | |
| | **Age** | | | |
| | **Education** | | | |
| | **BMI** | | | |
| | **HIV status** | | | |
| | **Baby is first child** | | | |
| | **Tribe** | | | |
| | **Risk aversion** | | | |
| | **Discount rate** | | | |
| **Household characteristics** | **Household size** | | | |
| | **# of children under 5** | | | |
| | **Asset index** | | | |
| | **HFIA score** | | | |
| | **Per capita expenditures** | | | |
| | **Sample size** | N | N | N |

| | |
|---|---|
| **R-Squared** | |

Statistical significance: *** ($p<0.01$), **($p<0.05$), and *($p<0.10$)

† For each respondent, the difference between their long-term WTP for LNS and their long-term WTP for *bonya*

The regressions above also included controls for month and year of enumeration, enumerator, contingent valuation group, and time between enrollment and enumeration (estimates not reported).

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 07: The impact of the interventions on iron status and inflammation (version 01.0, 12 June 2014, prepared by Josh Jorgensen)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 12.05.2014 | Josh | Original document Appendix added |

## 2. Study objectives

The trial has three sets of objectives, defined at various phases of the trial. The originally defined objective is to determine whether LNS consumed by women during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The objectives of the secondary analyses are to determine the main effect of intervention on hemoglobin (Hb), iron status, and inflammation. Details of this objectives are as follows:

2.1. Main effect of intervention on Hb, iron status, and inflammation

a. To determine if there are differences in mean Hb and markers of iron status (zinc protoporphyrin (ZPP) and soluble transferrin receptor (sTfR)) at 36 weeks of pregnancy between groups of women who were provided either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.
b. To determine if differences exist in the prevalence of low iron status and low and high Hb at 36 gestational weeks (gw) between groups of women who were provided either LNS, MMN, or IFA during pregnancy.
c. To determine the effect of the intervention on the prevalence of elevated C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP).
d. To determine whether baseline iron status is an effect modifier for the effect of group assignment on inflammation and birth outcomes.

## 3. Hypotheses

3.1. Main effect of intervention on Hb, iron status, and inflammation

a. Women who receive IFA during pregnancy will have higher mean blood Hb and lower plasma sTfR and ZPP at 36 gw compared to the MMN and LNS groups.
b. The percentage of women with Hb below -100 -g/L will be lower in the IFA group at 36 gw compared to the MMN and LNS groups.
c. The percentage of women with Hb above 130 -g/L will be higher in the IFA group at 36 gw compared to the MMN and LNS groups.

d. The percentages of women with elevated ZPP will be lower in the IFA group compared to the MMN and LNS groups.
e. The percentages of women with elevated sTfR will be lower in the IFA group compared to the MMN and LNS groups.
f. At 36 gw, the prevalence of elevated plasma concentration of CRP or AGP will be greater in the IFA group than the MMN or LNS groups.

## 4. Definition of outcome variables

Hemoglobin

Hb was analyzed by Hemocue autoanalyzer and reported as g/L. The lower cutoff used to define anemia is 100 g/L, while the upper cutoff used to define excessive Hb is 130 g/L. In exploratory analyses, the proportion of participants with Hb < 90 g/L or < 110 g/L, and > 145 g/L, will also be examined.

Zinc protoporphyrin (ZPP)

ZPP was analyzed on washed erythrocytes by an Aviv hematofluorometer. The upper cutoff, representing iron deficiency, is 60 µmol/mol heme.

Soluble transferrin receptor (sTfR)

sTfR was analyzed immunoturbidimetrically on a Roche Integra autoanalyzer. The upper cutoff used to define iron deficiency is 4.4 mg/L.

C-reactive protein (CRP)

CRP was analyzed immunoturbidimetrically on a Roche Integra autoanalyzer. The upper cutoff used to define an inflammatory response is 5.0 mg/L.

Alpha-1 acid glycoprotein (AGP)

AGP was analyzed immunoturbidimetrically on a Roche Integra autoanalyzer. The upper cutoff used to define an inflammatory response is 1.0 g/L.

## 5. Basis for the analysis: Intention to treat and per protocol

The primary analysis will be by intention-to-treat. That is, results for all women enrolled will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on participants~~,~~ who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available.

## 6. Time points

Blood samples were collected for Hb, ZPP, sTfR, CRP and AGP analyses at enrollment and 36 gw.

**7. Statistics software**

Analyses will be performed using SAS version 9.3.

**8. Outliers**

Outliers will be visually inspected by creating box and whisker plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept.

**9. Data transformation**

Distribution of outcome variables and key baseline variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

**10. Interaction**

Interactions will be examined between the intervention group and selected variables on their association with maternal iron status. If a statistically significant interaction ($p<0.05$) is found, group means will be examined at different levels of the predictor variable, either by category for categorical predictors, or at the $10^{th}$, $50^{th}$, and $90^{th}$ percentiles for continuous variables. Variables that show no interaction with the intervention group can be used as covariates in the main analysis. Variables included (as continuous variables where possible) in this analysis include:

1. Maternal BMI at baseline
2. Inflammatory markers (CRP and AGP) at baseline
3. Malaria at baseline
4. HIV status at baseline
5. Number of previous pregnancies
6. Maternal education
7. Site of enrollment
8. Season at enrollment
9. Hb at enrollment
10. ZPP and sTfR at enrollment

## 11. Covariates

The covariates to be included in the ANCOVA or logistic regression models will be derived from the list below. Each variable that show a statistically significant association with each outcome (P<0.1), will be included in the model.

1. Maternal BMI at baseline
2. Inflammatory markers (CRP and AGP) at baseline
3. Malaria at baseline
4. HIV status at baseline
5. Number of previous pregnancies
6. Maternal education
7. Site of enrollment
8. Season at enrollment
9. Hb at enrollment
10. ZPP and sTfR at enrollment

## 12. Confidence intervals

The calculated ratios and differences in between-group comparisons will be complemented with confidence intervals (at 95% level), for descriptive purposes. For continuous outcomes, confidence intervals will be based on ANOVA or ANCOVA and for binary outcomes CI's will be based on logistic regression.

## 13. Presentation of study findings

13.1. Main effect of intervention on Hb and iron status

Group means and standard deviations for Hb, and medians and 1$^{st}$ and 3$^{rd}$ quartiles for ZPP and sTfR will be tabulated by intervention group and presented in Table 1. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if P<0.05. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be Tukey-Kramer test for ANOVA. For all pairwise comparisons with P<0.05, the null-hypothesis of no difference in means between groups will be rejected.

The proportion of women with Hb, ZPP, and sTfR above or below specified cutoffs will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups will be tested with chi-square test or Fisher's exact test. Pairwise comparisons between groups will be done in the context of logistic regression

if global null-hypothesis is rejected with P<0.05. Risk ratios between intervention groups are also presented in Table 2.

13.2. Effect of intervention on inflammatory markers

The proportion of women with CRP and AGP above the specified cutoffs will be tabulated by intervention group as shown in Table 3. Global null hypothesis of no differences between groups will be tested with chi-squared test or Fisher's exact test. Pairwise comparisons between groups will be done in the context of logistic regression if global null-hypothesis is rejected with P<0.05. Risk ratios between intervention groups are also presented in Table 3.

**Reference**

Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstetrics and gynecology 1996;87(2):163-8. doi: 10.1016/0029-7844(95)00386-X.

## 14. Tables

Table 1. Differences between groups in baseline and change from baseline mean (SD) Hb and median (quartiles) ZPP, and sTfR.

| **Variable** | | **IFA [n]** | **MMN [n]** | **LNS [n]** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** |
| Hb (g/L) **($\bar{x}$ ± SD) [n]** | Baseline | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |
| | Change | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |
| ZPP µmol/mol heme (median (quartiles)) [n] | Baseline | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |
| | Change | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |
| sTfR (mg/L) (median (quartiles)) [n] | Baseline | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |
| | Change | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | x.xxx | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) |

Table 2. Differences between groups in the proportions of women with Hb, ZPP, and sTfR above or below specified cutoffs.

| | | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Hb < 100 g/L | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx | | 0.xx | | 0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| Hb > 130 g/L | BaselineBaseline | x (x.x)x (x.x) | x (x.x)x (x.x) | x (x.x)x (x.x) | 0.xx0.xx | | 0.xx0.xx | | 0.xx0.xx | | 0.xx0.xx |
| | 36 gw36 gw | x (x.x)x (x.x) | x (x.x)x (x.x) | x (x.x)x (x.x) | 0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx |
| ZPP > 60.0 µmol/mol heme | 36 gw36 gw36 gw | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | 0.xx0.xx 0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx |
| | BaselineBaselineBaseline | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | 0.xx0.xx 0.xx | x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | | 0.xx0.xx0.xx | | 0.xx0.xx0.xx |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| sTfR > 4.4 mg/L | 36 gw36 gw36 gw | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | 0.xx0.xx 0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx |
| | BaselineBaselineBaseline | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | x (x.x)x (x.x)x (x.x) | 0.xx0.xx 0.xx | x.xx (x.xx - x. xx)x.xx (x.xx - x. xx) | 0.xx0.xx0.xx | | 0.xx0.xx0.xx | | 0.xx0.xx0.xx |

Table 3. Differences between groups in the proportions of women with CRP or AGP above specified cutoffs.

| | | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| CRP > 5.0 mg/L | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx | | 0.xx | | 0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| AGP > 1.0 g/L | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx | | 0.xx | | 0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 08: Characterisation of microbial communities in the placenta, chorion, amnion, vagina and oral cavity (version 01.0, prepared by Ronan Doyle)

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## 1. Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The aim of the secondary analyses described in appendix 6 is to compare the prevalence of bacteria and histological chorioamnionitis in both the placenta and fetal membranes (chorion and amnion) between the three intervention groups. Further secondary analyses will look to compare the composition of the microbiota at two further sites, the vagina and oral cavity between the intervention groups. The following outcomes will be used to represent this:

1. Prevalence of bacteria in placenta and fetal membranes;
2. Prevalence of histological chorioamnionitis in the placenta and fetal membranes;
3. Mean bacterial load in the placenta and fetal membranes;
4. Comparison of alpha diversity (Chao1 and Shannon indexes) in all four sites;
5. Comparison of beta diversity (weighted and unweighted UniFrac distances) in all four sites;
6. Mean relative abundance of organisms associated with bacterial vaginosis (*Atopbium spp*., BVAB 1, 2, 3, *Escherichia coli*, *Gardnerella vaginalis*, Group B *Streptococcus*, *Mobiluncus spp.*, *Mycoplasma spp.*, *and Ureaplasma spp.*) in the vagina;
7. Mean relative abundance of red complex and orange complex bacteria (see Table 3) in the oral cavity.

## 2. Hypotheses to be tested

1. Prevalence of bacteria in placenta and fetal membranes of mothers provided with LNS during pregnancy will be lower than that of mothers who received either iron-folate or micronutrient supplementation.

2. Prevalence of histological chorioamnionitis in the placenta and fetal membranes of mothers provided with LNS during pregnancy will be lower than that of mothers who received either iron-folate or micronutrient supplementation.

3. Mean bacterial load in the placenta and fetal membranes of mothers provided with LNS during pregnancy will be lower than that of mothers who received either iron-folate or micronutrient supplementation.

4. Mean alpha diversity indexes in the placenta, fetal membranes, vagina and oral cavity of mothers provided with LNS during pregnancy will be higher than that of mothers who received either iron-folate or micronutrient supplementation.

5. Beta diversity distances in the placenta, fetal membranes, vagina and oral cavity will be smaller among mothers provided with LNS during pregnancy compared with mothers who received either iron-folate or micronutrient supplementation.

6. Mean relative abundance of *Lactobacillus spp.,* in the vagina of mothers provided with LNS during pregnancy will be higher than that of mothers who received either iron-folate or micronutrient supplementation.

7. Mean relative abundance of *Atopbium spp.,* BVAB, *Gardnerella vaginalis,* Group B *Streptococcus, Mobiluncus spp., Mycoplasma spp., Porphymonas spp., Peptostreptococcus spp.* and *Ureaplasma spp.* in the vagina of mothers provided with LNS during pregnancy will be lower than that of mothers who received either iron-folate or micronutrient supplementation.

8. Mean relative abundance of red complex and orange complex bacteria in oral cavity of mothers provided with LNS during pregnancy will be lower than that of mothers who received either iron-folate or micronutrient supplementation.

## 3. Definition of infection, inflammation and ecological characterisation of microbial communities

*The prevalence of bacteria in tissue*

Presence of bacteria is defined by any level of fluorescence above the lower limit of detection on the 16S rDNA broad-range SYBR green qPCR assay.

*Histological chorioamnionitis*

Chorioamnionitis is defined as ≥ 5 neutrophil granulocytes on average per 10 high power fields present in either the chorionic plate or the amniotic membrane.

*Bacterial load*

Bacterial load is quantified against a standard curve of extracted DNA from a pure *Escherichia coli* culture. Variable gene copy number between different bacterial species is adjusted for using relative abundances from microbiome data.

*Alpha diversity*

Alpha diversity is calculated on a per sample basis using both the Chao1 and Shannon metrics. Chao1 measures species richness from a rarefaction of observed species, whereas Shannon takes into account both overall species richness but also the evenness of those species within each sample. Individual sample alpha diversity scores are calculated from 100 subsamples without replacement at the same depth between samples. The value is then expressed as an average ± SEM for each group.

*Beta diversity*

Beta diversity will be measured using both weighted and unweighted UniFrac measures. This will take into account the phylogenetic differences between each lineage in each sample and collate this information in a matrix that contains each sample-to-sample difference. The greater the phylogenetic difference between sample communities, the larger distance score it is given. Weighted UniFrac gives increased weight to species that dominate a sample compared to those occurring less frequently, whereas unweighted treats all lineages as if they were represented equally. Average within group distances can be tested against between group distances to show if distinct phylogenetic differences exist between microbial communities found in each intervention group.

*Relative abundances of bacterial species*

Bacterial species will be identified by clustering Operational Taxonomic Units at 97% similarity against a reference database with known typed species' 16S rDNA sequences. The number of sequences will be rarified to an even level across all samples. The relative abundance will be taken as the mean abundance of each bacterial OTU within each intervention group. Bacteria associated with bacterial vaginosis were amalgamated from reviews of recent molecular studies showing an association with a drop in *Lactobacillus spp.* and a rise in strictly anaerobic bacteria mostly from the order *Clostridiales*[1,2]. Pathogens in the oral cavity were chosen based on the two complexes that associate most strongly with periodontal disease[3].

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes.

## 5. Time points for the analyses

The above analyses will use placenta and fetal membranes collected at delivery and vaginal and dental swabs collected one week after delivery.

## 6. Presentation of the study findings and hypothesis testing

### 6.1 Comparison of dichotomous bacterial prevalence and chorioamnionitis outcomes between the three intervention groups.

The proportion of mothers positive for bacteria of histological chorioamnionitis will be tabulated by intervention group as shown in Table 1. Global null hypothesis of no difference between the three groups will be calculated using Fisher Exact test. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with $P<0.05$. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected. Odds ratios between intervention groups are also presented in Table 1.

### 6.2 Comparison of bacterial load between the three intervention groups.

Median bacterial loads and interquartile ranges will be tabulated by intervention group as shown in Table 2. The difference between the 3 groups will be tested using the Kruskal-Wallis one-way ANOVA. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with $P<0.05$. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in medians between groups will be rejected.

### 6.3 Alpha diversity comparisons between the three intervention groups.

Mean alpha diversity scores ± SEM will be plotted against each other. Differences between the three groups will be tested using the one-way ANOVA method. Pairwise comparisons between groups will be done if global null-hypothesis is rejected with $P<0.05$. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

### 6.4 Beta diversity comparisons between the three intervention groups.

Within group beta diversity distances will be shown in a box-and-whisker plot, as well as collated between group distances. Differences in all groupings will be tested using the one-way ANOVA method. Pairwise comparisons for within and between group scores will be done if global null-hypothesis is rejected with $P<0.05$. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

### 6.5 Comparison of relative abundances of different bacteria between the three intervention groups.

Mean relative abundances ± SD will be tabulated by intervention group as shown in Table 3 and 4. Periodontal associated bacteria will be shown in Table 3 and bacterial vaginosis associated

bacteria will be shown in Table 4. The difference between means and the 95% confidence interval will be shown after resampling the data with replacement.

The difference between the 3 groups will be tested using the one-way ANOVA method. Multiple t-tests will be used for between group differences if one-way ANOVA returns a $P<0.05$. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

Due to the multiple tests employed to compare relative abundances, the Benjamin-Hochberg method will be used to adjust the p-values for multiple comparisons.

## 7. General notes on statistical methods

### 7.1 Software

All analysis will either be done on SPSS version 21, except the sequence data which will be analysed using various scripts found in the Quantitative Insights Into Molecular Ecology (QIIME) package and R.

### 7.2 Preparing anthropometric data for analysis

The same as that for the primary outcome analyses.

### 7.3 Multiple comparisons

The same as that for the primary outcome analyses, except in the case of the large scale comparisons of bacterial species, where the Benjamin and Hochberg method will be used to control the false discovery rate[4]. This method is essentially a sequential Bonferroni type procedure that provides greater statistical power than the Bonferroni calculation by controlling the false discovery rate without removing as many true positive results.

### 7.4 Confidence intervals

The same as that for the primary outcome analyses.

### 7.5 Interaction and effect modification

The same as that for the primary outcome analyses.

### 7.6 Covariate adjustment

The same adjustments will be done as for the main analyses.

## 8. Legends to the figures

Figure 1. Distribution of Chao1 index scores by intervention group.

Figure 2. Distribution of Shannon index scores by intervention group.

Figure 3. Within group and between group weighted UniFrac distances.

Figure 4. Within group and between group unweighted UniFrac distances.

## 9. Figures

Figure 1. Distribution of Chao1 index scores by intervention group.

Figure 2. Distribution of Shannon index scores by intervention group.

Figure 3. Within group and between group weighted UniFrac distances.

Figure 4. Within group and between group unweighted UniFrac distances.

## 10. Tables

Table 1. Dichotomous bacterial prevalence and chorioamnionitis outcomes between groups.

| **Outcome** | **Number of outcomes / infants with outcome data** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **LNS** | **MMN** | **IFA** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** | **Odds ratio (95 % CI)** | **P-value** |
| Bacteria prevalence in the placenta | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Bacteria prevalence in the fetal membrane | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Bacteria prevalence in both the placenta and fetal membrane | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Mild histological chorioamnionitis in chorionic plate (5-10 cells per 10 high power fields) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Moderate | xxx/xxx | xxx/xxx | xxx/xxx | x.xxx | x.xx (x.xx- | x.xxx | x.xx (x.xx- | x.xxx | x.xx (x.xx- | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| histological chorioamnionitis in chorionic plate (11-25 cells per 10 high power fields) | (xx.x %) | (xx.x %) | (xx.x %) | | x.xx) | | x.xx) | | x.xx) | |
| Severe histological chorioamnionitis in chorionic plate (>25 cells per 10 high power fields) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Mild histological chorioamnionitis in the amnion | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Moderate histological chorioamnionitis in the amnion | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Severe histological chorioamnionitis in the amnion | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Histological chorioamnionitis in chorionic plate | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Histological chorioamnionitis in amniotic membrane | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |
| Histological | xxx/xxx | xxx/xxx | xxx/xxx | x.xxx | x.xx (x.xx- | x.xxx | x.xx (x.xx- | x.xxx | x.xx (x.xx- | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| chorioamnionitis in either chorionic plate or amniotic membrane | (xx.x %) | (xx.x %) | (xx.x %) | | x.xx) | | x.xx) | | x.xx) | |
| Prevalence of both bacteria and chorioamnionitis in either tissue | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | xxx/xxx (xx.x %) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx | x.xx (x.xx-x.xx) | x.xxx |

Table 2. Bacterial load by intervention groups.

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | **Comparison between LNS and IFA group** | **Comparison between MMN and IFA group** |
|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **P-value** | **P-value** | **P-value** |
| Median (IQR) bacterial load in the placenta (copies $\mu l^{-1}$) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xxx | x.xxx | x.xxx |
| Median (IQR) bacterial load in the fetal membranes (copies $\mu l^{-1}$) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xxx | x.xxx | x.xxx |

Table 3. Relative abundance of periodontal associated bacteria in the oral cavity by intervention group.

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) relative abundance of *Treponema denticola* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Porphyromonas gingivalis* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Bacteroides forsythus* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Fusobacterium nucleatum* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Prevotella intermedia* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean (SD) relative abundance of *Prevotella nigrescens* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Peptostreptococcus micros* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Eubacterium nodatum* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Campylobacter rectus* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Campylobacter showae* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Streptococcus constellatus* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Campylobacter gracilis* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

Table 4. Relative abundance of bacteria associated with bacterial vaginosis found in the vagina by intervention group.

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) relative abundance of *Lactobacillus spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Atopbium spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of BVAB *spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Porphyromonas* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of *Gardnerella vaginalis* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance of Group B *Streptococcus* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative | x.xx | x.xx | x.xx | x.xxx | x.xx (xx to | x.xxx | x.xx (xx to | x.xxx | x.xx (xx to | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| abundance of *Mobiluncus spp.* | (x.xx) | (x.xx) | (x.xx) | | xx) | | xx) | | xx) | |
| Mean (SD) relative abundance *Ureaplasma spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance *Mycoplasma spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Mean (SD) relative abundance *Peptostreptococcus spp.* | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

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**Supplementing maternal and infant diet with micronutrient fortified lipid-based nutrient supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan: The impact of LNS on maternal salivary cortisol concentration

July 4, 2014

Prepared by Brietta Oaks and Christine Stewart

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## 1. Study objective

The primary objective for the main trial is to determine whether LNS consumed by women during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

This statistical analysis plan addresses the following secondary objective: to determine if there are differences in mean salivary cortisol concentration at either 28 weeks or 36 weeks gestation among groups of women who were provided either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.

## 2. Hypotheses

H1. Women who receive LNS during pregnancy will have a lower mean salivary cortisol concentration at 28 wk and 36 wk gestation compared to the MMN and IFA groups.

H2. Women who receive LNS during pregnancy will have a lower prevalence of high salivary cortisol at 28 wk and 36 wk gestation compared to the MMN and IFA groups.

## 3. Outcome variables

Cortisol at 28 wk gestation

Cortisol was analyzed using Salimetrics high-sensitivity salivary cortisol enzyme immunoassay, which can detect cortisol levels ranging from 0.193 to 82.77 nmol/L (0.007-3.0 µg/dL). High cortisol will be defined as cortisol concentrations >75$^{th}$ percentile and low cortisol as cortisol concentration < 25$^{th}$ percentile at 28 wk of the IFA group.

Cortisol at 36 wk gestation

High cortisol will be defined as cortisol concentrations >75$^{th}$ percentile and low cortisol as cortisol concentration < 25$^{th}$ percentile at 36 wk of the IFA group.

## 4. Basis for the analysis: Intention to treat

The primary analysis will be by intention-to-treat. That is, results for all women enrolled will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on participants, who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available.

## 5. Time points

Saliva samples for cortisol analysis were collected at enrollment, 28 wk, and 36 wk gestation.

## 6. Statistics software

Analyses will be performed using SAS version 9.3.

**7. Outliers**

Outliers will be visually inspected by creating box and whisker plots and scatterplots. Outliers which are clearly implausible will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible will be kept.

**8. Data transformation**

Distribution of cortisol will be log transformed and key baseline variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created. Cortisol will also be categorized into high vs. low values using the $90^{th}$ percentile of the control group (IFA) as a cutoff.

**9. Covariates and effect modifiers**

The covariates to be included in the ANCOVA model will be derived from the list below. Each variable that shows a statistically significant association with each outcome ($P<0.1$), will be included in the model. Time since waking and time since last meal will be included in all models regardless of their association with the outcome variables.

Interactions will be examined between the intervention group and the variables listed below on their association with cortisol concentration. If a statistically significant interaction ($p<0.1$) is found, group means will be examined at different levels of the predictor variable, either by category for categorical predictors, or at selected percentile cutoffs for continuous variables. Variables that show no interaction with the intervention group can be used as covariates in the main analysis. Variables to be examined as covariates include:

1. Cortisol at baseline
2. Maternal perceived stress
3. Maternal BMI at baseline
4. Maternal height
5. Gestational age at enrolment
6. Parity (primiparous vs. multiparous)
7. Maternal education
8. Maternal age
9. Site of enrollment
10. Season at baseline
11. Malaria at baseline
12. HIV status at baseline
13. Hb at baseline
14. Iron status (ZPP and sTfR) at baseline
15. Inflammatory markers (CRP and AGP) at baseline
16. Infant gender
17. Household food insecurity score at baseline, adjusted for month of enrolment
18. Asset index at baseline

Variables to be examined as effect modifiers include:

1. Maternal age
2. Parity

3. Infant gender
4. Baseline BMI

A standard ANCOVA model and a repeated measures ANCOVA model will be used for analysis of results.

**10. Presentation of study findings**

Group means and standard deviations for salivary cortisol concentration will be tabulated by intervention group and presented in Table 1. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups.
The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, pairwise comparisons of the three intervention groups will be Tukey-Kramer test for ANOVA. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

**11. Tables**

Table 1. Mean (SD) salivary cortisol concentration by supplement group at baseline, 28 wk, and 36 wk gestation.

| | IFA | MMN | LNS | Overall ANCOVA | Comparison of LNS vs. IFA | | Comparison of LNS vs. MMN | | Comparison of MMN vs. IFA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n= | n= | n= | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value |
| Baseline cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |
| 28 wk cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |
| 36 wk Cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |

Table 2. Differences between groups in the proportions of women with high and low cortisol at 28 wk and 36 wk gestation.

| | | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of LNS vs. IFA** | | **Comparison of LNS vs. MMN** | | **Comparison of MMN vs. IFA** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Cortisol > 75$^{th}$ percentile of IFA group | 28 wk | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| | 36 wk | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| Cortisol < 25$^{th}$ percentile of IFA group | 28 wk | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| | 36 wk | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan: The impact of LNS on maternal cholesterol and triglycerides in plasma and fatty acids in plasma and breast milk

July 4, 2014

Prepared by Brietta Oaks

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## 1. Study objectives

The primary objective for the main trial is to determine whether LNS consumed by women during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

This statistical analysis plan addresses the following secondary objectives:

a. To determine if there are differences in mean plasma total cholesterol concentration, triglyceride concentration, fatty acid levels (arachidonic acid (AA); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA); sum of DHA and EPA; and sum of all long chain omega-3 fatty acids: DHA, EPA, and docosapentaenoic acid (DPA)), or fatty acid ratios (linoleic acid (LA):AA; α-linolenic acid (ALA):DHA; AA:EPA; omega-6 fatty acid:omega-3 fatty acid) at 36 weeks of pregnancy between groups of women who were provided either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.
b. To determine if differences exist in the prevalence of high or low cholesterol concentration and high triglyceride concentration measured in plasma at 36 weeks gestation between groups of women who were provided either LNS, MMN, or IFA during pregnancy.
c. To determine if there are differences in mean breast milk fatty acid levels (listed above) or ratios (listed above) at 6 mo postpartum between groups of women who were provided either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.

## 2. Hypotheses

a. Women who receive LNS during pregnancy will have higher mean total cholesterol , triglyceride, and fatty acid levels (AA, EPA, DHA, and DPA) and a lower prevalence of low total cholesterol (< $10^{th}$ percentile of IFA group) in plasma at 36 wk gestation compared to the IFA and MMN groups
b. Women who receive LNS during pregnancy and lactation will have higher fatty acid levels (AA, EPA, DHA and DPA) in breast milk at 6 mo postpartum compared to the IFA and MMN groups.

## 3. Outcome variables

Total plasma cholesterol

High total cholesterol concentration will be defined as ≥ 6.2 mmol/L (240 mg/dL). {Roth, 2011 #434}

Low total cholesterol concentration will be defined as < $10^{th}$ percentile of the IFA group.

Plasma triglcyerides

High triglycerides concentration will be defined as ≥ 200 mmol/L.{Miller, 2011 #436}

Plasma fatty acids (as a percentage of total fatty acids)

1. AA
2. EPA
3. DHA

4. The sum of EPA+DHA
5. The sum of all long chain omega-3 fatty acids (EPA+DHA+DPA)

Plasma fatty acid ratios

1. linoleic acid:AA
2. α-linolenic acid:DHA
3. AA:EPA
4. omega-6 fatty acid:omega-3 fatty acid.

Breast milk fatty acids

Same as those measured in plasma.

Breast milk fatty acid ratios

Same as those measured in plasma.

**4. Basis for the analysis: Intention to treat**

The primary analysis will be by intention-to-treat. That is, results for all women enrolled will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on participants who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available.

**5. Time points**

Blood samples were collected for cholesterol, triglyceride, and fatty acid analyses at enrollment and 36 wk gestation. Breast milk samples were collected at 6 mo postpartum.

**6. Statistics software**

Analyses will be performed using SAS version 9.3.

**7. Outliers**

Outliers will be visually inspected by creating box and whisker plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept.

**8. Data transformation**

Distribution of outcome variables and key baseline variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

**9. Covariates and effect modifiers**

The covariates to be included in the ANCOVA models will be derived from the list below. Each variable that shows a statistically significant association with each outcome ($P<0.1$), will be included in the model.

Interactions will be examined between the intervention group and the variables listed below on their association with cholesterol, triglyceride and fatty acid concentrations. If a statistically significant interaction ($p<0.1$ is found, group means will be examined at different levels of the

predictor variable, either by category for categorical effect modifiers, or at selected percentile cutoffs for continuous variables. Variables that show no interaction with the intervention group can be used as covariates in the main analysis. Variables to be examined as covariates and effect modifiers include:

1. Maternal BMI at baseline
2. Maternal height
3. Gestational age at enrollment
4. Inflammatory markers (CRP and AGP) at baseline
5. Malaria at baseline
6. HIV status at baseline
7. Parity (primiparous vs. multiparous)
8. Maternal education
9. Site of enrollment
10. Season at enrollment
11. Baseline value for the outcome variable
12. Household food insecurity score
13. Asset index
14. Infant gender

## 10. Presentation of study findings

10.1. Main effect of intervention on plasma cholesterol, triglycerides, and fatty acids

Group means and standard deviations for plasma total cholesterol, triglycerides, fatty acid levels (AA, DHA, and EPA), and ratios will be tabulated by intervention group and presented in Table 1. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be Tukey-Kramer test for ANOVA. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

The proportion of women with cholesterol < $10^{th}$ percentile will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups will be tested with chi-square test or Fisher's exact test. Pairwise comparisons between groups will be done in the context of logistic regression if global null-hypothesis is rejected with $P<0.05$. Risk ratios between intervention groups are also presented in Table 2.

10.2. Effect of intervention on breast milk fatty acids

Group means and standard deviations for breast milk fatty acid levels (AA, EPA, DHA) and ratios will be tabulated by intervention group and presented in Table 3. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be Tukey-Kramer test for ANOVA. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

## 11. Tables

Table 1. Mean (SD) plasma total cholesterol concentration, triglyceride concentration, and fatty acids by supplement group at baseline and 36 wk gestation.

| | IFA | MMN | LNS | Overall ANCOVA | Comparison of LNS vs. IFA | | Comparison of LNS vs. MMN | | Comparison of MMN vs. IFA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n= | n= | n= | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value |
| Baseline Total cholesterol (mmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |
| 36 wk total cholesterol (mmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |
| Baseline Triglycerides (mmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |
| 36 wk triglycerides (mmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | p-value | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BaselineAA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| 36 wk AA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| Baseline EPA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| 36 wk EPA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| Baseline DHA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| 36 wk DHA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| Baseline omega 3:omega 6 | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| 36 wk omega 3:omega 6 | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |

Table 2. Differences between groups in the proportions of women with cholesterol or triglycerides above or below specified cutoffs.

| | | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| Cholesterol < 10$^{th}$ percentile of IFA group | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx0.xx | | 0.xx0.xx | | 0.xx0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| Cholesterol ≥ 6.2 mmol/L | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx0.xx | | 0.xx0.xx | | 0.xx0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| Triglycerides ≥ 200 mmol/L | Baseline | x (x.x) | x (x.x) | x (x.x) | 0.xx | | 0.xx0.xx | | 0.xx0.xx | | 0.xx0.xx |
| | 36 gw | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |

Table 3. Mean (SD) breast milk fatty acids by supplement group at 6 mo.

| | LNS | MMN | IFA | Overall ANCOVA | Comparison of IFA and MMN | | Comparison of IFA and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n= | n= | n= | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value |
| AA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| EPA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| DHA | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |
| omega 3:omega 6 | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | | | | |

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan, Version 01.0 (25.07.2014)

Appendix 11: Developmental outcomes at age 18 months

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**Version History Log**

This table will detail the version history for this document. It will detail the key elements of the changes to the versions.

| **Version** | **Date implemented** | **Details of significant changes** |
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## 1. Study objectives

The main aim of the trial was to determine whether LNS consumed by the mother during pregnancy and the first 6 months of lactation, and by the child from age 6-18 months, improves foetal and child growth, micronutrient status and neurobehavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The aim of the analyses described in this addendum is to compare infants in 3 different intervention groups:

a) Daily iron and folic acid during pregnancy, and calcium (Ca) only (akin to a placebo) during the first 6 months postpartum, with no supplementation for offspring during infancy
b) Daily multiple micronutrients (1-2 RDA of 18 vitamins and minerals) during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy
c) Daily LNS during pregnancy and the first 6 months postpartum (LNS-P&L with similar vitamin and mineral content as the daily multiple micronutrients, plus Ca, P, K, Mg and essential fatty acids), with LNS for offspring (LNS-20gM with 22 vitamins and minerals with concentrations based on RNIs for infants) during infancy

on the following outcomes:

1. 18-month motor development, language development, socio-emotional development, executive function, and interaction with caregivers
2. Prevalence of severe and moderate to severe delay in motor development, language development, socio-emotional development, and executive function

## 2. Hypotheses to be tested

1. 18-month scores in gross and fine motor development, language development, socio-emotional development, executive function, and interaction with caregivers of infants provided with LNS during pregnancy and from 6 to 18 months of age will be greater than that of infants of mothers who received either iron-folate or multiple micronutrient supplementation. A secondary analysis will also test the difference in scores between the MMN and IFA groups.

2. The same hypotheses will also be examined with regard to the prevalence of severe and moderate to severe delay in motor development, language development, socio-emotional development, and executive function.

## 3. Definition of the 18-month developmental outcomes

The gross motor score is calculated as the sum of 35 Kilifi Developmental Inventory (KDI) gross motor items, each scored 0 or 1 (sum of *Form 41a Q 5.1-5.2* and *5.6-7.12*). Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.

The fine motor score is calculated as the sum of 34 KDI fine motor items, each scored 0 or 1 following Abubakar et al. (2008). Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.

The psychomotor score is calculated as the sum of 69 KDI fine and gross motor items, each scored 0 or 1. Severe delay is defined in two ways: (1) the bottom 10% of our sample and (2) <-3 SD below the mean according to published norms from Kenya (Abubakar et al. 2008). Moderate to severe delay is defined in two ways: (1) the bottom 25% of our sample and (2) <-2 SD below the mean according to published norms from Kenya (Abubakar et al. 2008).

Language development is quantified as

a. Vocabulary score, calculated as the sum of *Form 41c LANGVOCAB1* through *LANGVOCAB100.* Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.
b. Expressive vocabulary > 10 words vs. <= 10 words, derived from the vocabulary score
c. Word combining (Has the child started combining words into sentences? 0 = not yet, 1 = sometimes, 2 = often) *Form 41c Q 4.1*

Socio-emotional development is calculated as the sum of *Form 41b PSED1* through *PSED19.* Severe delay is defined as the top 10% of our sample (a lower score indicates more advanced socio-emotional development). Moderate to severe delay is defined as the top 25% of our sample.

Executive function is calculated as

a. A not B task total number correct, *Form 41a Q16.2.* Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.
b. A not B task total errors after set 1, *Form 41a Q16.3*
c. A not B task total trials completed, *Form 41a Q 16.1*. If this variable is not normally distributed, another statistical approach will be used, such as creating a dichotomous variable

Interaction with caregivers is calculated as the sum of the activities with adults in the past three days (*Form 41d Q 4.1.1* through *Q 4.5.3*).

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes. In addition to the intention to treat analysis, we will also perform a per protocol analysis by examining the interaction between treatment group and adherence to supplement consumption. If the interaction term is significant at $p < 0.1$, we will further explore the nature of the interaction by examining the effect of treatment group at the $10^{th}$, $50^{th}$, and $90^{th}$ percentile of adherence.

## 5. Presentation of the study findings and hypothesis testing

The group means and standard deviations for the gross motor score, fine motor score, psychomotor score, vocabulary score, socio-emotional score, and A not B task total number correct, total errors after set 1, and total trials completed, and the interaction with caregivers score will be presented as indicated in Table 1. The results of pairwise comparisons will be indicated by superscripts. Means that are significantly different from each other will be marked by different letters (e.g., a and b). Means that are not significantly different from each other will be marked by the same letter.

The analysis will begin with testing the null hypothesis of no difference between the three treatment groups using ANCOVA or logistic regression, and controlling for pre-specified covariates (see below). For all analyses, if the global null hypothesis is rejected at 0.05 level, then we will perform pairwise comparisons of all three groups using Tukey-Kramer adjustment (for continuous variables) or the “Contrast” statements (for categorical variables) in SAS. We will also use Scheffe’s test to assess whether the LNS group differs from the non-LNS groups.

## 6. General notes on statistical methods

### 6.1 Software

SAS for Windows Release 9.3 (Cary, NC) will be used for all analyses.

### 6.2 Calculating scores and z-scores

If a large percentage of data is missing for any item, we will exclude that item from the total score. For all other missing item scores, we will impute the scores based on the other items in the same subscale. We will use the imputation method described in Raghunathan et al. (2001).

Z-scores of developmental variables will be calculated based on the distribution of the iLiNS-DYAD-M sample, by standardizing the distribution to a mean of 0 and standard deviation of 1.

### 6.3 Multiple comparisons

The Tukey-Kramer adjustment method is used.

### 6.4 Confidence intervals

The same as that for the primary outcome analyses.

### 6.5 Interaction and effect modification

We will examine the same factors as that for the primary outcome analyses. In addition, we will examine the following effect modifiers:

1. Family care indicators z-score
2. Household Food Insecurity Access (HFIA) Index, adjusted for season

### 6.6 Covariate adjustment

For each hypothesis, three models will be estimated:

1. No covariate adjustment
2. Adjustment for child age at developmental assessment
3. Adjustment for child age at developmental assessment and for any of the variables presented in Table 1 of the primary outcome Statistical Analysis Plan (SAP) showing statistically significant association (at $p<0.1$ level) with the developmental score

In addition to the variables in Table 1 of the primary outcome SAP, we will consider the following variables for inclusion:

1. Child sex
2. Household Food Insecurity Access (HFIA) Index, adjusted for season
3. Season at enrolment
4. Number of persons in the household
5. Children < age 5 years in the household
6. Family care indicators score, if this score is not different between supplement groups.
7. For the KDI scores, the child's mood, interaction with the tester, and activity level during testing, if they are not different between supplement groups (*Form 41a Q 3.1 - 3.3*).
8. For the language scores, the child's primary language (Chichewa, Chiyao, English, or other) and the number of languages to which the child had been exposed (*Form 25c Q 1.7-1.8*).
9. Data collector

## 7. References

Abubakar, A., Holding, P. A., Van Baar, A., Newton, C. R. J. C., & Van de Vijver, F. J. R. (2008). Monitoring psychomotor development in a resource-limited setting: An evaluation of the Kilifi Developmental Inventory. *Annals of Tropical Pediatrics*, 28, 217-226.

Raghunathan, T. E., Lepkowski, J. M., Van Hoewyk, J., & Solenberger, P. (2001). A multivariate technique for multiply imputing missing values using a sequence of regression models. Survey Methodology, 27(1), 85-95.

## 8. Tables

*Table 1*. Mean Motor, Language, Socio-emotional, Executive Function, and Interaction Z-Scores at the End of the Intervention Period

| | IFA | MMN | LNS | | | LNS vs MMN | | LNS vs IFA | | MMN vs IFA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean (SD)* | *Mean (SD)* | *Mean (SD)* | *p-value for the difference between the 3 trial groups* | *Covariate-adjusted p-value for the difference between the 3 trial groups* | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* |
| Fine Motor z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[b] | | | | | | |
| Gross Motor z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[c] | | | | | | |
| Language z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[d] | | | | | | |
| Socio-emotional z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[e] | | | | | | |
| A not B correct z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[f] | | | | | | |
| A not B perseverative errors z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[g] | | | | | | |
| Interaction with caregivers z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[h] | | | | | | |

***p < 0.001

[a]Adjusted for child age at developmental assessment.

[b]Adjusted for child age and ….

[c]Adjusted for child age and ….

[d]Adjusted for child age and ….

[e]Adjusted for child age and ….

[f]Adjusted for child age and ….

[g]Adjusted for child age and ….

*Table 2*. Effect of Intervention on Categorical Outcomes

| | IFA | MMN | LNS | *p-value for the difference between the 3 trial groups* |
|---|---|---|---|---|
| | *n/total (%)* | *n/total (%)* | *n/total(%)* | |
| Children who had an expressive vocabulary of at least 10 words | xxx/xxx (xx%) | xxx/xxx (xx%) | xxx/xxx (xx%) | x.xx |
| Children who had started combining words into phrases | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children in the lowest quartile of language scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children in the lowest decile of language scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (x%) | x.xx |
| Children in the lowest quartile of motor scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children in the lowest decile of motor scores | xx/xxx (x%) | xx/xxx (x%) | xx/xxx (x%) | x.xx |
| Children in the lowest quartile of socio-emotional scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children in the lowest decile of socio-emotional scores | xx/xxx (x%) | xx/xxx (x%) | xx/xxx (x%) | x.xx |
| Children in the lowest quartile of A not B correct scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children in the lowest quartile of A not B correct scores | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Children who completed all 10 trials of the A not B task | xxx/xxx (xx%) | xxx/xxx (xx%) | xxx/xxx (xx%) | x.xx |

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan, Version 01.0 (25.07.2014)

Appendix 12: Maternal cognition and mother-infant interaction at 6 months post-partum

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| Version date | July 28, 2014 |
| Author | Elizabeth Prado |
| Implementation date of current version | |

**Version History Log**

This table will detail the version history for this document. It will detail the key elements of the changes to the versions.

| **Version** | **Date implemented** | **Details of significant changes** |
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## 1. Study objectives

The main aim of the trial was to determine whether LNS consumed by the mother during pregnancy and the first 6 months of lactation, and by the child from age 6-18 months, improves foetal and child growth, micronutrient status and neurobehavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The aim of the analyses described in this addendum is to compare infants in 3 different intervention groups:

a) Daily iron and folic acid during pregnancy, and calcium (Ca) only (akin to a placebo) during the first 6 months postpartum, with no supplementation for offspring during infancy
b) Daily multiple micronutrients (1-2 RDA of 18 vitamins and minerals) during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy
c) Daily LNS during pregnancy and the first 6 months postpartum (LNS-P&L with similar vitamin and mineral content as the daily multiple micronutrients, plus Ca, P, K, Mg and essential fatty acids), with LNS for offspring (LNS-20gM with 22 vitamins and minerals with concentrations based on RNIs for infants) during infancy

on the following outcomes:

1. Maternal cognitive scores at 6 months post-partum
2. Mother-infant interaction scores at 6 months post-partum

## 2. Hypotheses to be tested

1. Maternal cognitive scores in working memory, verbal fluency, visuospatial ability, and functional health literacy will be greater among mothers provided with LNS during pregnancy and postpartum compared to mothers who received either iron-folate or multiple micronutrient supplementation. A secondary analysis will also test the difference in scores between the MMN and IFA groups.

2. Mother-infant interaction, as measured by the Home Observation for the Measurement of the Environment (HOME) Inventory scores, will be greater among mothers provided with LNS during pregnancy and postpartum compared to mothers who received either iron-folate or multiple micronutrient supplementation. A secondary analysis will also test the difference in scores between the MMN and IFA groups.

## 3. Definition of the maternal cognition and mother-infant interaction outcomes

The digit span forward and backward scores are calculated as the total number of sequences of digits, correctly repeated (digit span forward) or repeated in reverse order (digit span backward), before an error was committed on two consecutive trials of the same length, derived from *Form 59 Sections 4 and 5*.

The verbal fluency scores for the category "food" and for the category "people's names" are calculated as the total number of instances of each category named in one minute, derived from *Form 59 Sections 6 and 7*.

The mental rotation score is calculated in two ways: the total percent correct, including rotated figures correctly marked and mirror images correctly left unmarked; and *d-prime*, which is the z-score for the number of hits (rotated figures correctly marked) minus the z-score for the number of false alarms (mirror images incorrectly marked), derived from *Form 59 Section 8*.

The overall cognition score is calculated as the mean z-score across these five cognitive tests z-scores (digit span forward and backward, verbal fluency for food and people's names, and mental rotation *d-prime*).

The functional health literacy test is calculated as the total number of correct responses to the functional health literacy questions, out of a maximum possible 36 points, in *Form 59 Section 2*.

The total HOME Inventory score is calculated as the sum of the HOME Inventory items in *Form 60 Section 2*. We will also calculate each subscale score: maternal responsivity (*Form 60 Q 2.1-2.11*), acceptance (*Form 60 Q 2.12-2.18*), and involvement (*Form 60 Q 2.28-2.31*) as well as the learning materials (*Form 60 Q 2.24-2.27*), variety (*Form 60 Q 2.32-2.36*), and organization (*Form 60 Q 2.19-2.23*) in the child's environment.

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes. In addition to the intention to treat analysis, we will also perform a per protocol analysis by examining the interaction between treatment group and adherence to supplement consumption. If the interaction term is significant at $p < 0.1$, we will further explore the nature of the interaction by examining the effect of treatment group at the $10^{th}$, $50^{th}$, and $90^{th}$ percentile of adherence.

## 5. Presentation of the study findings and hypothesis testing

The group means and standard deviations will be presented as indicated in Tables 1 and 2. The results of pairwise comparisons will be indicated by superscripts. Means that are significantly different from each other will be marked by different letters (e.g., a and b). Means that are not significantly different from each other will be marked by the same letter.

The analysis will begin with testing the null hypothesis of no difference between the three treatment groups using ANCOVA or logistic regression, and controlling for pre-specified covariates (see below). For all analyses, if the global null hypothesis is rejected at 0.05 level, then we will perform pairwise comparisons of all three groups using Tukey-Kramer adjustment (for continuous variables) or the "Contrast" statements (for categorical variables) in SAS. We will also use Scheffe's test to assess whether the LNS group differs from the non-LNS groups.

## 6. General notes on statistical methods

### 6.1 Software

SAS for Windows Release 9.3 (Cary, NC) will be used for all analyses.

### 6.2 Calculating scores and z-scores

If a large percentage of data is missing for any item, we will exclude that item from the total score. For all other missing item scores, we will impute the scores based on the other items in the same subscale. We will use the imputation method described in Raghunathan et al. (2001).

Z-scores of cognitive variables will be calculated based on the distribution of the iLiNS-DYAD-M sample, by standardizing the distribution to a mean of 0 and standard deviation of 1.

### 6.3 Multiple comparisons

The Tukey-Kramer adjustment method is used.

### 6.4 Confidence intervals

The same as that for the primary outcome analyses.

### 6.5 Interaction and effect modification

We will examine the same factors as that for the primary outcome analyses. In addition, we will examine Household Food Insecurity Access (HFIA) Index, adjusted for season.

### 6.6 Covariate adjustment

For each hypothesis, two models will be estimated:

1. No covariate adjustment
2. Adjustment for any of the variables presented in Table 1 of the primary outcome Statistical Analysis Plan (SAP) showing statistically significant association (at $p<0.1$ level) with the cognitive or HOME score

In addition to the variables in Table 1 of the primary outcome SAP, we will consider the following variables for inclusion:

1. Child sex
2. Household Food Insecurity Access (HFIA) Index, adjusted for season
3. Season at enrolment
4. Data collector

## 7. References

Raghunathan, T. E., Lepkowski, J. M., Van Hoewyk, J., & Solenberger, P. (2001). A multivariate technique for multiply imputing missing values using a sequence of regression models. Survey Methodology, 27(1), 85-95.

## 8. Tables

*Table 1*. Mean Maternal Cognitive Z-Scores at the End of the Intervention Period

| | IFA | MMN | LNS | | *Covariate-adjusted p-value for the difference between the 3 trial groups* | LNS vs MMN | | LNS vs IFA | | MMN vs IFA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean (SD)* | *Mean (SD)* | *Mean (SD)* | *p-value for the difference between the 3 trial groups* | | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* |
| Overall cognitive z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[a] | | | | | | |
| Digit span forward z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[b] | | | | | | |
| Digit span backward z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[c] | | | | | | |
| Verbal fluency: food z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[d] | | | | | | |
| Verbal fluency: names z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[e] | | | | | | |
| Mental rotation z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[f] | | | | | | |
| Functional health literacy z-score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[g] | | | | | | |

[a]Adjusted for ….

[b]Adjusted for ….

[c]Adjusted for ….

[d]Adjusted for ….

[e]Adjusted for ….

[f]Adjusted for ….

[g]Adjusted for ….

*Table 2*. Mean Mother-infant Interaction Scores at the End of the Intervention Period

| | IFA | MMN | LNS | | | LNS vs MMN | | LNS vs IFA | | MMN vs IFA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean (SD)* | *Mean (SD)* | *Mean (SD)* | *p-value for the difference between the 3 trial groups* | *Covariate-adjusted p-value for the difference between the 3 trial groups* | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* | *Difference in means (95% CI)* | *p-value* |
| Total HOME Inventory score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[a] | | | | | | |
| Maternal responsivity score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[b] | | | | | | |
| Maternal acceptance score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[c] | | | | | | |
| Maternal involvement score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[e] | | | | | | |
| Learning materials score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[d] | | | | | | |
| Variety score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[e] | | | | | | |
| Organization score | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx | x.xx[f] | | | | | | |

[a]Adjusted for ….

[b]Adjusted for ….

[c]Adjusted for ….

[d]Adjusted for ….

[e]Adjusted for ….

[f]Adjusted for ….

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 13: The impact of intervention on maternal anthropometry and placental weight

Version 01.0 (19.08.2014)

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## 1. Version history

## 2. Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 months of lactation, and by the child from 6-18 months, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid (FA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The aims of the secondary analyses described in appendix XX are to compare maternal anthropometry in three different intervention groups between enrollment and 36 weeks gestation and to compare placental weight in the three different intervention groups. Details of the objectives are as follows:

2.1 Main effect of the intervention on pregnancy weight gain and blood pressure.

1. To determine if there are differences in the weekly weight gain between enrollment and 36 weeks gestation between groups of women who were provided either LNS, MMN, or IFA during pregnancy.
2. To determine if there are differences in placental weight at delivery between groups of women who were provided either LNS, MMN, or IFA during pregnancy.

## 3. Hypotheses to be tested

Hypothesis 1: Women who receive LNS during pregnancy will have higher mean weekly change in weight compared to the IFA and MMN groups.

Hypothesis 2: The proportion of women with placental weight below the $10^{th}$ centile of a reference population's placental weight for gestational age and birth weight will be lower among women who received LNS than among women who received either MMN or IFA[1].

Hypothesis 3: The proportion of women with placental weight below the $10^{th}$ centile of a reference population's placental weight to birth weight ratio will be lower among women who received LNS than among women who received either MMN or IFA.

[1] Almog B, Shehata F, ALjabbri S, Levin I, Shalom-Paz E and Shrim A. (2011). Placental weight percentile curves for singleton and twin deliveries. Placenta 32:58-62.

## 4. Definition of outcome variables

a) Maternal weight at enrollment, 32 gestation weeks and 36 gestation weeks
Maternal weight will be taken from weight measurements done at enrollment, around 32 gestation weeks and around 36 gestation weeks. Number of weeks between enrollment and anthropometric measurements will be calculated by following formula: weeks in study = (date of measurement – date of enrollment)/7. The final result will be expressed in grams per week. *The data will be extracted from Form 04: Q1.2, Q2.4; Form 06a: Q1.2, Q7.6*

b) Mean placental weight
Placental weight will be defined as a weight measured after delivery, expressed in grams, rounded to the nearest 1 g and with no decimals. *The data will be extracted from Form 23: Q4.6.*

c) Mean duration of pregnancy at delivery
The duration of pregnancy will be calculated from gestational age at enrollment, date of enrolment and date of delivery, using the following formula: The duration of pregnancy at birth = the duration of pregnancy at enrolment + (date of delivery – date of enrolment)/7. Women with twin pregnancy will be considered not having valid data on this outcome (because ultrasound dating of pregnancy is unreliable for twin pregnancies) and hence they will be excluded from this analysis. The values will be expressed as gestation weeks, with two decimals. *The data will be extracted from Form06a: Q1.2, Q7.6.1, Q7.6.2, Q7.7; Form 23: Q2.1.*

d) Birth weight
Birth weight will be defined as a weight measured within 48 hours from delivery, expressed in grams, rounded to the nearest 10 g and with no decimals. *The data will be extracted from Form 23: Q2.1, Form 24: Q1.2, Q2.4.*

e) Placental weight for gestation and placental weight for birth weight centiles.

Placental weight for gestation and birth weight centiles will be assessed using normograms and tables produced for North American population as described in Almog et al.[2] Low placental weight for gestation and low placental weight for birth weight will be defined as placental weight for gestation/birth weight below the $10^{th}$ centile. Individual centile values will be expressed as a percentage, with one decimal.

[2] Almog B, Shehata F, ALjabbri S, Levin I, Shalom-Paz E and Shrim A. (2011). Placental weight percentile curves for singleton and twin deliveries. Placenta 32:58-62.

f) Placental weight to birth weight ratio
Placental weight to birth weight ratio will be calculated by dividing placental weight by birth weight, expressing the value as a fraction to 2 decimal places

## 5. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes.

## 6. Time points for the analyses

The anthropometry analyses will cover the time period from enrollment to 36 weeks gestation and the placental weight data will be collected immediately after delivery.

## 7. Presentation of the study findings and hypothesis testing

*7.1 Success of enrollment and follow-up*

All enrolled participants and the success of their follow-up will be described in a flow chart (figure 1) according to the CONSORT guidelines. For additional information the drop-out rate between groups will be tested with Fisher's exact test and baseline characteristics of drop-outs compared to those who completed the study will be tested with t-test or chi square. P-values for these tests will be described in the text.

*7.2 Baseline information*

Maternal characteristics at enrollment will be tabulated by study group as indicated in Table 1. The following characteristics will be described:

Number of enrolled participants; mean (sd) age in years; mean (sd) completed years of education; mean (sd) gestational age at enrollment in weeks; mean (sd) number of previous pregnancies; proportion of primigravid women; mean (sd) weight in kilograms; mean (sd) height in centimeters; mean (sd) body mass index (BMI) in kilograms per meter squared; mean (sd) mid-upper arm circumference (MUAC) in millimeters; mean (sd); proportion of women with anaemia; proportion of women with HIV, and proportion of women with malaria.

*7.3 Comparisons of the outcomes of anthropometry and placental weight*

The group mean and standard deviation of maternal pregnancy weight gain and placental weight will be tabulated by intervention group as shown in Table 2. The table will also indicate the differences in means and their 95 % confidence intervals between the intervention groups.

The difference between the three groups will be tested with ANOVA (model without covariates) and null-hypothesis of no difference between groups will be rejected if P<0.05. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be done (Stata command *pwcompare*). For all pairwise comparisons with P<0.05, the null-hypothesis of no difference in means between groups will be rejected.

For testing hypothesis on maternal weight gain during pregnancy we will create a mixed model of the following formula:

$$W_{ij} = b_0 + b_1 \times GA_{ij} + b_2 \times IFA_i + b_3 \times MMN_i + b_4\left(IFA_i \times GA_{ij}\right) + b_5(MMN_i \times GA_{ij})$$

where the mixed model includes random intercept and random slope which are allowed to correlate with each other. Coefficient $b_1$ will tell the mean weight gain in LNS group per week and coefficients $b_4$ and $b_5$ will tell the difference in weight gain over time between LNS compared to IFA and MMN groups, respectively. We will test global null hypothesis of no differences between groups by testing $b_4 = b_5 = 0$ and reject null hypothesis if P<0.05. Hypothesis of no differences between groups will be tested with Stata command "*test*". For pairwise comparisons with P<0.05, the null hypothesis of no differences between groups will be rejected only if global null hypothesis is also rejected. For comparison of IFA and MMN groups we will create another mixed model of the same formula with MMN as reference group. Interpretation of the results will be done similarly as described above.

The Institutes of Medicine guidelines on appropriate gestational weight gain will be used to determine whether participants gained insufficient or sufficient weight during pregnancy. Considering that weight gains in this population may well be below the IOM minimum, we will consider revising the minimum weight gain to 80% of the IOM minimum weight gain. The guidelines vary based on the pre-pregnancy BMI; however, pre-pregnancy BMI is not available, so a proxy for pre-pregnancy weight gain will be established by use of regression modeling. The technique is described below.

a) First we will determine the best transformation of maternal BMI that achieves normal distribution by regressing BMI with gestational age. BMI, log BMI, and inverse BMI will be regressed with age, age squared, and age cubed. The regression with the highest r-square will be the best model for predicting BMI based on gestational age.

b) Using the best transformation as chosen above, we will regress BMI on age and save both the predicted value and the residual as separate variables within the data table. In SAS, this is done by the following command, which saves both the predicted value and the residual (actual – predicted value) in a separate file (named bmi1 in this case) for use later in the analysis.

```
proc glm data=bmi;
      model invbmi = age age*age age*age*age;
      output out=bmi1 p=predict r=resid;
   run;
```

c) We will visually inspect the regression curve above to determine the youngest gestational age before the confidence intervals expands. Ideally this age is young enough that a substantial weight gain has not yet been achieved, yet still fits well along the regression curve. The predicted mean BMI is calculated at the age of interest. This is done in SAS by the following, which uses the data set that includes the predicted values saved in step 2 above. The mean BMI at 12.6, 13.7, and 17.0 gestational weeks is determined.

```
proc means data=bmi1 n mean std;
     where age in (12.6, 13.7, 17.0);
     class age;
     var predict;
  run;
```

d) Create adjusted values for each of the ages inspected in step 3 above by adding the residual saved in step 2 above, and perform the back-transformation (if log or inverse were used as transformations above). This is done in SAS by the following command, where adjBMI12_6 is the adjusted BMI at 12.6 gestational weeks, 0.0476412 is the mean from step 3 above, and the resid is the residual value as determined by step 2 above.

```
data bmi1;
    set bmi1;
    adjBMI12_6 = 1/(0.0476412  + resid);
    adjBMI17_0 = 1/(0.0458771  + resid);
  run;
```

The prevalence of low placental weight for gestation/ birth weight will be calculated by dividing the number of placentas < $10^{th}$ centile for gestation / birth weight by the number of all babies with valid data on this outcome. The proportions of placental weight below the $10^{th}$ percentile of expected placental weight for gestation age / birth weight will be tabulated as shown in Table 3. Global null hypothesis of no difference between the groups will be tested with chi squared test or Fischer's exact test. Pairwise comparisons between the groups will be done in the context of

logistic regression if the global null hypothesis is rejected with $p<0.05$. Risk ratios between intervention groups will be presented as shown in Table 3.

## 8. General notes on statistical methods

### 7.1 Software

The same as that for the primary outcome analyses

### 7.2 Preparing anthropometric data for analysis

The same as that for the primary outcome analyses

### 7.3 Multiple comparisons

The same as that for the primary outcome analyses.

### 7.4 Confidence intervals

The same as that for the primary outcome analyses.

### 7.5 Interaction and effect modification

There will be two sets of tests for interaction between the intervention group and selected other variables on their association with the maternal anthropometry and the placental outcomes. All tests will be done using the likelihood ratio test.

The first set of analyses will be hypothesis-driven and will include unambiguous predefined variables that could logically modify the effect of the nutritional intervention on pregnancy. Variables included (as continuous variables where possible) in this analysis include:

1. Maternal BMI at enrolment
2. Gestational age at enrollment
3. Maternal age at enrollment
4. Maternal education
5. Number of previous pregnancies
6. Anemia at enrollment
7. Malaria at enrollment
8. HIV at enrollment

The second set of analyses will be exploratory in nature and will include variables that can be constructed in several ways or that cannot *a priori* be logically linked to an effect modification. Themes or variables included in this analysis include:

1. Household wealth
2. Syphilis at enrollment

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as stratified by the respective predictor variable. Variables that show no interaction with the intervention group can be used as covariates in the main analysis.

### 7.6 Covariate adjustment

The same adjustments will be done as for the main analyses.

## 9. Legends to the figures

Figure 1: Participant flow in CONSORT recommended format

## 10. Figures

Figure 1

Tables

Table 1. Baseline characteristics of the participating women at enrolment, by study group

| **Characteristic** | **IFA** | **MMN** | **LNS** | **Test** |
|---|---|---|---|---|
| Number of participants | xxx | xxx | xxx | |
| Mean (SD) maternal age, years | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) maternal education, competed years at school | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) gestational age at enrolment, weeks | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) number of previous pregnancies | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) weight, kg | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) MUAC, cm | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) BMI, kg/m² | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of women with a low BMI (< 18.5 kg/m²) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of anemic women (Hb < 100 g/l) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive HIV test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive malaria | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |

| | | | | |
|---|---|---|---|---|
| test (RDT) | | | | |
| Number (%) of women with a positive syphilis antibody test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |

Table 2. Differences between groups in mean (SD) weight gain, and placental weight.

| | **Result by study group** | | | | **Comparison between LNS and IFA group** | | **Comparison between LNS and MMN group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **IFA(n=xxx)** | **MMN (n=xxx)** | **LNS(n=xxx)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** | **Difference in means (95 % CI)** | **P-value** |
| Mean (SD) maternal weight gain (kg / week) | x.xx (x.x) | x.xx (x.x) | x.xx (x.x) | x.xxx | x.xx (x.xx to x.xx) | x.xxx | x.x (xx to xx) | x.xxx | x.x (xx to xx) | x.xxx |
| Mean (SD) placental weight (g) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| | | | | | | | | | | |

Table 3. Differences between groups in proportions of pregnancy weight gain and placental weight below specified cut off points.

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **LNS (n=xxx)** | **MMN (n=xxx)** | **IFA (n=xxx)** | **P-value** | **Risk ration (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** | **Risk ration (95 % CI)** | **P-value** |
| | | | | | | | | | | |
| Number (%) pregnancy weekly weight gain below predicted expected gain | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) pregnancy weekly weight gain above predicted expected gain | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) Placental weight by gestation age below 10$^{th}$ percentile | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Number (%) Placental weight by birth weight below 10$^{th}$ percentile | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number (%) Placental weight to birth weight ratio below 10$^{th}$ percentile | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 14: The impact of the interventions on maternal vitamin A status (version 01.0, 31 August 2014, prepared by Andrew Hall and Marjorie Haskell)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 08.31.2014 | Andrew Hall and Marjorie Haskell | Initial version |

## 2. Study objectives

2.1. Main effect of intervention on maternal vitamin A status and prevalence of vitamin A deficiency

a. To determine the effects of supplementation with LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy on maternal plasma retinol concentration at 36 weeks gestation.

b. To determine the effects of supplementation with LNS, MMN capsules, or IFA capsules during pregnancy on the prevalence of low plasma retinol concentration at 36 weeks gestation.

c. To determine whether baseline plasma retinol concentration is an effect modifier for the effect of group assignment on birth outcomes.

d. To determine the effects of supplementation with LNS, multiple micronutrient (MMN) capsules, or placebo (calcium) capsules during lactation on maternal plasma and breast milk retinol concentration at 6 months postpartum.

e. To determine the effects of supplementation with LNS, MMN capsules, or placebo (calcium) during lactation on the prevalence of low plasma and breast milk retinol concentrations at 6 months post-partum.

## 3. Hypotheses

3.1. Main effect of intervention on vitamin A status

a. Gestational supplementation with LNS will lead to a greater improvement in vitamin A status compared with MMN or IFA, and gestational supplementation with MMN will lead to a greater improvement in vitamin A status compared with IFA. Specifically,

i. Women receiving LNS during pregnancy will have higher plasma retinol concentration at 36 weeks gestation compared with those receiving MMN or IFA.
ii. Women receiving MMN during pregnancy will have higher plasma retinol concentration at 36 weeks gestation compared with those receiving IFA.

b. Gestational supplementation with LNS will lead to a greater reduction in the prevalence of maternal vitamin A deficiency compared with MMN or IFA, and gestational supplementation with MMN will lead to a greater reduction in the prevalence of vitamin A deficiency compared with IFA. Specifically,
   i. Women receiving LNS during pregnancy will have a lower prevalence of plasma retinol below 1.05 µmol/L at 36 weeks gestation compared with those receiving MMN or IFA.
   ii. Women receiving MMN during pregnancy will have lower prevalence of plasma retinol below 1.05 µmol/L at 36 weeks gestation compared with those receiving IFA.

c. Post-partum supplementation with LNS will lead to a greater improvement in vitamin A status compared with MMN or placebo (calcium), and post-partum supplementation with MMN will lead to a greater improvement in vitamin A status compared with placebo (calcium). Specifically,
   i. Women receiving LNS during lactation will have higher plasma and breast milk retinol concentrations at 6 months post-partum compared with those receiving MMN or placebo (calcium).
   ii. Women receiving MMN during lactation will have higher plasma retinol and breast milk retinol concentrations at 6 months post-partum compared with those receiving placebo (calcium).

d. Post-partum supplementation with LNS will lead to a greater reduction in the prevalence of maternal vitamin A deficiency compared with MMN or placebo (calcium), and post-partum supplementation with MMN will lead to a greater reduction in the prevalence of vitamin A deficiency compared with placebo (calcium). Specifically,
   i. Women receiving LNS during lactation will have lower prevalence of plasma retinol below 1.05 µmol/L and breast milk retinol below 28 nmol/g fat at 6 months post-partum compared with those receiving MMN or placebo (calcium).
   ii. Women receiving MMN during lactation will have lower prevalence of plasma retinol below 1.05 umol/L and breast milk retinol below 28 nmol/g fat at 6 months post-partum compared with those receiving placebo (calcium).

**4.** Definition of outcome variables

Plasma retinol

Retinol is extracted from plasma into hexane and measured by HPLC using retinyl acetate as an internal standard. Plasma retinol is expressed as µmol retinol per L of plasma.

Breast milk retinol

Retinyl esters in breast milk are converted to retinol by saponification, then retinol is extracted into hexane and measured by HPLC using retinal oxime as an internal standard. Breast milk retinol is expressed as nmol retinol per g milk fat.

Breast milk fat

Milk fat is determined by using the crematocrit method, and expressed as g fat per L milk.

**5. Basis for the analysis: Intention to treat and per protocol**

The primary analysis will be by intention-to-treat, i.e. analysis according to original group assignment regardless of protocol violations, with the inclusion of all available data from participants lost to follow-up.

**6. Time points**

Blood samples for plasma retinol analyses are collected at enrollment, 36 weeks gestation, and 6 months postpartum. Breast milk samples for milk retinol and milk fat analyses are collected at 6 months postpartum.

**7. Statistics software**

All statistical analyses are performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

**8. Outliers**

Outliers will be visually inspected by creating box and whisker plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept.

**9. Data transformation**

Distribution of outcome variables and key baseline variables will be inspected for normality and transformed as necessary to achieve normal distribution prior to analysis. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

**10. Interaction**

Interactions will be examined between the intervention group and selected variables on their association with maternal vitamin A status. If a statistically significant interaction ($p<0.05$) is found, group means will be examined at different levels of the predictor variable, either by category for categorical predictors, or at the $10^{th}$, $50^{th}$, and $90^{th}$ percentiles for continuous variables. Variables included (as continuous variables where possible) in this analysis include:

1. Maternal BMI at baseline
2. Inflammatory markers (CRP and AGP) at baseline
3. Malaria at baseline
4. HIV status at baseline
5. Number of previous pregnancies
6. Maternal education
7. Site of enrollment
8. Season at enrollment
9. Plasma retinol at enrollment
10. Receipt of post-partum high-dose vitamin A capsule (if available), lactation timepoint only

**11. Covariates**

Each of the following variables that show a statistically significant association with the outcome ($P<0.1$), will be included as covariates in the ANCOVA or logistic regression models.

Pregnancy timepoints:

1. Maternal BMI at baseline
2. Inflammatory markers (CRP and AGP) at baseline
3. Malaria at baseline
4. HIV status at baseline
5. Number of previous pregnancies
6. Maternal education
7. Site of enrollment
8. Season at enrollment
9. Plasma retinol at enrollment

**12. Confidence intervals**

The calculated ratios and differences in between-group comparisons will include confidence intervals (at 95% level), for descriptive purposes. For continuous outcomes, confidence intervals will be based on ANOVA or ANCOVA and for binary outcomes confidence intervals will be based on logistic regression.

## 13. Presentation of study findings

13.1. Main effect of intervention on vitamin A status

Group means and 95% confidence intervals for plasma retinol, and breast milk retinol per gram fat are tabulated by intervention group and presented in Table 1.

The difference between intervention groups is tested by ANOVA and ANCOVA, with rejection of the null-hypothesis of no difference between groups if $P<0.05$. If the null-hypothesis is rejected, the Tukey-Kramer test is used for pair-wise comparisons between groups.

The proportion of women with plasma retinol < 1.05 µmol/L is tabulated by intervention group in Table 2. The null hypothesis of no differences between groups will be tested with chi-square test or Fisher's exact test. Pairwise comparisons between groups will be done in the context of logistic regression if global null-hypothesis is rejected with $P<0.05$. Risk ratios between intervention groups are also presented in Table 2.

## 14. Tables

Table 1. Maternal Plasma and Breast Milk Retinol.

| Variable | Timepoint | IFA (pregnancy) or calcium (lactation) [n] | MMN [n] | LNS [n] | P-value | Comparison of IFA (or calcium) and MMN | | Comparison of IFA (or calcium) and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | P-value | Difference in means (95 % CI) | P-value | Difference in means (95 % CI) | P-value | Difference in means (95 % CI) |
| Plasma retinol (µmol/L) (mean ( 95% CI)) [n] | Baseline | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) |
| | 36 weeks gestation | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) |
| | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) |
| Breast milk retinol (nmol/g fat) (mean ( 95% CI)) [n] | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) |

Table 2. Proportions of women with plasma retinol < 1.05 µmol/L and breast milk retinol <28 nmol/g fat.

| Cutoff | Timepoint | IFA (pregnancy) or calcium (lactation) n (%) | MMN n (%) | LNS n (%) | P-value | Comparison of IFA (or calcium) and MMN | | Comparison of IFA (or calcium) and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value |
| Plasma retinol <1.05 µmol/L | Baseline | x (x.x) | x (x.x) | x (x.x) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx |
| | 36 weeks gestation | x (x.x) | x (x.x) | x (x.x) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx |
| | 6 months postpartum | x (x.x) | x (x.x) | x (x.x) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx |
| Breast milk retinol <28 nmol/g fat | 6 months postpartum | x (x.x) | x (x.x) | x (x.x) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx | x.xx (x.xx, x.xx) | x.xxx |

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 15, version 2.0: Comparison of the main effect of treatment group on change in maternal vitamin B12 and folate status during pregnancy, in maternal and infant B12 and folate status at 6 months postpartum, in infant B12 and folate status at 18 months, and in vitamin B12 in breast milk at 6 months postpartum.

Prepared by Juliana Haber and Lindsay Allen on June 19, 2016

Version history:

| | | |
|---|---|---|
| 2014-09-11 | Version 01.0 | Original document |
| 2016-06-19 | Version 02.0 | Added analyses on maternal Vitamin B12 and folate status at 6 months post-partum and infant status at 6 & 18 mo |

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**6.5** Evaluating whether maternal B12 in early pregnancy, late pregnancy, or at 6 months postpartum is most highly correlated with breast milk B12 and infant B12 at 6 months.

7. General notes on statistical methods

8. Tables and Figures

Table 1. Comparison of maternal plasma B12, folate and homocysteine at <20 wk gestation, at 36 wk gestation, and at 6 months postpartum between intervention groups. Baseline plasma B12 will be controlled for in later time points.

Table 2. Comparison of infant plasma B12 & folate at 6 months postpartum & 18 months postpartum between intervention groups, unadjusted analyses.

Table 3. Prevalence of abnormal maternal plasma B12, folate and homocysteine and breast milk vitamin B12 homocysteine at <20 wk gestation, at 36 wk gestation, and at 6 months postpartum.

Table 4. Prevalence of abnormal infant plasma B12 & folate values at 6 and 18 months.

Figure 1: Path analysis of milk B12 at 6 months.

Figure 2: Path analysis of infant B12 at 6 months.

1) **Study Objectives.**

This analysis falls under the iLiNS-DYAD-M trial, the primary aims of which is to evaluate the efficacy of lipid-based nutrient supplements (LNS) for pregnant women and their infants. A secondary aim is to study the impact of LNS on breast milk B12 concentrations at 6 mo postpartum. This sub-study analysis will compare the change in plasma B12, folate and homocysteine from enrolment (before 20 wk gestation) through 36 wk gestation and 6 months postpartum between similar groups of women randomly assigned to receive daily antenatal supplements in one of the following three intervention groups:

a. 60 mg iron and 400g folic acid (IFA)

b. 20 mg iron and multiple micronutrients tablet (MMN) or

c. 20 mg iron and multiple micronutrients in a lipid-based nutrient supplement (LNS)

This sub-study will also compare changes in infant plasma B12 and folate at 6 and 18 months postpartum in the different intervention groups. Infants of mothers who received the LNS intervention were supplemented with LNS between 6 and 18 mo postpartum.

In addition, we will evaluate the strength of the association between maternal status in early pregnancy, at 36 wk gestation, and at 6 mo postpartum and i) B12 breast milk concentrations at 6 mo and ii) infant plasma B12 at 6 mo.

**2. Study Description**. Pregnant women were randomly assigned to receive one of three daily supplements throughout pregnancy. Blood samples were collected at the time of enrollment (<20 wk gestation) at 36 wk gestation, as determined by ultrasonography, as well at 6 mo postpartum. Breast milk samples were collected at 6 months postpartum. Infant blood samples were collected at 6 and 18 months postpartum. Concentrations of the main outcomes, plasma B12, folate and homocysteine were quantified by chemiluminescence for B12 and folate and high performance liquid chromatography HPLC for homocysteine. Breast milk B12 was assessed by chemiluminescence. The concentrations of B12, folate and homocysteine at each time point will be compared between groups.

**3. Hypotheses to be tested**

The primary hypotheses A1- D make 1 assumption: that there is not a significant difference between the LNS and MMN groups. If LNS and MMN treatment groups significantly differ, then we will do a 3 group comparison.

. Primary hypothesis A1: The combined MMN and LNS intervention group will have significantly higher maternal plasma B12 concentrations at 36 weeks compared to the IFA group in women in Malawi

. Primary hypothesis A2: The combined MMN and LNS intervention group will have significantly higher maternal plasma B12 concentrations at 6 months postpartum compared to the IFA group in women in Malawi

. Primary hypothesis B1: The combined MMN and LNS intervention group will have significantly lower maternal plasma homocysteine concentrations at 36 weeks gestation compared to the IFA group in women in Malawi

. Primary hypothesis B2: The combined MMN and LNS intervention group will have significantly lower maternal plasma homocysteine concentrations at 6 months postpartum compared to the IFA group in women in Malawi

. Primary hypothesis C1: The combined MMN and LNS intervention group will have significantly higher infant plasma B12 concentrations at 6 months postpartum compared to the IFA group in Malawi

. Primary hypothesis C2: The LNS intervention group will have significantly higher infant plasma B12 concentrations at 18 months postpartum, compared to the IFA and MMN groups in Malawi

. Primary hypothesis D: The combined MMN and LNS intervention group will have significantly higher breast milk B12 concentrations at 6 months postpartum compared to the IFA group in women in Malawi

. Secondary/exploratory hypothesis E: The impact of the treatment on breast milk B12 at 6 months is positively and partially mediated by the change in maternal plasma B12 between baseline and 36 wk gestation and positively and partially mediated by the change in maternal plasma B12 from 36 wk gestation to 6 months postpartum.

. Secondary/exploratory hypothesis F: The impact of the treatment on infant B12 at 6 months is positively and partially mediated by the change in maternal plasma B12 between baseline and 36 wk gestation and positively and partially mediated by breast milk B12 at 6 months postpartum.

Secondary/exploratory hypothesis G: When controlling for <20 wk plasma and 36 wk plasma, B12 in milk at 6 mo is significantly correlated with maternal plasma B12 at 6 mo, and not with maternal plasma at <20 wk or 36 wk gestation.

Secondary/exploratory hypothesis H: When controlling for maternal plasma at <20 wk gestation and at 6 months postpartum, infant plasma B12 at 6 mo is significantly correlated with maternal plasma B12 at 36 wk gestation, and not with maternal plasma at <20 wk or 6 mo postpartum.

**4. Definition of the sub study outcomes**

Outcomes

a. Concentrations of maternal plasma B12, folate and homocysteine at <20 weeks gestation, 36 wk gestation and 6 months postpartum

b. Concentrations of infant plasma B12 and folate at 6 & 18 months postpartum.

c. Breast milk B12 concentrations at 6 mo postpartum.

d. Prevalence of abnormal values of B12, folate and homocysteine at baseline, 36 wk gestation and 6 months postpartum in mothers.

e. Percent abnormal values of B12 and folate at 6 &18 months postpartum in infants.

***Cutoffs used to calculate percent of abnormal values:***

| | Mothers | | | Infants | |
|---|---|---|---|---|---|
| Biomarker | <20 wk Pregnancy | 36 wk Pregnancy | 6 mo Postpartum | 6 mo | 18 mo |
| Plasma B12 | <150 pmol/L | <100 pmol/L | <150 pmol/L | Plasma B12 <150 pmol/L | Plasma B12 <150 pmol/L |
| Plasma Folate | <10 nmol/L | <10 nmol/L | <10 nmol/L | Plasma folate <10 nmol/L | Plasma folate <10 nmol/L |
| Plasma Homocysteine Elevated | <10 μmol/L | <10 μmol/L | <10 μmol/L | | |
| Breast milk B12 Deficient | | | <362 pmol/L | | |

**5. Basis for the analysis: Intention to treat and per protocol**

The basis for the analysis will be the same as that of the primary outcomes. Subjects lost to follow-up will not contribute data to the final time point. Subjects that complete the study will be included in the analysis regardless of adherence to the study protocol.

In addition to the intention to treat analysis, a per protocol analysis will be performed including subjects meeting criteria for good adherence to study protocol. Adherence is recorded biweekly by interview of study subject and verified by collection and count of remaining intervention supplements. Good adherence will be defined as consumption on > 70% of supplement days. There is no adherence data for infants in the control group at 18 mo. As such, infant adherence at 18 mo will be based upon maternal adherence.

Furthermore, we will test for differences in adherence based upon maternal baseline characteristics.

**6. Time points for the analyses**

Biological samples will be collected at baseline (<20 wk gestation) at term, before delivery (36 wk gestation), and at 6 months postpartum in mothers and at 6 & 18 months postpartum in infants. Breast milk samples will be collected at 6 mo postpartum.

**7.Statistical software**

All statistical analyses will be performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

**8. Presentation of the study findings and hypothesis testing**

ANOVA(baseline)/ANCOVA (36wk, 6mo) or logistic regression will be conducted to evaluate whether there is a significant difference between the LNS and MMN groups for hypotheses A1-D and for maternal plasma folate. If a significant difference is found between the groups, the groups will be analyzed separately in a 3 group test, rather than a 2 group test.

For Hypotheses A1-D and for maternal plasma folate, group means and standard deviations for each time point will be presented in tables 1 (maternal) & 2 (infant). In addition, the prevalence of abnormal values for mothers at baseline, 36 weeks gestation, and 6 months postpartum and for infants at 6 and 18 mo in each intervention group will be presented in Tables 3 & 4, respectively. Both risk ratios (RR) and

adjusted odds ratios (OR) will be calculated and presented in Tables 3 and 4.

An overall ANOVA(baseline)/ANCOVA (36wk, 6mo) will be conducted to generate the p values for continuous variables, while logistic regression will be used to generate p values for categorical variables. Logistic regression will utilize adjusted OR and differences between groups will be based upon the Wald-chi squared p value. In cases where LNS & MMN groups were not combined, (see note above,) Tukey's test will be conducted to evaluate pairwise differences between the groups for ANOVA/ANCOVA tests.

Hypotheses E and F will then be examined using a path analysis and displayed in Figure 1 and Figure 2, (not shown.) The LNS and MMN groups will be combined for this analysis.

Exploratory hypotheses G and H will be examined using multiple linear regression models. A total of 4 models will be evaluated for each hypothesis: 3 2-factor models and 1 3-factor model. The 2-factor models will include the independent variables i) maternal plasma at <20 wk gestation & 36 wk gestation, ii) maternal plasma at <20 wk gestation & 6 mo postpartum, or iii) maternal plasma at <20 wk gestation & 6 mo postpartum. The 3-factor model will include maternal plasma at <20 wk gestation, 36 wk gestation, and 6 mo postpartum.

Outcome variables will be assessed for conformance to the normal distribution and transformed if needed. If no suitable transformation can be found, non parametric testing will be used.

The covariates to be included in the ANOVA, ANCOVA, and logistic regression models will be derived from the list below. Each variable that shows a statistically significant association with each outcome ($P<0.1$), will be included in the adjusted model. The difference between the three groups will be tested with ANOVA (model without covariates), ANCOVA (model with covariates), or logistic regression (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$.

**9. Description of covariates**

. a) Initial maternal B12, folate and homocysteine (baseline)

. b) Initial maternal C-reactive protein (CRP)

. c) Initial maternal alpha-1-glycoprotein (AGP)

. d) Initial maternal body mass index (BMI)

. e) Maternal malaria at baseline

. f) Maternal HIV status at baseline

. g) Parity

. h) Maternal age

. i) Season of enrollment

. j) Maternal ARV use at baseline

. k) Maternal height

. l) Maternal weight

. m) Maternal education

Table 1. Maternal plasma B12, folate and homocysteine and breast milk vitamin B12.

| Variable | Time point | IFA [n] | MMN / LNS [n] | Comparison of IFA and pooled MMN/LNS | |
|---|---|---|---|---|---|
| | | | | P-value | Difference in means (95 % CI) |
| Plasma B12 (pmol/L) (mean ( 95% CI)) [n] | Baseline | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xx | x.xx (x.xx, x.xx) |
| | 36 weeks gestation | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| Plasma folate (nmol/L) (mean ( 95% CI)) [n] | Baseline | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 36 weeks gestation | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| Plasma homocysteine (umol/L) (mean ( 95% CI)) [n] | Baseline | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 36 weeks gestation | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| Breast milk B12 (pmol/L) (mean ( 95% CI)) [n] | 6 months postpartum | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |

Table 2. Infant plasma B12 & folate

| Variable | Time point | IFA [n] | MMN / LNS [n] | Comparison of IFA and pooled MMN/LNS | |
|---|---|---|---|---|---|
| | | | | P-value | Difference in means (95 % CI) |
| Plasma B12 (pmol/L) (mean ( 95% CI)) [n] | 6 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 18 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| Plasma folate (nmol/L) (mean ( 95% CI)) [n] | 6 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 18 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| Plasma homocysteine (umol/L) (mean ( 95% CI)) [n] | 6 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |
| | 18 months | x.xx (x.xx, x.xx) [n] | x.xx (x.xx, x.xx) [n] | x.xxx | x.xx (x.xx, x.xx) |

Table 3 Proportions of women with abnormal biochemical values

| **Cutoff** | **Time point** | **IFA n (%)** | **MMN / LNS n (%)** | **Comparison of IFA and pooled MMN/LNS** | |
|---|---|---|---|---|---|
| | | | | **Risk ratio (95 % CI)** | **P-value** |
| Plasma B12 <150 pmol/L | Baseline | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma B12 <100 pmol/L | 36 wk | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma B12 <150 pmol/L | 6 months postpartum | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma folate <10 nmol/L | Baseline | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma folate <10 nmol/L | 36 wk | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma folate <10 nmol/L | 6 months postpartum | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma tHcy >10 umol/L | Baseline | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma tHcy >10 umol/L | 36 wk | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma tHcy >10 umol/L | 6 months postpartum | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |

breast milk B12 concentrations controlling for baseline B12 status, and assessing the effect of parity and maternal age as covariates. (Table to be completed later).

Table 4 Proportions of infants with abnormal biochemical values

| **Cutoff** | **Time point** | **IFA n (%)** | **MMN / LNS n (%)** | **Comparison of IFA and pooled MMN/LNS** | |
|---|---|---|---|---|---|
| | | | | **Risk ratio (95 % CI)** | **P-value** |
| Plasma B12 <150 pmol/L | 6 months | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xx |
| | 18 months | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| Plasma folate <10 nmol/L | 6 months | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |
| | 18 months | x (x.x) | x (x.x) | x.xx (x.xx, x. xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 16: The impact of intervention on maternal reproductive tract infections and malaria (version 01.0, 13 September, prepared by Minyanga Nkhoma)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 13.09.2014 | M. Nkhoma | Original document |

## 2. Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The objectives of the secondary analyses described in this appendix are to determine the effect of the intervention on maternal reproductive tract infections (candidiasis, trichomoniasis), urinary tract infections and malaria. Details of these objectives are as follows:

2.1 Effect of intervention on maternal candidiasis, trichomoniasis, urinary tract infections and malaria

a) To determine if there are differences in the prevalence of candidiasis, trichomoniasis and urinary tract infection at delivery between groups of women who received LNS, MMN or IFA.
b) To determine if differences exist in the prevalence of maternal malaria parasitemia at 32 gestation weeks (RDT), 36 gestation weeks (PCR) and at delivery (RDT and PCR) between groups of women who received LNS, MMN and IFA.

## 3. Hypotheses

3.1 The prevalence of candidiasis, trichomoniasis and urinary tract infection at delivery will be lower among women who received LNS than among women who received either IFA or MMN.

3.2 The prevalence of maternal malaria parasitemia at 32 gestation weeks (RDT), 36 gestation weeks (PCR) and at delivery (RDT and PCR) will be lower among women who received LNS than among women who received either IFA or MMN.

## 4. Definition of outcome variables

a) *Candidiasis*
Candidiasis was diagnosed from direct microscopy of vaginal fluid smear obtained at one week after delivery. These data will be extracted from *F25, Q4.3.*

b) *Trichomoniasis*
Trichomoniasis was diagnosed as the presence of viable T vaginosis protozoa from direct microscopy of vaginal fluid smear obtained at one week after delivery. These data will be extracted from *F25, Q4.2*.

c) *Urinary tract Infection*
Urinary tract infection was diagnosed as the presence of nitrite on urine dipstick analysis. These data will be extracted from *F25, Q2.8.*

d) *Malaria Parasitemia*
Malaria parasitemia was diagnosed as a positive P falciparum test on rapid diagnostic test using Clearview Malaria Combo, British Biocell International Ltd., Dundee, UK at 32 gestation weeks and at delivery. PCR was used to diagnose asymptomatic malaria at 36 gestation weeks and at delivery. These data will be extracted from *F06b, Q4.2 and F23, Q4.1* and also from laboratory result forms.

## 5. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes.

## 6. Time points for the analyses

Vaginal swabs and urine were collected at one week after delivery. Blood for asymptomatic malaria was collected at 32 and 36 gestation weeks and at delivery.

## 7. Presentation of the study findings and hypothesis testing

*7.1 Success of enrollment and follow-up*

All enrolled participants and the success of their follow-up will be described in a flow chart (figure 1) according to the CONSORT guidelines. For additional information the drop-out rate between groups will be tested with Fisher's exact test and baseline characteristics of drop-outs compared to those who completed the study will be tested with t-test or chi square. P-values for these tests will be described in the text.

*7.2 Baseline information*

Maternal characteristics at enrollment will be tabulated by study group as indicated in Table 1.

7.3 Comparisons of dichotomous outcomes in each intervention group

The proportions of mothers with reproductive tract infections (candidiasis, trichomoniasis), urinary tract infections and malaria will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups will be tested with Fisher's exact test. Pairwise comparisons between groups will be done in the context of log-binomial regression (Stata glm) if global null-hypothesis is rejected with $P<0.05$. Risk ratios between intervention groups are also presented in Table 2.

## 8. General notes on statistical methods

8.1 Software

All analyses will be done using STATA version 12

8.2 Multiple comparisons

The same as that for the primary outcome analyses.

8.3 Confidence intervals

The same as that for the primary outcome analyses.

### 8.4 Interaction and effect modification

There will be two sets of tests for interaction between the intervention group and selected other variables on their association with maternal reproductive tract infections, urinary tract infection and malaria parasitemia. All tests will be done using the likelihood ratio test.

The first set of analyses will be hypothesis-driven and will include unambiguous predefined variables that could logically modify the effect of the nutritional intervention on these outcomes. Variables included (as continuous variables where possible) in this analysis include:

1. Number of previous pregnancies
2. Age at enrollment
3. Malaria at enrollment
4. HIV at enrollment
5. Gestational age at enrollment
6. Maternal education

The second set of analyses will be exploratory in nature and will include variables that can be constructed in several ways or that cannot *a priori* be logically linked to an effect modification. Themes or variables included in this analysis include:

1. Syphilis at enrollment
2. BMI at enrollment

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as stratified by the respective predictor variable. Variables that show no interaction with the intervention group can be used as covariates in the main analysis.

### 8.5 Covariate adjustment

The covariates to be included in the logistic regression models will be derived from the list below. Each variable that shows a statistically significant association with each outcome ($P<0.1$), will be included in the model.

1. Maternal BMI at baseline
2. Malaria at baseline
3. HIV status at baseline
4. Number of previous pregnancies
5. Maternal education
6. Site of enrollment
7. Season at enrollment
8. Hb at enrollment

## 9. Legends to the figures

*Figure 1: Participant flow chart according to the CONSORT guidelines*

## 10. Figures

*Figure 1: Participant Flow Chart*

## 11. Tables

Table 1. Baseline characteristics of the participating women at enrolment, by study group

| **Characteristic** | **IFA** | **MMN** | **LNS** | **Test** |
|---|---|---|---|---|
| Number of participants | xxx | xxx | xxx | |
| Mean (SD) maternal age, years | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) maternal education, competed years at school | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) gestational age at enrolment, weeks | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) number of previous pregnancies | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) height, cm | xxx.x (xx.x) | xxx.x (xx.x) | xxx.x (xx.x) | ANOVA |
| Mean (SD) weight, kg | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) MUAC, cm | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) blood hemoglobin concentration, g/l | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number (%) of anemic women (Hb < 100 g/l) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive HIV test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive malaria test (RDT) | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |
| Number (%) of women with a positive syphilis antibody test | xxx (xx.x%) | xxx (xx.x%) | xxx (xx.x%) | Chi-squared |

Table 2. Maternal reproductive tract infections, urinary tract infection and asymptomatic malaria by intervention group

| | **Number of outcomes / Number mothers with outcome data** | | | | **Comparison between LNS and IFA group** | | **Comparison between LNS and MMN group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **IFA**<br>**N (%)** | **MMN**<br>**N (%)** | **LNS**<br>**N (%)** | **P-value** | **Risk Ratio (95 % CI)** | **P-value** | **Risk Ratio (95 % CI)** | **P-value** | **Risk Ratio (95 % CI)** | **P-value** |
| Candidiasis | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (x.xx to x.xx) | x.xxx | x.x (xx to xx) | x.xxx | x.x (xx to xx) | x.xxx |
| Trichomoniasis | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| Urinary tract infection | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| malaria parasitemia at 32 gw (RDT) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| malaria parasitemia at 36 gw (PCR) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |
| malaria parasitemia at delivery (RDT or PCR) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | xx/xxx (xx.x %) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx | x.xx (xx to xx) | x.xxx |

**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 17: Effect on breastfeeding practices from birth to six months (version 01.0, prepared by Mary Arimond, Kathryn Dewey and Jan Peerson, 16 Oct 2014)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 16.10.2014 | Arimond, Dewey Peerson | Original document, added 16 Oct 2014 |
| | | | |

## 2. Overview and study objectives

The analysis presented here is nested within a pre-existing iLiNS-DYAD-G and iLiNS-DYAD-M analysis plans for primary and other secondary outcomes. Refer to the main analysis plans for: inclusion and exclusion criteria for the trial; data cleaning protocols; procedures for breaking code; and procedures for modifying this protocol.

The main objective of data collection related to breastfeeding practices before six months of age is to compare neonatal practices and exclusive and predominant breastfeeding practices across intervention groups. Analysis will be within (not across) site.

The intervention could impact practices through affecting the mother's health and/or her perceptions of her own: health; nutritional status; or breast milk quality. All of these could impact her perception of her ability to exclusively or predominantly breastfeed her infant up to 6 months of age. The intervention could also impact breastfeeding practices through impacts on the infant (appetite, vigor and/or demand for breastfeeding).

IYCF practices we will compare across groups include: early breastfeeding practices (early initiation, use of prelacteals); exclusive and predominant breastfeeding for infants under 6 mo of age

**Specific objectives of analysis**

1.1 Primary objective

To compare specified breastfeeding practices up to 6 months of age across intervention groups.

1.2 Secondary objective

To provide additional descriptive data on breastfeeding practices to contextualize results, and to aid readers in comparing to other settings.

## 3. Hypotheses to be tested

Provision of LNS to mothers during pregnancy will increase early initiation of breastfeeding and decrease use of prelacteals during the first week after birth.

Provision of LNS to mothers during pregnancy and the first six months will increase exclusive and predominant breastfeeding during the first six months, compared to the IFA group.

## 4. Description of breastfeeding outcome variables, infants under 6 months

All outcomes are based on maternal recall of practices in response to structured survey questions.

**Planned timing of outcome assessment:**

**Ghana**

Early breastfeeding practices were assessed via survey within 1-2 days of birth (recorded on child anthropometry form) and/or on day 8 or later (recorded on "delivery details" form). Exclusive and predominant breastfeeding were assessed based on monthly visits at ~1-5 months of age (allowed up to ± 1 week of the planned visit date). In addition, data from later time points (at ~6 mo and ~9 mo) will be used for survival analyses (see outcomes, below).

**Malawi**

Early breastfeeding practices were assessed via survey either immediately after delivery (newborn details questionnaire; late collection was allowed for this form) and/or in a home visit with target timing of 7 days (± 7 days) after birth (postnatal care practices questionnaire). Exclusive and predominant breastfeeding were assessed based on four-weekly visits at 4, 8, 12, 16, 20, and 24 weeks (allowed up to ± 1 week of the planned visit date). In addition, data from later time points (at ~6 mo and ~9 mo) will be used for survival analyses.

**Outcomes:**

The original intention was to create summary variables for exclusive and predominant breastfeeding across time, to better reflect the desired practices since birth. However, this results in substantial loss of sample size in both sites and given the high proportion of missing data (59%) some outcomes will not be constructed for Malawi; several cross-sectional outcomes have been added (#3-8 below).

1. Infant breastfed immediately or within 1 hr[1] (%)
2. Infant not fed any prelacteal[2] in ~ first week (%)
3. Exclusive breastfeeding[3] at 16 weeks (Malawi) or 4 months (Ghana) (%)
4. Predominant breastfeeding at 16 weeks (Malawi) or 4 months (Ghana) (%)
5. Exclusive breastfeeding at 20 weeks (Malawi) or 5 months (Ghana) (%)
6. Predominant breastfeeding at 20 weeks (Malawi) or 5 months (Ghana) (%)
7. Exclusive breastfeeding at 24 weeks (Malawi) (%)
8. Predominant breastfeeding at 24 weeks (Malawi) (%)
9. Mean or median # time points w/exclusive breastfeeding (Ghana only)
10. Mean or median # time points w/predominant breastfeeding (Ghana only)
11. Exclusively breastfed at all 5 time points (%, Ghana only)
12. Predominantly breastfed at all 5 time points (%, Ghana only)
13. Age at first time point not reported to be exclusively breastfed (survival analysis)
14. Age at first time point not reported to be either exclusively or predominantly breastfed (survival analysis)

## 5. Approach to analysis and exclusions specific to this analysis

All tests will be two-sided, at 5% level of significance.

[1] We considered analyzing also for breastfeeding within the first 24 hours but there is little variability.

[2] The definition of prelacteals was strict; any non-breastmilk liquid or food, regardless of quantity, was considered a prelacteals (e.g in Ghana, infants (usually male) may be given a drop of lemon or lime juice; we classified this as a prelacteals).

[3] In Ghana, the gripe water is sold in sealed bottles, is recommended by and sometimes sold by clinic nurses, and is generally given in very small quantities. While we did consider this a prelacteal, for definition of exclusive breastfeeding, after consultation with the local team, given both the very small volume and the low likelihood of contamination of gripe water, we allowed gripe water under exclusive breastfeeding (i.e., treated as a "medicine"). We also allowed drops of lemon/lime juice, but note this was given in only five instances across all data collection time points (~1-5 mo) used to assess exclusive breastfeeding. In Malawi the opposite decision was taken, and gripe water was not allowed under exclusive breastfeeding because unlike in Ghana, the source, ingredients, quantity and hygiene of gripe water are highly variable. On the questionnaire, it was grouped with water and sugar water and cannot be separated.

Since varying numbers of observations are available depending on the time point (i.e., there were a substantial number of missed visits), sample sizes by group will be reported for each time point. If specific outcome variables are missing for more than 10% of infants (with denominator being total records available for the time point) we will report the number of observations used per specific outcome analysis.

Analysis will be in the first place by intention-to-treat. Data on subjects who were lost to follow-up (either temporarily or permanently) will be included in the analysis for all time points where data are available. This will be followed by a per protocol analysis, with "per protocol" as defined in the main trial analysis plans.

Data available in the DYAD-Ghana trial are divided into three "periods" based on their relationship to an error in allocation of treatments. Women in "period 1" received the same supplement throughout pregnancy, though it was not the intended supplement (reversal of MMN and IFA groups); women in "period 2" received the incorrect supplement at enrollment, but started receiving the intended supplement at some point during the pregnancy; women in "period 3" received the correct supplement throughout pregnancy and lactation. At no point was LNS confused with the two tablets (IFA and MMN).

Questions on neonatal practices were captured at two time points to minimize missing data; data will be taken at from the earliest time point available (for example, in Ghana, data on the delivery details form will be used only if the newborn anthropometry form is missing or incomplete).

For visits at 4 weeks of age and older, observations more than 14 days from the median age per visit will be excluded from all analyses (median age was very close to target age for these time points in each site).

Twins are excluded from all analyses of breastfeeding outcomes under 6 months of age.

## 6. Statistical methods

### 5.1 Software

All analyses will be done using SAS version 9.3 (SAS Inst. Cary, NC, USA) or Stata version 10.1 or higher (StataCorp, TX, USA).

### 5.2 Background characteristics

Selected background characteristics will be examined by group for analysis samples.

### 5.3 Analysis of the effect of the intervention

General comments:

Analysis of the effect of the intervention will follow these steps:

a. In Ghana only, we will test group-by-period interactions for each outcome. In the absence of group-by-period interactions, observations from participants in all periods will be included in the analysis, and analysis will be performed both for groups as allocated (reflecting the supplement received during early lactation up to six months post-partum) and for groups based on first supplement received. If there are significant group-by-period interactions for a specified outcome, period 3 data only will be used for that outcome.

b. In each site, we will assess pre-specified covariates (see below) for relationship to each outcome.

c. We will test the null hypothesis of no difference among the three treatment groups using ANCOVA or logistic regression, with and without controlling for significant covariates.

d. If the global null hypothesis is rejected at 0.05 level for any outcome, then we will perform post-hoc pairwise comparisons of all three groups using appropriate adjustments for multiple comparisons to examine contrasts of interest.

e. The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Each interaction will be assessed separately, in models including all significant covariates.

f. Significant interactions ($p < 0.10$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification.

g. Confidence intervals will be adjusted for multiple comparisons.

5.5 Covariates in main effects models

In theory, a variety of community-, household-, maternal-, and child-level characteristics could affect child feeding practices independently of the intervention. Data are available for the covariates listed below.

All covariates are as measured at baseline, with the exception of season, and child sex and age. Season of measurement is included in models for outcomes 3-8 as it is conceptualized to impact ease of exclusive/predominant breastfeeding through impacting women's workload. Since child age at each visit can vary (see exclusions above) and since feeding practices change rapidly in early infancy, child age at time of measure will be included in models for cross-sectional outcomes numbers 3-8.

Before making final decisions on inclusion of covariates, completeness of data for the covariates will be considered and covariates will be excluded if loss of sample size is judged too large.

- Enrollment site (Malawi only)
- Season of measurement (cross-sectional outcomes 3-8 only)
- Characteristics of households
    - Baseline HH asset score
    - Baseline HH food security (HFIA score)
- Characteristics of mother
    - BMI[4]
    - Age
    - Parity (dichotomous – any previous live birth, or none)
    - Education
    - HIV status (Malawi only)
- Child's characteristics
    - Child age (cross-sectional outcomes 3-8 only)
    - Child sex

5.6 List of potential effect modifiers to be examined

With the exception of study site and child age, the covariates identified will also be evaluated for their potential to interact with intervention group.

[4] Predicted BMI at 13.7 wk of gestation, for Malawi; BMI at enrollment for Ghana, because baseline BMI was not related to gestational age at enrollment (R-squared = 0.007)

## 7. Design of tables and figures

The following tables and example figures will be examined by the manuscript writing group:

Table 1. Background characteristics of study participants

Table 2. Breastfeeding practices, by intervention group

Figure 1. Participant flow

Additional figures to illustrate results from survival analysis, and, as needed, to illustrate interactions.

**Table 1. Example table for background characteristics of study participants in analysis sub-sample (possibly, separate tables per outcome)[a,b]**

| | | **IFA N = XXX** | **MMN N = XXX** | **LNS N = XXX** | **p-value[c]** |
|---|---|---|---|---|---|
| Site (%) | | | | | |
| | Lungwena | | | | |
| | Malindi | | | | |
| | Mangochi | | | | |
| Season of measure (%) | | | | | |
| | (Describe) | | | | |
| | (Describe) | | | | |
| | Etc. | | | | |
| Asset index (mean) | | | | | |
| HFIA score (mean) | | | | | |
| Mother's BMI | | | | | |
| Mother's age (y) | | | | | |
| Primigravid at enrollment (%) | | | | | |
| Mother's education (y) | | | | | |
| Mother HIV positive (%) | | | | | |
| Child male (%) | | | | | |
| Child age at *[X visit]* (mo) | | | | | |

[a] *[Will evaluate how different the sub-samples comparisons are for various outcomes, and decide how to handle in presenting results. If the comparisons are similar across outcomes, we will select one to present and note that others are similar.]*

[b] IFA=iron folic acid group (standard care); MMN=multiple micronutrient group; LNS=lipid-based nutrient supplement group.

[c] Comparison between intervention groups; p-value for ANOVA (continuous and quasi-continuous variables) or chi-square test (categorical variables).

**Table 2. Breastfeeding practices, by intervention group**

| | N[a] | (missing) | IFA[b] | MMN | LNS | All | P-value[c] |
|---|---|---|---|---|---|---|---|
| Infant breastfed immediately or within 1 hr (%) | | | | | | | |
| Infant not fed any prelacteal in ~ first week (%) | | | | | | | |
| Exclusive breastfeeding at 16 weeks (Malawi) or 4 months (Ghana) (%) | | | | | | | |
| Predominant breastfeeding at 16 weeks (Malawi) or 4 months (Ghana) (%) | | | | | | | |
| Exclusive breastfeeding at 20 weeks (Malawi) or 5 months (Ghana) (%) | | | | | | | |
| Predominant breastfeeding at 20 weeks (Malawi) or 5 months (Ghana) (%) | | | | | | | |
| Exclusive breastfeeding at 24 weeks (Malawi) (%) | | | | | | | |
| Predominant breastfeeding at 24 weeks (Malawi) (%) | | | | | | | |
| Mean or median # time points w/exclusive breastfeeding (Ghana only) | | | | | | | |
| Mean or median # time points w/predominant breastfeeding (Ghana only) | | | | | | | |
| Exclusively breastfed at all 5 time points (%, Ghana only) | | | | | | | |
| Predominantly breastfed at all 5 time points (%, Ghana only) | | | | | | | |

[a] Number of infants not permanently lost to follow-up at time of measure for each outcome, and at final time of measure for outcomes summarized across time.

[b] IFA=iron folic acid group (standard care); MMN=multiple micronutrient group; LNS=lipid-based nutrient supplement group.

[c] Values presented are unadjusted means (SD) or medians (I-Q ranges), or prevalence. Decision on presenting means or medians will be made after examination of distributions. Statistical tests are for adjusted analyses; analysis of covariance and logistic regression, controlling for…..

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 18: Developmental milestones (version 01.0, prepared by Elizabeth Prado, 14 Dec, 2014)

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| | |
|---|---|
| Version number | 01.0 |
| Version date | December 14, 2014 |
| Author | Elizabeth Prado |
| Implementation date of current version | |

**Version History Log**

This table will detail the version history for this document. It will detail the key elements of the changes to the versions.

| **Version** | **Date implemented** | **Details of significant changes** |
|---|---|---|
| | | |
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## 1. Study objectives

The main aim of the trial was to determine whether LNS consumed by the mother during pregnancy and the first 6 months of lactation, and by the child from age 6-18 months, improves foetal and child growth, micronutrient status and neurobehavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The aim of the analyses described in this addendum is to compare infants in 3 different intervention groups:

a) Daily iron and folic acid during pregnancy, and calcium (Ca) only (akin to a placebo) during the first 6 months postpartum, with no supplementation for offspring during infancy
b) Daily multiple micronutrients (1-2 RDA of 18 vitamins and minerals) during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy
c) Daily LNS during pregnancy and the first 6 months postpartum (LNS-P&L with similar vitamin and mineral content as the daily multiple micronutrients, plus Ca, P, K, Mg and essential fatty acids), with LNS for offspring (LNS-20gM with 22 vitamins and minerals with concentrations based on RNIs for infants) during infancy

on the following outcomes:

1) the timing of the acquisition of certain developmental milestones, monitored monthly from birth through 18 months of age
2) the proportion of children who had achieved certain motor milestones by 6, 12, and 18 months of age.

## 2. Hypotheses to be tested

1. The timing of developmental milestone acquisition of infants whose mothers were provided with LNS during pregnancy and who were provided with LNS from 6 to 18 months of age will be earlier than that of infants of mothers who received either iron-folate or multiple micronutrient supplementation. A secondary analysis will also test the difference between the MMN and IFA groups.

2. The proportion of children who had achieved motor milestones at 6, 12, and 18 months of age will be higher in infants provided with LNS during pregnancy and from 6 to 18 months of age as compared to infants of mothers who received either iron-folate or multiple micronutrient supplementation. A secondary analysis will also test the difference between the MMN and IFA groups.

## 3. Definition of the 18-month developmental outcomes

### 3.1 Timing of milestone acquisition

The following milestones were monitored monthly by interview with a caregiver:

Pronouncing single words
Waving goodbye
Drinking from a cup
Eating by self
Running
Walking alone
Standing alone
Walking with assistance
Hands and knees crawling
Standing with assistance
Sitting without support
Shouts for attention
Sits with slight support
Friendly to strangers
Laughs aloud
Smiles
Visually recognizes mother
Startled by sound

A milestone is considered to be achieved when the child was recorded to have achieved the skill on two consecutive visits. We assume that the child acquired the skill before the first of these two visits, so the age of acquisition is the mean age between the first of the two consecutive visits and the previous visit on which the child had not yet achieved the skill.

We will use right censoring if the child was not recorded to have achieved the milestone by the last visit during the intervention period.

### 3.2 Proportion of children who had achieved milestones at 6, 12, and 18 months of age

The following milestones were assessed at 6, 12, and 18 months of age.

Running
Walking alone
Standing alone
Walking with assistance
Hands and knees crawling
Standing with assistance
Sitting without support

We will include all children for which these milestones were assessed at the target age plus or minus one month. Thus for milestones assessed at 6 months of age, we will include children assessed at age 5.0 to 7.0 months, for milestones assessed at 12 months of age, we will include children assessed at age 11.0 to 13.0 months, for milestones assessed at 18 months of age, we will include children assessed at age 17.0 to 19.0 months. The outcome is a binary variable indicating whether or not the child had achieved the milestone at that age.

## 4. Basis for the analysis: Intention to treat and per protocol

The basis for the analysis will be the same as that for the primary outcomes. In addition to the intention to treat analysis, we will also perform a per protocol analysis by examining the effect of the intervention in participants with self-reported high adherence. The cut-off to define high adherence will be determined to be consistent with the analyses on birth outcomes and growth.

## 5. Presentation of the study findings and hypothesis testing

### 5.1 Timing of milestone acquisition

We will use the survival analysis procedure in SAS (PROC LIFEREG) to estimate the means for normally distributed variables and geometric means for skewed variables. This method takes censoring into account in the calculation of the means and geometric means. The means or geometric means and standard deviations for each milestone will be presented as indicated in Table 1. The results of pairwise comparisons will be indicated by superscripts. Means that are significantly different from each other will be marked by different letters (e.g., a and b). Means that are not significantly different from each other will be marked by the same letter.

The analysis will begin with testing the null hypothesis of no difference between the three treatment groups using survival analysis by SAS PROC LIFEREG and controlling for pre-specified covariates (see below).

If the global null hypothesis is rejected at p=0.05 level, then we will perform pairwise comparisons of all three groups using Tukey-Kramer adjustment. We will also use Scheffe's test to assess whether the LNS group differs from the non-LNS groups.

### 5.2 Proportion of children who had achieved milestones at 6, 12, and 18 months of age

The proportion of children who had achieved each milestone by 6, 12, and 18 months in each group will be presented as shown in Table 2. Proportions that are significantly different from each other will be marked by different letters (e.g., a and b). Proportions that are not significantly different from each other will be marked by the same letter.

We will use logistic regression to test the null hypothesis of no difference between the three treatment groups. If the global null hypothesis is rejected at p=0.05 level then we will calculate odds ratios and/or relative risk for the differences between groups.

## 6. General notes on statistical methods

### 6.1 Software

SAS for Windows Release 9.4 (Cary, NC) will be used for all analyses.

### 6.2 Multiple comparisons

The Tukey-Kramer adjustment method is used.

### 6.3 Confidence intervals

The same as that for the primary outcome analyses.

### 6.4 Interaction and effect modification

We will examine the same factors as that for the primary outcome analyses. In addition, we will examine the following effect modifiers:

1. Family care indicators z-score
2. Household Food Insecurity Access (HFIA) Index, adjusted for season

### 6.5 Covariate adjustment

For the first hypothesis, two models will be estimated:

1. No covariate adjustment
2. Adjustment for any of the variables presented in Table 1 of the primary outcome Statistical Analysis Plan (SAP) showing statistically significant association (at $p<0.1$ level) with the age of acquisition of the milestone

For the second hypothesis, three models will be estimated:

1. No covariate adjustment
2. Adjustment for child age at assessment
3. Adjustment for child age at assessment and any of the variables presented in Table 1 of the primary outcome Statistical Analysis Plan (SAP) showing statistically significant association (at $p<0.1$ level) with the achievement of the milestone

In addition to the variables in Table 1 of the primary outcome SAP, we will consider the following variables for inclusion:

1. Child sex
2. Household Food Insecurity Access (HFIA) Index, adjusted for season
3. Season at enrolment
4. Number of persons in the household
5. Children < age 5 years in the household
6. Family care indicators score, if this score is not different between supplement groups.

## Tables

*Table 1*. Geometric mean of age of achievement of motor, language, and personal-social milestones in each supplement group

| | IFA | MMN | LNS | | | LNS vs MMN | | LNS vs IFA | | MMN vs IFA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean or Geometric Mean (SD)* | *Mean or Geometric Mean (SD)* | *Mean or Geometric Mean (SD)* | *p-value for the difference between the 3 trial groups* | *Covariate-adjusted p-value for the difference between the 3 trial groups* | *Difference (95% CI)* | *p-value* | *Difference (95% CI)* | *p-value* | *Difference (95% CI)* | *p-value* |
| Pronouncing single words | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[b] | | | | | | |
| Waving goodbye | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[c] | | | | | | |
| Drinking from a cup | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[d] | | | | | | |
| Eating by self | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[e] | | | | | | |
| Running | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[f] | | | | | | |
| Walking alone | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[g] | | | | | | |
| Standing alone | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx[a] | x.xx[h] | | | | | | |
| Walking with assistance | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | | | | | | | | |
| Hands and knees crawling | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | | | | | | | | |
| Standing with assistance | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | | | | | | | | |
| Sitting without support | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | | | | | | | | |
| Shouts for attention | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | | | | | | | | |

| | | | |
|---|---|---|---|
| Sits with slight support | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |
| Friendly to strangers | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |
| Laughs aloud | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |
| Smiles | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |
| Visually recognizes mother | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |
| Startled by sound | x.xx<br>(x.xx) | x.xx<br>(x.xx) | x.xx<br>(x.xx) |

***$p < 0.001$

[a]Adjusted for child age at developmental assessment.

[b]Adjusted for child age and ….

[c]Adjusted for child age and ….

[d]Adjusted for child age and ….

[e]Adjusted for child age and ….

[f]Adjusted for child age and ….

[g]Adjusted for child age and ….

*Table 2*. Proportion of children in each group who had achieved motor milestones by 6, 12, and 18 months of age

| | IFA | MMN | LNS | *p-value for the difference between the 3 trial groups* |
|---|---|---|---|---|
| | *n/total (%)* | *n/total (%)* | *n/total(%)* | |
| Sitting without support | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Hands and knees crawling | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Standing with assistance | | | | |
| 6 months | xx/xxx (xx%) | xxx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Walking with assistance | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Standing alone | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Walking alone | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| Running | | | | |
| 6 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 12 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |
| 18 months | xx/xxx (xx%) | xx/xxx (xx%) | xx/xxx (xx%) | x.xx |

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 19: Effect of lipid-based nutrient supplements on infant and young child feeding practices at age 18 months (version 01.0, prepared by Mary Arimond, Kathryn Dewey, Janet Peerson, Souheila Abbeddou, Harriet Okronipa, and Chiza Kumwenda, 20 Dec, 2014)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 20.12.2014 | Arimond, Dewey, Peerson, Abbeddou, Okronipa, Kumwenda | Original document added 20 Dec 2014.<br><br>This cross-site analysis plan replaces earlier single site plans for iLiNS-DOSE and iLiNS-DYAD-Ghana. Relative to the earlier plans, the analytic approach is the same, but changes are:<br><br>Analysis is restricted to endline and per protocol analysis is dropped;<br><br>Details of variable construction are dropped and are captured elsewhere in codebooks;<br><br>Covariate selection is harmonized with Burkina site (addition of several candidate co-variates);<br><br>Presentation of results is harmonized across sites. |
| | | | |

## 2. Overview and study objectives

The analysis presented here is nested within a pre-existing iLiNS analysis plans for primary and other secondary outcomes. Refer to the four main analysis plans (iLiNS-DOSE (Malawi), iLiNS-ZINC (Burkina Faso), iLiNS-DYAD-Ghana, iLiNS-DYAD-Malawi) for: inclusion and exclusion criteria for the trial; data cleaning protocols; procedures for breaking code; and procedures for modifying this protocol.

The main objective of data collection related to infant and young child feeding (IYCF) practices at 18 months is to compare a range of practices across intervention groups. Analysis will be within (not across) sites but a cross-site manuscript will be prepared.

This analysis is motivated by concerns that energy-dense LNS may displace breastfeeding and/or nutrient-dense local foods and/or impede dietary diversification with local foods, thus negatively impacting infant feeding practices and development of infant dietary preferences and habits. Effects on IYCF practices could be mediated either by maternal perceptions of different needs

for breast milk or local foods for infants receiving supplements and/or by a change in appetite, demand for breastfeeding, or preference for local foods among infants who consume the supplement.

IYCF practices to be compared across groups include: continued breastfeeding, frequency of breastfeeding, frequency of feeding solid/semi-solid foods[1], consumption of nutrient-dense food groups yesterday and last week, and food group diversity yesterday. We also assessed consumption of other fortified products (other than the project LNS) but preliminary analysis showed that consumption of such products was very rare in two of three sites, so this is not included as an outcome in this analysis.

**Specific objectives of analysis**

1.1 Primary objective

To compare infant and young child feeding practices and summary diet quality variables across intervention groups.

1.2 Secondary objectives

To provide descriptive data on IYCF practices to contextualize results of the trials, and to aid readers in comparing to IYCF in other settings

## 3. Hypotheses to be tested

Stated qualitatively: Provision of LNS would not impact infant and young child feeding practices. More specifically, provision of LNS would not cause a change in:

- Breastfeeding (prevalence of any breastfeeding, reported frequency of breastfeeding the previous day)
- Frequency of feeding other solids foods (meals and snacks, or feeding episodes);
- Dietary diversity yesterday measured as food group diversity at or above the WHO cut-off[2];
- Number of nutrient-dense food groups (animal-source foods, fruits and vegetables) consumed yesterday or last week

[1]Frequency of feeding data are available for iLiNS-ZINC (Burkina Faso) and iLiNS-DYAD-Malawi only.

[2] WHO (2008) Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA.

## 4. Timing and description of IYCF outcome variables at 18 months (endline)

All outcomes are based on caregiver recall of practices in response to structured survey questions. Dietary diversity and consumption of nutrient-dense food groups were assessed through a guided free recall of foods consumed by the child yesterday, and a list-based recall of the number of days food groups were consumed in the 7 days preceding the interview.

This analysis is of endline data in all study sites, with a common target age of 18 months.

Tolerance for late endline visits varied by site and study, and child age (expressed as a deviation from the site-specific median age for endline) will be included as a potential covariate in all sites.

In Malawi, late endline visits were allowed, to minimize loss to follow-up for primary outcomes. For this analysis, data are excluded if collected more than 28 days early or more than 42 days late. The rationale for allowing a wide tolerance is that feeding practices may change more slowly by 18 months of age compared to earlier time points. Also, in Malawi the endline clinic visit was planned to occur exactly one year after the date of enrollment. However, so long as it occurred within one month of the target date, the child received a 2 week supply of LNS. So, if the endline FFQ occurred within ~6 weeks (42 days) of target, the child should have had LNS in the week prior to the FFQ.

In Ghana, IYCF practices data were usually measured within a week of the infant turning 18 months of age. In instances where the mother/caregiver travelled, field workers were allowed to complete data collection up to one month from the scheduled date. This was rare at endline and there are no exclusions in Ghana.

In Burkina Faso, the target for endline visits was 39 +/- 2 weeks, after enrollment, with enrollment at age ~9 months. In special cases, early endline visits were allowed from 35 weeks onward (e.g. if the family planned to travel) and late visits were allowed up to 43 weeks after enrollment; as in Malawi, in these cases the child continued to receive LNS until the endline visit where IYCF practices were surveyed.

**Outcomes:**

1. Infant still breastfed at 18 months (%)
2. Infant breastfed 6 or more times yesterday (%)

3. Frequency of feeding adequate (WHO indicator, %)[3]
4. 4 or more food groups yesterday (WHO indicator; %)
5. Mean or median # ASF groups yesterday (of 5)[4]

[3] Burkina and DYAD-Malawi only.

[4] The 5 ASF groups are: 1) organ meats; 2) other meat/poultry; 3) fish; 4) eggs; and 5) dairy.

6. Mean or median # fruit/vegetable groups yesterday (of 5)[5]

7. Mean ASF score, last seven days (of 28)[6]
8. Lowest ASF score tertile (%)
9. Mean fruit/vegetable score, last seven days (of 35)[7]
10. Lowest fruit/vegetable score tertile (%)

## 5. Approach to analysis and exclusions specific to this analysis

All tests will be two-sided, at 5% level of significance.

If specific outcome variables are missing for more than 10% of infants (with denominator being total records available for the time point) we will report the number of observations used per specific outcome analysis.

Analysis will be by intention-to-treat.

Observations outside the tolerances for visit timing stated above will be excluded from analysis. There will be no other exclusions.

Note: Data available in the DYAD-Ghana trial are divided into three "periods" based on their relationship to an error in allocation of treatments. Women in "period 1" received the same supplement throughout pregnancy, though it was not the intended supplement (reversal of MMN and IFA groups); women in "period 2" received the incorrect supplement at enrollment, but started receiving the intended supplement at some point during the pregnancy; women in "period 3" received the correct supplement throughout pregnancy and lactation. At no point was LNS confused with the two tablets (IFA and MMN).

Note: For iLiNS-DOSE, comparisons will be made across groups receiving different quantities of LNS, but "milk" and "no milk" LNS of the same quantity (20g and 40g) will be grouped together.

[5] The 5 fruit and vegetables groups are: 1) vitamin A-rich orange/yellow vegetables; 2) dark green leafy vegetables; 3) other vegetables; 4) vitamin A-rich fruits; and 5) other fruits.

[6] Score sums four groups over seven days; groups are similar to those for yesterday, but organ meats and other flesh foods are grouped together.

[7] Score sums five groups over seven days; groups are the same as those for yesterday.

## 6. Statistical methods

5.1 Software

All analyses will be done using SAS version 9.3 (SAS Inst. Cary, NC, USA) or Stata version 10.1 or higher (StataCorp, TX, USA).

5.2 Background characteristics and loss to follow-up

Selected background characteristics will be examined for the analysis sample compared to those lost to follow-up. Differential attrition will be assessed with chi-square tests. The same background characteristics will be examined by group for analysis samples.

5.3 Analysis of the effect of the intervention

General comments:

a. The hypothesis stated in section 3 is a non-equivalence hypothesis. However, the study was not powered for IYCF practices outcomes and we are severely underpowered for equivalence analyses, particularly for dichotomous outcomes which comprise the majority of outcomes in this analysis. Therefore the more traditional approach in the nutrition literature of analyzing for significant differences will be followed in the first instance. This limitation will be clearly explained in the discussion section of any publication.

b. For quasi-continuous variables, we will supplement this with an equivalence approach to hypothesis testing, to help inform conclusions from this analysis (see below).

Analysis of the effect of the intervention will follow these steps:

a. In Ghana only, we will test group-by-period interactions for each outcome. In the absence of group-by-period interactions, observations from participants in all periods will be included in the analysis, and analysis will be performed both for groups as allocated (reflecting the supplement received during early lactation up to six months post-partum) and for groups based on first supplement received. If there are significant group-by-period interactions for a specified outcome, period 3 data only will be used for that outcome.

b. We will check for collinearity by running models with all covariates (see below) and examining variance inflation factors (VIF). VIF above 10 are problematic and one or more covariates will be removed after discussion of which to drop; this decision can be made considering subject matter and/or data constraints (e.g. number of missing values per covariate).

c. In each site, we will assess pre-specified covariates (see below) for relationship to each outcome. Covariates significantly associated with an outcome (criterion: $p <$

0.10) will be included in models for that outcome; final covariates can vary by outcome.

d. We will test the null hypothesis of no difference among the three treatment groups using ANCOVA or logistic regression, with and without controlling for significant covariates.

e. If the global null hypothesis is rejected at 0.05 level for any outcome, then we will perform post-hoc pairwise comparisons of all three groups (Ghana) or four groups (Malawi DOSE) using appropriate adjustments for multiple comparisons to examine contrasts of interest.

f. In the DYAD trials, if the IFA and MMN groups are not different for a specific outcome, we will test differences between LNS and non-LNS groups (IFA and MMN, grouped together) (using all data from periods 1-3 in Ghana). In iLiNS-DOSE, if the global null hypothesis and pairwise comparisons by dose are not significant, we will test differences between all LNS groups (together) and non-LNS group.

g. The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Each interaction will be assessed separately, in models including all significant covariates. In DYAD and DOSE trials, if two-group comparisons are tested under f., effect modifiers will be tested within two-group models only.

h. Significant interactions ($p < 0.05$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification.

i. Confidence intervals will be adjusted for multiple comparisons.

j. Equivalence analysis: For quasi-continuous outcomes (number of time points with exclusive or predominant breastfeeding; number of fruit/vegetable groups consumed yesterday; number of animal-source food groups consumed yesterday, and fruit/vegetable and animal-source food scores for last week) equivalence will be assessed based on defined margins. Margins for food groups yesterday will be ±1.0 (one more or one fewer fruit/vegetable group yesterday; one more or one fewer animal-source food group yesterday). For scores for last week, the margin will be ±4 points (~= a difference of one fruit/vegetable or animal-source food group on four or more of the last seven days). We will assess equivalence in the context of ANCOVA models, controlling for the same pre-specified covariates as noted above. Equivalence will be determined to exist if the 90% confidence

interval for the difference between the means is entirely contained within the negative and the positive values of the equivalence margin. In DYAD and DOSE trials, if two-group comparisons are tested under f., equivalence testing will be between two groups.

5.5 Covariates in main effects models

In theory, a variety of community-, household-, maternal-, and child-level characteristics could affect child feeding practices independently of the intervention. Data are available for the covariates listed below.

All covariates are as measured at baseline, with the exception of season, and child sex (DYAD studies) and age. Season of measurement is included due to potential seasonal effects on access to diverse foods (and through this, on feeding practices).

Since child age at each visit can vary (see exclusions above), child age at time of measure (deviation from median age at endline) will be included as a potential covariate in models for all outcomes.

Before making final decisions on inclusion of covariates, completeness of data for the covariates will be considered and covariates will be excluded if loss of sample size is judged too large.

- Enrollment site (DYAD-Malawi only)
- Season of measurement (definition of season varies by site)
- Characteristics of households
    - Distance to nearest weekly market, in meters[8]
    - Baseline HH asset score[9]
    - Baseline HH small livestock score[10]
    - Baseline HH food security category from HFIAS[11]

[8] For continuous covariates highly skewed in one or more sites, we will test for trend by grouping the variables in 4 groups, and assigning the group median as the value for a new variable for each case in the group. This new variable will be the covariate. The grouping rule will be quartiles where possible; in cases where there is very heavy lumping on "0", the first group will be all 0's and groups 2-4 will be tertiles among the non-zero values.

[9] As above, for distance to market.

[10] As above.

[11] Categories as in Coates et al., 2007.

  - Number of under-fives in the HH at baseline (categorical variable, differing by site)[12]

- Characteristics of mother
  - BMI[13]
  - Age
  - Education (categorical variable, differing by site)
  - HIV status (DYAD-Malawi only; unknown in DOSE and ZINC; excluded if known to be positive in DYAD-Ghana)
  - Ethnicity or language (categorical variable, differing by site)
  - Marital status (categorical variable, differing by site)
- Child's characteristics
  - Child age (deviation from median age at endline)
  - Child sex
  - HAZ at baseline (DOSE and ZINC only)[14]
  - WHZ at baseline (DOSE and ZINC only)

5.6 List of potential effect modifiers to be examined

Most of the covariates identified will also be evaluated for their potential to interact with intervention group; exceptions are distance to market, maternal BMI, and child age deviation.

[12] A high proportion of data are missing for DYAD-Malawi, and parity at baseline (nulliparous Y/N) will be used instead.

[13] In DYAD studies: will use predicted BMI at 13.7 wk of gestation, for Malawi; BMI at enrollment for Ghana, because baseline BMI was not related to gestational age at enrollment (R-squared = 0.007).

[14] As DYAD interventions began antenatally, there are no baseline values for infant anthropometry.

## 7. Design of tables and figures

Tables will vary slightly by site reflecting different study designs and covariate categories. The tables listed below will be examined by the manuscript writing group, and final decisions on how to best consolidate results across sites for presentation in a manuscript will follow later.

Table 1. Comparison of analysis sample to those lost to follow-up

Table 2. Comparison of baseline characteristics and two concurrent covariates, by intervention group

Table 3. Associations of covariates to outcomes

Table 4. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values[15]

Figure 1. Participant flow

Additional Tables and Figures as needed to describe or illustrate interactions. For the manuscript, Table 4 may be supplemented by selected descriptive Figures for cross-site comparison of outcomes.

On the following pages, Tables show an example format from the DYAD-Ghana design.

[15] For DYAD-Ghana, this table will be presented for groups as assigned, groups based on first type of supplement received, and potentially also for a two-group comparison (LNS vs. non-LNS). For DYAD-Malawi, this will be presented for three groups and potentially also for a two-group comparison (LNS vs. non-LNS). For ZINC, this table will show a two-group comparison. For DOSE, this table will show a four-group comparison (0, 10, 20, and 40 g LNS groups) and potentially also a two-group comparison (LNS vs. non-LNS).

**Table 1. Comparison of analysis sample to those lost to follow-up**

| | | **Lost to follow-up** n= | | **In analysis sample** n= | | **All enrolled** n= | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean/% | SD | Mean/% | SD | Mean/% | SD | P-value | Test |
| **Distance to market** | Score[a] | | | | | | | | Chi-sq |
| **Asset index** | Score[a] | | | | | | | | Chi-sq |
| **Small livestock score** | Score[b] | | | | | | | | Chi-sq |
| **HFIA category (%)** | | | | | | | | | Chi-sq |
| | Food secure | | | | | | | | |
| | Mildly food insecure | | | | | | | | |
| | Moderately food insecure | | | | | | | | |
| | Severely food insecure | | | | | | | | |
| **Other U5 at baseline (%)** | Yes | | | | | | | | Chi-sq |
| **Maternal education (%)** | | | | | | | | | Chi-sq |
| | None | | | | | | | | |
| | Some 1° (1-5 y) | | | | | | | | |
| | Completed 1°, some 2° (6-8 y) | | | | | | | | |
| | Upper 2° (9-11 y) | | | | | | | | |
| | Completed 2° or more (12+ y) | | | | | | | | |
| **Maternal age (y)** | | | | | | | | | ANOVA |
| **Maternal BMI (kg/m2)** | | | | | | | | | ANOVA |
| **Language spoken in HH (%)** | | | | | | | | | Chi-sq |
| | Krobo/Ga | | | | | | | | |
| | Ewe | | | | | | | | |
| | Other | | | | | | | | |
| **Marital status (%)** | Married | | | | | | | | Chi-sq |
| **Child sex (%)** | Male | | | | | | | | Chi-sq |

[a] Source variables were highly skewed. Quartiles were created and the median score for the quartile was assigned to all households within each quartile.

[b] The source variable was heavily lumped on "0" and highly skewed. Four groups were created for zero, and tertiles of none-zero values. The median score for the group was assigned to all households in the group (0, or tertile medians).

**Table 2. Comparison of baseline characteristics and two concurrent covariates, by intervention group (as assigned)**

| | | **IFA** n= | | **MMN** n= | | **LNS** n= | | **ALL** n= | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean/ % | SD | Mean/ % | SD | Mean/ % | SD | Mean/ % | SD | P-value | Test |
| **Season of interview (%)** | Dry season | | | | | | | | | | Chi-sq |
| **Distance to market** | Score[a] | | | | | | | | | | Chi-sq |
| **Asset index** | Score[a] | | | | | | | | | | Chi-sq |
| **Small livestock score** | Score[b] | | | | | | | | | | Chi-sq |
| **HFIA category (%)** | | | | | | | | | | | Chi-sq |
| | Food secure | | | | | | | | | | |
| | Mildly food insecure | | | | | | | | | | |
| | Moderately food insecure | | | | | | | | | | |
| | Severely food insecure | | | | | | | | | | |
| **Other U5 at baseline (%)** | Yes | | | | | | | | | | Chi-sq |
| **Maternal education (%)** | | | | | | | | | | | Chi-sq |
| | None | | | | | | | | | | |
| | Some 1° (1-5 y) | | | | | | | | | | |
| | Completed 1°, some 2° (6-8 y) | | | | | | | | | | |
| | Upper 2° (9-11 y) | | | | | | | | | | |
| | Completed 2° or more (12+ y) | | | | | | | | | | |
| **Maternal age (y)** | | | | | | | | | | | ANOVA |
| **Maternal BMI (kg/m2)** | | | | | | | | | | | ANOVA |
| **Main language in HH (%)** | | | | | | | | | | | Chi-sq |
| | Krobo/Ga | | | | | | | | | | |
| | Ewe | | | | | | | | | | |
| | Other | | | | | | | | | | |
| **Marital status (%)** | Married | | | | | | | | | | Chi-sq |
| **Child sex (%)** | Male | | | | | | | | | | Chi-sq |
| **Age deviation (d)** | | | | | | | | | | | ANOVA |

[a] Source variables were highly skewed. Quartiles were created and the median score for the quartile was assigned to all households within each quartile.

[b] The source variable was heavily lumped on "0" and highly skewed. Four groups were created for zero, and tertiles of none-zero values. The median score for the group was assigned to all households in the group (0, or tertile medians).

**Table 3. Associations of covariates to outcomes**

| | | stillbf | bf_6 | dd24GE4 | asf24sum | asf24sum | frveg24sum | frveg24sum | asf7sum | frveg7sum | i18_asfT1 | i18_frvegT1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | logit | logit | logit | ANOVA | OLOGIT | ANOVA | OLOGIT | ANOVA | ANOVA | logit | logit |
| **Season of interview** | dryseas | | | | | | | | | | | |
| **Distance to market** | distance | | | | | | | | | | | |
| **Asset index** | asset | | | | | | | | | | | |
| **Small livestock score** | smlivestock | | | | | | | | | | | |
| **HFIA category** | hfia_cat | | | | | | | | | | | |
| **Other U5 at baseline** | other_u5yn | | | | | | | | | | | |
| **Maternal education** | schooling | | | | | | | | | | | |
| **Maternal age** | moth_age | | | | | | | | | | | |
| **Maternal BMI** | mombmi | | | | | | | | | | | |
| **Main language in HH** | HHlanguage | | | | | | | | | | | |
| **Marital status** | marital | | | | | | | | | | | |
| **Child sex** | male | | | | | | | | | | | |
| **Age deviation** | age_dev | | | | | | | | | | | |

P-values are from simple bivariate ANOVA or logit or ologit models. Covariates included in models testing for effect of group are:

Still breastfed (not reported fully weaned) (for each outcome, list covariates significant at P < 0.10)
Breastfed 6+ times/yesterday
4+ food groups yesterday (WHO indicator)
# ASF food groups yesterday, range 0-5
# fruit/veg food groups yesterday, range 0-5
ASF score last 7 d, range 0-28
Fruit/vegetable score last 7 d, range 0-35

Lowest tertile for 7 d ASF score

Lowest tertile for 7 d fruit/vegetable score

**Table 4a. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values - groups as assigned[a]**

| | IFA (n=) | | MMN (n=) | | LNS (n=) | | ALL (n=) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Proportion or mean | SD | Proportion or mean | SD | Proportion or mean | SD | Proportion or mean | SD | Unadjusted p-value[b] | Adjusted p-value[c] |
| Still breastfed | | | | | | | | | | |
| Breastfed 6+ times/yesterday | | | | | | | | | | |
| 4+ food groups yesterday (WHO)[d] | | | | | | | | | | |
| # ASF food groups yesterday, range 0-5[e] | | | | | | | | | | |
| # fruit/veg food groups yesterday, range 0-5[f] | | | | | | | | | | |
| ASF score last 7 d, range 0-28[g] | | | | | | | | | | |
| Fruit/veg score last 7 d, range 0-35[h] | | | | | | | | | | |
| Lowest tertile for 7 d ASF score | | | | | | | | | | |
| Lowest tertile for 7 d fruit/veg score | | | | | | | | | | |

**Table 4b. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values - "flipped" groups (as received at enrollment for IFA and MMN)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Still breastfed | | | | | | | | | | |
| Breastfed 6+ times/yesterday | | | | | | | | | | |
| 4+ food groups yesterday (WHO)[d] | | | | | | | | | | |
| # ASF food groups yesterday, range 0-5[e] | | | | | | | | | | |
| # fruit/veg food groups yesterday, range 0-5[f] | | | | | | | | | | |
| ASF score last 7 d, range 0-28[g] | | | | | | | | | | |
| Fruit/veg score last 7 d, range 0-35[h] | | | | | | | | | | |
| Lowest tertile for 7 d ASF score | | | | | | | | | | |
| Lowest tertile for 7 d fruit/veg score | | | | | | | | | | |

**Table 4c.Two-group analyses: Outcomes at 18 months, unadjusted proportions and means, unadjusted and adjusted P-values - groups as assigned**

| | IFA + MMN (n=) | | LNS (n=) | | ALL (n=) | | | |
|---|---|---|---|---|---|---|---|---|
| | Proportion or mean | SD | Proportion or mean | SD | Proportion or mean | SD | Unadjusted p-value[b] | Adjusted p-value[c] |
| Still breastfed | | | | | | | | |
| Breastfed 6+ times/yesterday | | | | | | | | |
| 4+ food groups yesterday (WHO)[d] | | | | | | | | |
| # ASF food groups yesterday, range 0-5[e] | | | | | | | | |
| # fruit/veg food groups yesterday, range 0-5[f] | | | | | | | | |
| ASF score last 7 d, range 0-28[g] | | | | | | | | |
| Fruit/veg score last 7 d, range 0-35[h] | | | | | | | | |
| Lowest tertile for 7 d ASF score | | | | | | | | |

Lowest tertile for 7 d fruit/veg score

[a] IFA=iron folic acid group (standard care); MMN=multiple micronutrient group; LNS=lipid-based nutrient supplement group; ASF=animal-source food.

[b] P-values from ANOVA and LOGIT models for dichotomous. P-values for OLOGIT for food groups yesterday were similar and all NS.

[c] Models adjusted for all significant covariates in bivariate models, see above.

[d] At least 4 out of the following 7 food groups: grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A rich fruit and vegetables; other fruits and vegetables.

[e] The 5 ASF groups are: 1) organ meats; 2) other meat/poultry; 3) fish; 4) eggs; and 5) dairy.

[f] The 5 fruit and vegetables groups are: 1) vitamin A-rich orange/yellow vegetables; 2) dark green leafy vegetables; 3) other vegetables; 4) vitamin A-rich fruits; and 5) other fruits.

[g] Score sums four groups over seven days; groups are similar to those for yesterday, but organ meats and other flesh foods are grouped together.

[h] Score sums five groups over seven days; groups are the same as those for yesterday.

**Table 5. Evaluation of effect modification – significance of interaction terms**

| | | **stillbf** | **bf_6** | **dd24GE4** | **asf24sum** | **asf24sum** | **frveg24sum** | **frveg24sum** | **asf7sum** | **frveg7sum** | **i18_asfT1** | **i18_frvegT1** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | logit | logit | logit | ANOVA | OLOGIT | ANOVA | OLOGIT | ANOVA | ANOVA | logit | logit |
| **Season of interview** | dryseas | | | | | | | | | | | |
| **Asset index** | asset | | | | | | | | | | | |
| **Small livestock score** | smlivestock | | | | | | | | | | | |
| **HFIA category** | hfia_cat | | | | | | | | | | | |
| **Other U5 at baseline** | other_u5yn | | | | | | | | | | | |
| **Maternal education** | schooling | | | | | | | | | | | |
| **Maternal age** | moth_age | | | | | | | | | | | |
| **Main language in HH** | Hhlanguage | | | | | | | | | | | |
| **Marital status** | marital | | | | | | | | | | | |
| **Child sex** | male | | | | | | | | | | | |

**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 20: Experience and Hypothetical Willingness-to-Pay for LNS-P&L and LNS-Child (version 01.0, prepared by Emy Reimao, Katie Adams, and Steve Vosti, 11 Feb, 2015)

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## 1. Overview and Study Objectives

In this paper we will present the results of analyses exploring the role of 'experience' in shaping hypothetical willingness-to-pay (hWTP) for LNS-P&L and LNS-Child over the course of the DYAD-M trial. Two local products are used for comparison: *bonya*, locally consumed small dried fish, for LNS-P&L and *Likuni Phala*, a maize-based porridge, for LNS-Child. Data on hWTP was collected multiple times over the course of the trial, enabling us to assess the influence of personal experiences at various points during the trail on stated WTP. The measures of experience, detailed in the Description of Variables section below, are meant to capture a respondent's experiences[1] during the trial that might influence his/her hWTP and include treatment group, the passage of time,[2] adherence to study protocol, and health.[3]

## 2. Description of the Study

A more detailed description of the iLiNS DYAD-M randomized trial, including the study population and inclusion and exclusion criteria is available in the main statistical analysis plan (iLiNS-DYAD-M Statistical Analysis Plan, version 16.0 with appendices 1-18, 2014-12-14). In short, screening, recruitment and enrollment of pregnant women into the randomized controlled trial were done on a rolling basis from February 2011 to June 2013. During this period, women receiving prenatal care in Mangochi, Malindi, Lungwena and Namwera were screened for potential participation in the trial. Eligible and willing women were then recruited to participate in the study and randomized into one of the trial's three equally-sized arms in which women received: (1) daily iron-folic acid tablets throughout pregnancy, (2) daily micronutrient tablets during pregnancy and the first six months of lactation, or (3) LNS-P&L during pregnancy and the first six months postpartum. The babies born to women randomized into the LNS-P&L group also received LNS-Child at 6 to18 months of age, while the babies born to women in the other two study arms did not receive any supplementation.

Using contingent valuation methods[4], we elicited hWTP for a week's supply of LNS-P&L three times during the trial (twice during pregnancy and once postpartum) from a random subsample of households participating in the iLiNS DYAD-M trial. We also elicited hypothetical WTP for a week's supply of LNS-Child twice during the trial. For comparison, each time hWTP for LNS-P&L was collected, we also elicited hWTP for a week's supply of *bonya*, and each time we collected information on hWTP for LNS-Child we also gathered data on the hWTP for *Likuni Phala*. The elicited values for a week's supply were then converted into daily values for comparability across studies. For all products (LNS-P&L, LNS-Child, *bonya*, and *Likuni Phala*), after eliciting hWTP for a week's supply of the product, we used a set of

[1] In the DYAD-M trial, respondents to the hWTP were either the iLiNS woman or the head of household. For heads of household, some experience variables will measure the influence of the iLiNS woman's experience (e.g., morbidity during pregnancy) on the head of household's hWTP. The influence of these indirect experience variables may be quite important if, for example, the head of household is the primary decision-maker with respect to household expenditures on food and/or health-related products.

[2] The passage of time encompasses both the amount of time in which households randomized into the LNS arm of the trial had to learn about LNS (i.e., use the product and learn about some of its private costs and benefits, etc.) as well as experiences not related to LNS that may also influence hWTP for LNS products, such as the weeks of gestation or the child's weeks of age.

[3] Note that most experience variables are endogenous. As such, our characterization of the role of experience in explaining hWTP will be based on measures of association (not causation).

[4] The questionnaire is described in more detail in SAP for the hWTP; the elicitation method was the same for all rounds: iLiNS DYAD-M SES Analysis - Baseline hypothetical WTP for LNS-P & L, 2014-03-20.

follow-up questions[5] to assess hWTP in the long-term (i.e., throughout pregnancy/throughout the first six months postpartum/throughout the period of six to 18 months of age).

Because hWTP was collected multiple times over the course of the trial, we have a panel for each household. However, for logistical reasons and difficulty locating some respondents (traveling, working away from home, etc.) the actual date of enumeration was often weeks or months from the planned date of enumeration.[6] Therefore, instead of comparing hWTP by round of survey administration, we treat time as a continuous variable measured in months from enrollment for the case of hWTP during pregnancy and months from the birth of the iLiNS baby for the case of hWTP postpartum. This set-up has implications for data analysis, which we describe in Section 5.5.

## 3. Hypotheses to be Tested

The table below summarizes the main hypotheses ($H_0$) to be tested.

Because hWTP was elicited at three distinct stages during the course of the mother/baby dyad's participation in the iLiNS trial (i.e., during pregnancy, when the iLiNS baby was less than 6 mo. of age, and when the baby was 6 mo. of age and older) and because some of the relevant experience variables are specific to those stages (e.g., child morbidity variables could not be considered experience variables in the pregnancy stage), the hypotheses listed below are stage-specific. Stage A refers to observations collected between maternal enrollment into the trial through the birth of the iLiNS baby. Stage B refers to observations collected during the first 6 mo. postpartum. Stage C refers to observations collected after the iLiNS baby turned 6 mo. of age.

Note that the term 'by group' indicates a comparison across households in which the mother/baby dyad received LNS (the LNS group) and those who did not (the non-LNS group). Also, because the relationship between the experience variables and hWTP may be quite different for those in the LNS group compared to those in the non-LNS group, some of the hypotheses listed below will be tested separately for each subgroup (as specified in the table). Finally, 'E' in Table 1 below is a vector of stage-specific experience variables as defined in Section 4.2, including time enrolled in the study, adherence to study protocol, maternal and child morbidity, maternal and child anthropometrics, and select knowledge, attitudes, and practices (KAP) variables.

[5] For WTP for LNS-P&L, these follow-up questions began with the following: "You have told me that you would be willing to pay [maximum WTP] today for week's supply of [product]. Would you be willing to pay every week for the remainder of your pregnancy/for up to six months of breastfeeding for yourself/for one year? If the answer was 'no', then the follow-up questions varied depending on the survey round. At pregnancy the question was "What price would you be willing to pay every week for [product] for the remainder of your pregnancy?". During lactation, "What price do you think you could pay every day for 1 day's worth of [product] up to six months of breastfeeding?". And once the child was born, "What price do you think you could pay every day for 1 day's worth of [product] throughout the year?"

[6] Kernel density estimation will be used to depict the number of months from enrollment to hWTP during pregnancy and the number of months from the iLiNS baby's birth to hWTP survey administration postpartum.

Table 1. Null Hypothesis Tests by Stage in Trial and by Sample

| **Sample** | **Stage A: Pregnancy** | **Stage B: Baby < 6mo** | **Stage C: Baby >= 6mo** |
|---|---|---|---|
| Full Sample | $H_0$1: There is no difference in short-term hWTP for LNS-P&L by group | $H_0$2: There is no difference in short-term hWTP for LNS-P&L by group | $H_0$3: There is no difference in short-term hWTP for LNS-Child by group |
| Full Sample | $H_0$4: There is no difference in long-term hWTP for LNS-P&L by group | $H_0$5: There is no difference in long-term hWTP for LNS-P&L by group | $H_0$6: There is no difference in long-term hWTP for LNS-Child by group |
| Full Sample | $H_0$7: There is no difference in the cross-product difference in short-term hypothetical WTP for LNS-P&L and *bonya* by group. That is, (WTP for LNS-P&L- WTP for *bonya*) | $H_0$8: There is no difference in the cross-product difference in short-term hypothetical WTP for LNS-P&L and *bonya* by group. That is, (WTP for LNS-P&L- WTP for *bonya*) | $H_0$9: There is no difference in the cross-product difference in short-term hypothetical WTP for LNS-Child and *Likuni Phala* by group. That is, (WTP for LNS-Child - WTP for *Likuni Phala*) |
| Full Sample | $H_0$10: There is no difference in the cross-product difference in long-term hypothetical WTP for LNS-P&L and *bonya* by group | $H_0$11: There is no difference in the cross-product difference in long-term hypothetical WTP for LNS-P&L and *bonya* by group | $H_0$12: There is no difference in the cross-product difference in long-term hypothetical WTP for LNS-Child and *Likuni Phala* by group |
| By LNS/Non-LNS Subgroups | $H_0$13: There is no systematic association between E and short-term hWTP for LNS-P&L | $H_0$14: There is no systematic association between E and short-term hWTP for LNS-P&L | $H_0$15: There is no systematic association between E and short-term hWTP for LNS-Child |
| By LNS/Non-LNS Subgroups | $H_0$16: There is no systematic association between E and long-term hWTP for LNS-P&L | $H_0$17: There is no systematic association between E and long-term hWTP for LNS-P&L | $H_0$18: There is no systematic association between E and long-term hWTP for LNS-Child |
| By LNS/Non-LNS Subgroups | $H_0$19: There is no systematic association between E and the cross-product difference in short-term hypothetical WTP for LNS-P&L and *bonya* | $H_0$20: There is no systematic association between E and the cross-product difference in short-term hypothetical WTP for LNS-P&L and *bonya* | $H_0$21: There is no systematic association between E and the cross-product difference in short-term hypothetical WTP for LNS-Child and *Likuni Phala* |
| By LNS/Non-LNS Subgroups | $H_0$22: There is no systematic association between E and the cross-product difference in long-term hypothetical WTP for LNS-P&L and *bonya* | $H_0$23: There is no systematic association between E and the cross-product difference in long-term hypothetical WTP for LNS-P&L and *bonya* | $H_0$24: There is no systematic association between E and the cross-product difference in long-term hypothetical WTP for LNS-Child and *Likuni Phala* |

Note: ‘E’ in the table above is a vector of experience variables as defined in Section 4.2 below.

For each of the hypotheses, we will also test for heterogeneity in the effect/association by survey respondent (iLiNS woman or head of household), heterogeneity by time (defined as months enrolled in iLiNS trial in Stage A and months from birth of iLiNS baby in Stages B and C), and heterogeneity by site of enrollment into the study.

## 4. Description of Variables

The following sections describe the dependent and explanatory variables that will be used to model the relationship between hWTP and the measures of experience.

### 4.1 Dependent Variables

By hypothesis:

- Hypothesis 1, 2, 13, and 14: Short-term WTP for a day's supply of LNS-P&L in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 4, 5, 16, and 17: Long-term WTP for a day's supply of LNS-P&L in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 7, 8, 19, and 20: Difference between short-term WTP for a day's supply of LNS-P&L and *bonya* in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 10, 11, 22, and 23: Difference between long-term WTP for a day's supply of LNS-P&L and *bonya* in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 3 and 15: Short-term WTP for a day's supply of LNS-Child in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 6 and 18: Long-term WTP for a day's supply of LNS-Child in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 9 and 21: Difference between short-term WTP for a day's supply of LNS-child and *Likuni Phala* in 4$^{th}$ quarter 2011 US dollars.
- Hypothesis 12 and 24: Difference between long-term WTP for a day's supply of LNS-child and *Likuni Phala* in 4$^{th}$ quarter 2011 US dollars.

Note: The distributions of WTP for each of the four products are right-skewed. To account for this in our models, we may transform WTP to ln(WTP).[7]

### 4.2 Experience Variables

The following table defines the set of variables meant to capture a respondent's experiences during the trial that might influence his/her hWTP.

[7] Because the natural log of zero is undefined, we will set all zero WTP values to a value slightly smaller than the minimum non-zero value of ln(WTP).

For all analyses of hWTP for LNS-P&L, morbidity and adherence data will come from Form 18, Maternal Biweekly Follow-up. For all analyses of hWTP for LNS-Child, morbidity and adherence data will come from Form 27 Child Weekly Morbidity Data. Data on maternal perception of sufficiency of food, growth of iLiNS baby, and ease of feeding LNS-Child to the baby will come from KAP data (Form 14b or 14c). The specific experience variables will vary by stage in the iLiNS trial when hWTP data were collected, as indicated in the third column of the table below.

| **Variable Name** | **Description** | **Relevant Stages** |
|---|---|---|
| Months Enrolled | Number of months from enrollment to hWTP survey administration. | A |
| Months from Birth | Number of months from the birth of the iLiNS baby to hWTP survey administration. | B, C |
| Inter-household LNS | A count variable indicating the number of women/children the respondent reported knowing outside his/her household who received LNS-P&L/LNS-Child. | A, B, C |
| Adherence | Percentage of supplements (sachets or tablets) consumed as prescribed during the 30-day period[8] immediately prior to the hWTP survey administration. | A, B, C |
| Poor Appetite | Count variable indicating the number of days of reported maternal (stages A and B) or baby (stages B and C) poor appetite in the week or 30-day period, respectively, immediately prior to the hWTP survey administration. | A, B, C |
| Nausea | Count variable indicating the number of days of reported maternal (stages A and B) nausea during the week immediately prior to the hWTP survey administration. | A, B |
| Vomiting, mother | Count variable indicating the number of days of reported maternal vomiting during the week immediately prior to the hWTP survey administration. | A, B |
| Vomiting, child | Count variable indicating the number of days of reported baby vomiting during the 30-day period immediately prior to the hWTP survey administration. | B, C |
| Nausea and Vomiting During Pregnancy[9] | Variable indicating the proportion of days since enrollment into the DYAD-M trial of reported maternal nausea or vomiting. | A |
| Diarrhea, mother | Count variable indicating the number of days of reported maternal diarrhea during the week immediately prior to the hWTP survey administration. | A, B |
| Diarrhea, child | Count variable indicating the number of days of reported baby diarrhea during the 30-day period immediately prior to the hWTP survey administration. | B, C |
| Child Gender | Gender of the iLiNS baby. | B, C |
| BMI | iLiNS baby's body mass index at birth. | B |

[8] If less than 30 days elapsed between enrollment and the first hWTP survey administration, the adherence and morbidity variables for this observation will be constructed based on the period from enrollment to hWTP survey administration.

[9] Maternal morbidity data is collected only every other week. We assume that the proportion calculated based on the weeks for which we have data does not differ systematically from the weeks for which we do not.

| | | |
|---|---|---|
| WLZ | iLiNS baby's weight-for-length z-score at the measurement closest to hWTP survey administration calculated using WHO Anthro, a Stata macro from the World Health Organization based on the updated WHO child growth standards. | C |
| LAZ | iLiNS baby's length-for-age z-score at birth (stage B) or at the measurement closest to hWTP survey administration (stage C) calculated using WHO Anthro, a Stata macro from the World Health Organization based on the updated WHO child growth standards. | B, C |
| Growing Well | Dummy variable = 1 if mother/caregiver indicated she thought the iLiNS baby was growing well and = 0 otherwise. | B, C |
| Reduced Activity | A count variable indicating the number of days the mother/caregiver reported the iLiNS baby experienced reduced activity in the 30-day period immediately prior to the hWTP survey administration. | B, C |
| Good Food | Dummy variable = 1 if mother/caregiver reported being able to feed the iLiNS baby the kind of food she thought was good for him/her and = 0 otherwise. | C |
| LNS-Child Difficult to Eat[10] | Dummy variable = 1 if the mother reported it was difficult for the iLiNS baby to eat LNS-Child and = 0 if mother reported it was easy. | C |

4.3 Time-Invariant Control Variables

- Age: Respondent's age in years at baseline.
- Education: Number of completed years of formal education by the respondent.
- Language: Set of dummy variables indicating primary language spoken at home.
- Maternal height: Mother's height in meters measured at enrollment.
- Maternal BMI: Mother's body mass index at enrollment.
- Primiparity: Dummy variable = 1 if iLiNS baby is mother's first child.
- Gestational age at enrollment: Number of weeks pregnant at enrollment.
- Site of Enrollment: Dummy variable = 1 if site of enrollment was Mangochi and = 0 otherwise.

4.4 Time-Varying Control Variables

- Months from enrollment to hWTP survey administration (relevant in Stage A)

[10] This information is only available for subset of children randomized into the LNS group, so it only applies to the LNS subgroup analysis.

- Months from birth of iLiNS baby to hWTP survey administration (relevant in Stages B and C)
- Lean Season: Dummy variables indicating whether the hWTP survey was administered during the lean season.
- Year: Dummy variables indicating the year the hWTP survey was administered.
- Enumerator: Set of enumerator control variables.
- Version of Questionnaire: Set of control variables for version of hWTP survey to control for starting 'bid' (described in more detail in the SAP on the baseline hWTP).

## 5. Statistical Methods

### 5.1 Data Cleaning

Cleaning of the SES data follows the same procedure outlined in the main analysis plan (iLiNS-DYAD-M Statistical Analysis Plan, version 16.0 with appendices 1-19, 2014-12-20).

### 5.2 Outliers

Identification and treatment of outliers in the SES data[11], maternal nutrition variables, and experience variables will follow the treatment described in the main statistical analysis plan (iLiNS-DYAD-M Statistical Analysis Plan, version 18.0 with appendices 1-19, 2014-12-20) and in consult with Jan Peerson.

### 5.3 Software

All statistical analyses will be performed with Stata 13 statistical package.

### 5.4 Basis for the Analysis

The basis for the analysis is an intent-to-treat framework. hWTP respondents who were lost to follow-up (either temporarily or permanently) will be included in the analysis for all time points where data are available, and the sample size will be clearly reported for each regression analysis/time point.

### 5.5 Analysis

#### *5.5.1 Summary Baseline Characteristics*

Summary statistics, including mean (count for dichotomous variables), standard deviation (percentage for dichotomous variables), minimum, and maximum for all baseline control variables (as described in section 4.3 above) will be presented in Table 1. As a check for the success of the randomization, we will report any differences in mean explanatory variables across treatment groups. Scatter plots, histograms, and/or kernel density estimates will also be presented.

[11] hWTP observations more than six standard deviations above the mean of non-zero observations will be omitted as outliers.

*5.5.2 Summary of Experience Variables*

Summary statistics, including mean, standard deviation, minimum, and maximum for the experience variables will be presented in Table 2. Scatter plots, histograms, and/or kernel density estimates will also be presented.

Table 3 will present summary statistics for the experience variables by treatment groups (i.e., LNS vs non-LNS). Statistically significant differences in the mean value of these experience variables by treatment group and by respondent will be indicated in the table.

*5.5.3 Summary of Short- and Long-Term hWTP*

Summary statistics, including mean, standard deviation, minimum, and maximum for short-term (i.e., a day's supply) hWTP for LNS-P&L, LNS-Child, and the difference in hWTP for those products and *bonya* or *Likuni Phala*, as relevant, will be presented in Table 5 both for the full sample and by group (LNS or non-LNS). Table 4 will present the same statistics for long-term hWTP (i.e., throughout pregnancy/throughout the first six months postpartum/throughout the period from 6-18 mo. after the birth of the iLiNS baby).

Kernel density estimates of hWTP will also be presented.

*5.5.4 Effect of Treatment Group on hWTP*

The following regression models will be estimated to test the hypotheses related to the effect of being randomized into the LNS group (vis-à-vis being randomized into the iron-folic acid or multiple micronutrient tablets group) on hWTP, which are hypotheses 1-12 in Section 3 above.

For Stage A (pregnancy), where we potentially have two observations of hWTP for each respondent, we will estimate the following random effects model for $i = 1, 2, \dots, N$ contingent valuation survey respondents and for $t = A1, A2$ rounds of hWTP data collection:

$$y_{it} = \alpha_i + \beta_1 LNS_i + \varphi T_{it} + \delta X_i + \varepsilon_{it}. \quad (1)$$

The dependent variable, $y_{it}$, is the hWTP variable of interest for respondent $i$ at time $t$. $LNS_i$ is an indicator variable equal to one if the mother in respondent $i's$ household was randomized to receive LNS and zero otherwise. The vector $T_{it}$ is composed of time-varying covariates defined in Section 4.4. To improve the precision of our estimates, we also include a vector of time-invariant baseline covariates, $X_i$, as defined in Section 4.3 above. To account for the fact that the error is likely correlated over time for a given respondent, we will cluster the standard errors $\varepsilon_{it}$ at the respondent level.

Heterogeneity in the effect of LNS on hWTP by time will be estimated as follows:

$$y_{it} = \alpha_i + \beta_1 LNS_i + \beta_2 t_{it} + \beta_3 (LNS_i * t_{it}) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (2)$$

where $t_{it}$ is months from enrollment to the date of hWTP enumeration.

Heterogeneity by survey respondent will be similarly estimated as:

$$y_{it} = \alpha_i + \beta_1 LNS_i + \beta_2 R_i + \beta_3 (LNS_i * R_i) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (3)$$

where $R_i$ = 1 if the survey respondent was the iLiNS woman and = 0 if head of household. [12]

Finally, heterogeneity by survey respondent will be estimated as:

$$y_{it} = \alpha_i + \beta_1 LNS_i + \beta_2 S_i + \beta_3 (LNS_i * S_i) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (4)$$

where $S_i$ = 1 if the site of enrollment is Mangochi and = 0 otherwise.

For Stage B (birth to <6mo), where we have just one hWTP observation per respondent, we will estimate the following model using OLS for $i = 1, 2, \dots, N$ contingent valuation survey respondents and for $t = B1$:

$$y_{it} = \alpha + \beta_1 LNS_i + \varphi T_{it} + \delta X_i + \varepsilon_{it} \quad (5)$$

Everything is as defined as in equation (1), but there is only one observation per respondent. We also allow for time, respondent, and site interactions, as in equations (2),(3) and (4), respectively.

Finally, at Stage C (>= 6mo), we again have potentially two observations per respondent so we estimate equation (1) for $t = C1, C2$. Heterogeneity by time, respondent, and site will be estimated by equations (2) -(4) for $t = C1, C2$.

*5.5.5 Relationship Between Experience and hWTP*

This section describes the regression equations that will be used to estimate the relationship between hWTP and the set of experience variables defined in section 4.2 (hypotheses 13-24 in Section 3 above). As noted, these regressions will be run separately on the subset of LNS and non-LNS households.

For Stage A (pregnancy), where we potentially have two observations of hWTP for each respondent, we will estimate the following pooled OLS models for $i = 1, 2, \dots, N$ contingent valuation survey respondents and for $t = A1, A2$ rounds of hWTP data collection:

$$y_{it} = \alpha + \beta_1 E_{it} + \varphi T_{it} + \varepsilon_{it}. \quad (6)$$

Here, $y_{it}$ is the hWTP variable of interest for respondent $i$ at time $t$. The experience variables relevant for Stage A as described in the table in Section 4.2 are contained in the vector $E_{it}$. The vector $T_{it}$ is composed of other time-varying covariates defined in Section 4.4, and $\varepsilon_{it}$ is an idiosyncratic error. To account for the fact that the error is likely correlated over time for a given respondent, we will cluster the standard errors at the respondent level.

Heterogeneity in the association between the experience variables over time will be estimated with interactions defined as:

$$y_{it} = \alpha + \beta_1 E_{it} + \beta_2 t_{it} + \beta_3 (E_{it} * t_{it}) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (7)$$

where $t_{it}$ is months from the birth of the iLiNS baby to hWTP survey administration.

[12] In cases where the iLiNS woman is also the head of household, this variable will be coded as =1 (iLiNS woman).

Heterogeneity by respondent will similarly be modeled using interactions as:

$$y_{it} = \alpha + \beta_1 E_{it} + \beta_2 R_i + \beta_3 (E_{it} * R_i) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (8)$$

where $R_i$ = 1 if the survey respondent was the iLiNS woman and = 0 if head of household.

Finally, heterogeneity by site of enrollment into the study will be modeled as:

$$y_{it} = \alpha + \beta_1 E_{it} + \beta_2 S_i + \beta_3 (E_{it} * S_i) + \varphi T_{it} + \delta X_i + \varepsilon_{it}, \quad (9)$$

where $S_i$ = 1 if the site of enrollment is Mangochi and = 1 otherwise.

For Stage B (birth to <6mo), where we have just one hWTP observation per respondent, we will estimate the following model using OLS for $i = 1, 2, \dots, N$ contingent valuation survey respondents and for $t = B1$:

$$y_{it} = \alpha + \beta_1 E_{it} + \varphi T_{it} + \delta X_i + \varepsilon_{it}. \quad (10)$$

Everything is as defined as in equation (6) except $E_{it}$ is limited to variables relevant to Stage B as defined in the table in Section 4.2. Also, given the single observation per respondent, we do not include the fixed effect term. As above, heterogeneity by time, by respondent, and by site will be assed using interaction terms as above.

Finally, at Stage C (>= 6mo), we again have potentially two observations per respondent so we estimate the pooled OLS models of equations (6)- (9) for $t = C1, C2$.

## 5.6 Other Statistical Notes

### 5.6.1 *Collinearity*

Collinearity among all covariates will be assessed using Stata's collin command. Variables with a high variance inflation factor (VIF > 10) will be assessed and the set of covariates will be reduced so that all covariates have a VIF < 10 (Chen et al. 2003).

### 5.6.2 *Missing Data*

For the main analyses, all missing data, including impossible/improbable outliers coded as missing, will be treated as missing (i.e., not imputed). As a robustness check of the results, we may also conduct sensitivity analyses with imputed data.

## 6. Design of Tables

Table 1. Description of Respondent, Maternal, and Household Characteristics

| | Variable | Definition | Mean | Std Dev | Min | Max |
|---|---|---|---|---|---|---|
| Respondent | Age | Age in years | | | | |
| | Education | Completed years of education | | | | |
| | Head of Household | = 1 if respondent is head of household (= 0 if iLiNS woman) | | | | |
| Maternal | Height | Height in centimeters | | | | |
| | BMI | Body mass index at enrollment | | | | |
| | Gestational Age | Gestational age in weeks at enrollment into iLiNS trial | | | | |
| | Primiparity | = 1 if iLiNS infant if mother's first pregnancy | | | | |
| Household | Children Under 5 | Number of children under age 5 | | | | |
| | Asset Index | Proxy measure of socioeconomic status based on asset ownership | | | | |
| | HFIA Score | Household Food Insecurity Access Score | | | | |
| | PC Food Expenditures | Per capita daily expenditures on food in 2011 USD | | | | |
| | PC Household Daily Income | Per capita household income per day in 2011 USD | | | | |
| | Chichewa | = 1 if Chichewa is primary language spoken in household | | | | |
| | Chiyao | = 1 if Chiyao is primary language spoken in household | | | | |
| | Mangochi | = 1 if the site of maternal enrollment into the study is Mangochi | | | | |

N=xxx

Significance codes for difference in means between LNS and non-LNS groups: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Table 2. Definitions of Experience Variables

| | Variable | Definition |
|---|---|---|
| Maternal | Months Enrolled | Number of months from enrollment to hWTP survey administration. |
| | Inter-household LNS-P&L | A count variable indicating the number of women the respondent reported knowing outside his/her household who received LNS-P&L. |
| | Maternal Adherence | Percentage of supplements (sachets or tablets) consumed as prescribed during the 30-day period immediately prior to the hWTP survey administration. |
| | Maternal Poor Appetite | Count variable indicating the number of days of reported maternal poor appetite during the week immediately prior to the hWTP survey administration. |
| | Maternal Nausea | Count variable indicating the number of days of reported maternal nausea during the week immediately prior to the hWTP survey administration. |
| | Maternal Vomiting | Count variable indicating the number of days of reported maternal vomiting during the week immediately prior to the hWTP survey administration. |
| | Nausea and Vomiting During Pregnancy | Variable indicating the proportion of days since enrollment into the DYAD-M trial of reported maternal nausea or vomiting.[13] |
| | Maternal Diarrhea | Count variable indicating the number of days of reported maternal diarrhea during the week immediately prior to the hWTP survey administration. |
| Infant | Months from Birth | Number of months from the birth of the iLiNS infant to hWTP survey administration. |
| | Inter-household LNS-Child | A count variable indicating the number of infants the respondent reported knowing outside his/her household who received LNS-Child. |
| | Infant Adherence | Percentage of sachets of LNS-Child consumed as prescribed during the 30-day period immediately prior to the hWTP survey administration. |
| | Infant Poor Appetite | Count variable indicating the number of days of reported infant poor appetite during the 30-day period immediately prior to the hWTP survey administration. |
| | Infant Vomiting | Count variable indicating the number of days of reported infant vomiting during the 30-day period immediately prior to the hWTP survey administration. |
| | Infant Diarrhea | Count variable indicating the number of days of reported infant diarrhea during the 30-day period immediately prior to the hWTP survey administration. |
| | Reduced Activity | A count variable indicating the number of days the mother/caregiver reported the infant experienced reduced activity in the 30-day period immediately prior to the hWTP survey administration. |
| | BMIZ at Birth | Infant's body mass index for age z-score at birth. |
| | LAZ at Birth | Infant's length-for-age z-score at birth. |
| | WLZ | Infant's weight-for-length z-score at the measurement closest to hWTP survey administration. |
| | LAZ | Infant's length-for-age z-score at the measurement closest to hWTP survey administration. |
| | Growing Well | Dummy variable = 1 if mother/caregiver indicated she thought the infant was growing well and = 0 otherwise. |
| | Good Food | Dummy variable = 1 if mother/caregiver reported being able to feed the infant the kind of food she though was good for him/her and = 0 otherwise. |
| | LNS-Child Difficult to Eat | Dummy variable = 1 if the mother/caregiver reported it was difficult for the infant to eat LNS-Child and = 0 if mother reported it was easy. |

[13] Proportion calculated is based on days for which data was collected (every other week), and assumes that the proportion for days for which data was not collected does not systematically differ from that of the days for which data was collected.

Table 3. Summary of Experience Variables by Treatment Group: Pregnancy

| | | LNS | | | Non-LNS | | |
|---|---|---|---|---|---|---|---|
| | Variable | Mean | Std Deviation | Min, Max | Mean | Std Deviation | Min, Max |
| Maternal | Months Enrolled | | | | | | |
| | Inter-household LNS-P&L | | | | | | |
| | Maternal Adherence | | | | | | |
| | Maternal Poor Appetite | | | | | | |
| | Maternal Nausea | | | | | | |
| | Maternal Vomiting | | | | | | |
| | Nausea and Vomiting During Pregnancy | | | | | | |
| | Maternal Diarrhea | | | | | | |

N=xxx

Table 4. Summary of Experience Variables by Treatment Group: 0-6 Months Postpartum

| | | LNS | | | Non-LNS | | |
|---|---|---|---|---|---|---|---|
| | Variable | Mean | Std Deviation | Min, Max | Mean | Std Deviation | Min, Max |
| Maternal | Inter-household LNS-P&L | | | | | | |
| | Maternal Adherence | | | | | | |
| | Maternal Poor Appetite | | | | | | |
| | Maternal Nausea | | | | | | |
| | Maternal Vomiting | | | | | | |
| | Maternal Diarrhea | | | | | | |
| Infant | Months from Birth | | | | | | |
| | Infant Poor Appetite | | | | | | |
| | Infant Vomiting | | | | | | |
| | Infant Diarrhea | | | | | | |
| | Reduced Activity | | | | | | |
| | BMIZ at Birth | | | | | | |
| | LAZ at Birth | | | | | | |
| | Growing Well | | | | | | |

N=xxx

Table 5. Summary of Experience Variables by Treatment Group: 6+ Months Postpartum

| | | LNS | | | Non-LNS | | |
|---|---|---|---|---|---|---|---|
| | Variable | Mean | Std Deviation | Min, Max | Mean | Std Deviation | Min, Max |
| Infant | Months from Birth | | | | | | |
| | Inter-household LNS-Child | | | | | | |
| | Infant Poor Appetite | | | | | | |
| | Infant Vomiting | | | | | | |
| | Infant Diarrhea | | | | | | |
| | Reduced Activity | | | | | | |
| | WLZ | | | | | | |
| | LAZ | | | | | | |
| | Growing Well | | | | | | |
| | Good Food | | | | | | |
| | LNS-Child Difficult to Eat | | | | | | |

N=xxx

Table 6. Average hWTP for a Day's Supply by Treatment Group: Pregnancy

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-P&L | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Bonya | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-P&L | | | | | |
| | Bonya | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-P&L – WTP for bonya).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 7. Average Long-Term hWTP by Treatment Group: Pregnancy

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-P&L | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Bonya | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-P&L | | | | | |
| | Bonya | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-P&L – WTP for bonya).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 8. Average hWTP for a Day's Supply by Treatment Group: 0-6 Months Postpartum

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-P&L | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Bonya | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-P&L | | | | | |
| | Bonya | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-P&L – WTP for bonya).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 9. Average Long-Term hWTP by Treatment Group: 0-6 Months Postpartum

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-P&L | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Bonya | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-P&L | | | | | |
| | Bonya | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-P&L – WTP for bonya).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 10. Average hWTP for a Day's Supply by Treatment Group: 6+ Months Postpartum

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-Child | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Likuni Phala | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-Child | | | | | |
| | Likuni Phala | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-Child – WTP for Likuni Phala).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 11. Average Long-Term hWTP by Treatment Group: 6+ Months Postpartum

| | Product | N | Mean† (Std Error) | Std Deviation | Min, Max* | Zero Max WTP/Difference |
|---|---|---|---|---|---|---|
| LNS Group | LNS-Child | xxx | x.xx (x.xx) | x.xx | x, x.xx | xx (x.x%) |
| | Likuni Phala | | | | | |
| | Difference | | | | | |
| Non-LNS Group | LNS-Child | | | | | |
| | Likuni Phala | | | | | |
| | Difference | | | | | |

†In 4th Quarter 2011 US Dollars.
*Observations > 6 SD above the mean were omitted as outliers.
Difference is defined as (WTP for LNS-Child – WTP for Likuni Phala).
Significance codes for difference in means between LNS and non-LNS groups: *** (p < .01), ** (p < .05), * (p < .1).

Table 12. Effect of Treatment Group on hWTP: Pregnancy

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 13. Heterogeneity by Months Enrolled in the Effect of Group on hWTP: Pregnancy

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Months Enrolled | | | | |
| LNS X Months Enrolled | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months Enrolled' indicates the number of months from maternal enrollment into the trial to hWTP survey administration. Cluster-robust standard errors in parentheses.

Table 14. Heterogeneity by Respondent in the Effect of Group on hWTP: Pregnancy

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mother | | | | |
| LNS X Mother | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 15. Heterogeneity by Site of Enrollment in the Effect of Group on hWTP: Pregnancy

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mangochi | | | | |
| LNS X Mangochi | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are

included in the model (unreported). The variable 'Mangochi' indicates whether site of enrollment into the study is Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 16. Effect of Treatment Group on hWTP: 0-6 Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from birth of infant to hWTP survey administration are included in the model (unreported).

Table 17. Heterogeneity by Months from Birth in the Effect of Group on hWTP: 0-6 Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Months from Birth | | | | |
| LNS X Months from Birth | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and

year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months from Birth' indicates the number of months from the birth of the infant to hWTP survey administration.

Table 18. Heterogeneity by Respondent in the Effect of Group on hWTP: 0-6 Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mother | | | | |
| LNS X Mother | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from the birth of the infant to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0).

Table 19. Heterogeneity by Site of Enrollment in the Effect of Group on hWTP: 0-6 Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mangochi | | | | |
| LNS X Mangochi | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education,

primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'Mangochi' indicates whether site of enrollment into the study is Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 20. Effect of Treatment Group on hWTP: 6+ Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (when infant is 6-18mo) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (when infant is 6-18mo ) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from the birth of the infant to hWTP survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 21. Heterogeneity by Months from Birth in the Effect of Group on hWTP: 6+ Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Months from Birth | | | | |
| LNS X Months from Birth | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (when infant is 6-18mo) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (when infant is 6-18mo ) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months from Birth' indicates the number of months from the birth of the infant to hWTP survey administration.

Table 22. Heterogeneity by Respondent in the Effect of Group on hWTP: 6+ Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mother | | | | |
| LNS X Mother | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (when infant is 6-18mo) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (when infant is 6-18mo ) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from the birth of the infant to hWTP survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 23. Heterogeneity by Site of Enrollment in the Effect of Group on hWTP: 6+ Months Postpartum

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| LNS | | | | |
| Mangochi | | | | |
| LNS X Mangochi | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are

included in the model (unreported). The variable 'Mangochi' indicates whether site of enrollment into the study is Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 24. Effect of Experience on hWTP: Pregnancy, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 25. Heterogeneity by Months Enrolled in Effect of Experience on hWTP: Pregnancy, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Months Enrolled X Inter-household LNS-P&L | | | | |
| Months Enrolled X Maternal Adherence | | | | |
| Months Enrolled X Maternal Poor Appetite | | | | |
| Months Enrolled X Maternal Nausea | | | | |
| Months Enrolled X Maternal Vomiting | | | | |
| Months Enrolled X Nausea and Vomiting During Pregnancy | | | | |
| Months Enrolled X Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months Enrolled' indicates the number of months from maternal enrollment into the trial to hWTP survey administration. Cluster-robust standard errors in parentheses.

Table 26. Heterogeneity by Respondent in Effect of Experience on hWTP: Pregnancy, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mother | | | | |
| Mother X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 27. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: Pregnancy, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable ‘Mangochi’ indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 28. Effect of Experience on hWTP: Pregnancy, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 29. Heterogeneity by Months Enrolled in Effect of Experience on hWTP: Pregnancy, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Months Enrolled X Inter-household LNS-P&L | | | | |
| Months Enrolled X Maternal Adherence | | | | |
| Months Enrolled X Maternal Poor Appetite | | | | |
| Months Enrolled X Maternal Nausea | | | | |
| Months Enrolled X Maternal Vomiting | | | | |
| Months Enrolled X Nausea and Vomiting During Pregnancy | | | | |
| Months Enrolled X Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months Enrolled' indicates the number of months from maternal enrollment into the trial to hWTP survey administration. Cluster-robust standard errors in parentheses.

Table 30. Heterogeneity by Respondent in Effect of Experience on hWTP: Pregnancy, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mother | | | | |
| Mother X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 31. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: Pregnancy, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable ‘Mangochi’ indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 32. Effect of Experience on hWTP: 0-6 Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported).

Table 33. Heterogeneity by Months from Birth in Effect of Experience on hWTP: 0-6 Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Months from Birth X | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |

Maternal Vomiting

Maternal Diarrhea

Infant Poor Appetite

Infant Vomiting

Infant Diarrhea

Reduced Activity

BMI at Birth

LAZ at Birth

Growing Well

Constant

N

Wald $Chi^2$

Prob > $Chi^2$

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months From Birth' indicates the number of months from the birth of the iLiNS infant to hWTP survey administration.

Table 34. Heterogeneity by Respondent in Effect of Experience on hWTP: 0-6 Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Mother | | | | |
| Mother X | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |

Maternal Vomiting

Maternal Diarrhea

Infant Poor Appetite

Infant Vomiting

Infant Diarrhea

Reduced Activity

BMI at Birth

LAZ at Birth

Growing Well

Constant

N

Wald $Chi^2$

Prob > $Chi^2$

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0).

Table 35. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: 0-6 Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable ‘Mangochi’ indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 36. Effect of Experience on hWTP: 0-6 Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported).

Table 37. Heterogeneity by Months from Birth in Effect of Experience on hWTP: 0-6 Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Months from Birth X | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |

| |
|---|
| Maternal Vomiting |
| Maternal Diarrhea |
| Infant Poor Appetite |
| Infant Vomiting |
| Infant Diarrhea |
| Reduced Activity |
| BMI at Birth |
| LAZ at Birth |
| Growing Well |
| Constant |
| N |
| Wald $Chi^2$ |
| Prob > $Chi^2$ |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months From Birth' indicates the number of months from the birth of the iLiNS infant to hWTP survey administration.

Table 38. Heterogeneity by Respondent in Effect of Experience on hWTP: 0-6 Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Maternal Diarrhea | | | | |
| Months from Birth | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| BMI at Birth | | | | |
| LAZ at Birth | | | | |
| Growing Well | | | | |
| Mother | | | | |
| Mother X | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |

| | |
|---|---|
| Maternal Nausea | |
| Maternal Vomiting | |
| Maternal Diarrhea | |
| Infant Poor Appetite | |
| Infant Vomiting | |
| Infant Diarrhea | |
| Reduced Activity | |
| BMI at Birth | |
| LAZ at Birth | |
| Growing Well | |
| Constant | |
| N | |
| Wald $Chi^2$ | |
| Prob > $Chi^2$ | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0).

Table 39. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: 0-6 Months Postpartum,Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable 'Mangochi' indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 40. Effect of Experience on hWTP: 6+ Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| LNS-Child Difficult to Eat | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 41. Heterogeneity by Months From Birth in Effect of Experience on hWTP:6+ Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| LNS-Child Difficult to Eat | | | | |
| Months from Birth X | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |

| | |
|---|---|
| Good Food | |
| LNS-Child Difficult to Eat | |
| Constant | |
| N | |
| Wald $Chi^2$ | |
| Prob > $Chi^2$ | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Chils, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months From Birth' indicates the number of months from the birth of the iLiNS infant to hWTP survey administration. Cluster-robust standard errors in parentheses.

Table 42. Heterogeneity by Respondent in Effect of Experience on hWTP: 6+ Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| LNS-Child Difficult to Eat | | | | |
| Mother | | | | |
| Mother X | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |

| | |
|---|---|
| Growing Well | |
| Good Food | |
| LNS-Child Difficult to Eat | |
| Constant | |
| N | |
| Wald $Chi^2$ | |
| Prob > $Chi^2$ | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 43. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: 6+ Months Postpartum, LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable 'Mangochi' indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

Table 44. Effect of Experience on hWTP: 6+ Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). Cluster-robust standard errors in parentheses.

Table 45. Heterogeneity by Months From Birth in Effect of Experience on hWTP:6+ Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| Months from Birth X | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |

| | |
|---|---|
| Constant | |
| N | |
| Wald $Chi^2$ | |
| Prob > $Chi^2$ | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for respondent, mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, and version of questionnaire are included in the model (unreported). 'Months From Birth' indicates the number of months from the birth of the iLiNS infant to hWTP survey administration. Cluster-robust standard errors in parentheses.

Table 46. Heterogeneity by Respondent in Effect of Experience on hWTP: 6+ Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-Child | LNS-Child-Likuni Phala | LNS-Child | LNS-Child-Likuni Phala |
| | (1) | (2) | (3) | (4) |
| Months from Birth | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |
| Good Food | | | | |
| Mother | | | | |
| Mother X | | | | |
| Inter-household LNS-Child | | | | |
| Infant Poor Appetite | | | | |
| Infant Vomiting | | | | |
| Infant Diarrhea | | | | |
| Reduced Activity | | | | |
| WLZ | | | | |
| LAZ | | | | |
| Growing Well | | | | |

| | |
|---|---|
| Good Food | |
| Constant | |
| N | |
| Wald $Chi^2$ | |
| Prob > $Chi^2$ | |

Significance codes: *** (p < .01), ** (p < .05), * (p < .1).
Notes: Dependent variables are (1) hWTP for a day's supply of LNS-Child, (2) difference in hWTP for a day's supply of LNS-Child and Likuni Phala, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-Child and Likuni Phala. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The variable 'mother' indicates whether the respondent to the hWTP survey was the iLiNS woman (=1) or head of household (=0). Cluster-robust standard errors in parentheses.

Table 47. Heterogeneity by Site of Enrollment in Effect of Experience on hWTP: 6+ Months Postpartum, Non-LNS-Group

| | Day's Supply | | Long-Term | |
|---|---|---|---|---|
| | LNS-P&L | LNS-P&L-Bonya | LNS-P&L | LNS-P&L-Bonya |
| | (1) | (2) | (3) | (4) |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Mangochi | | | | |
| Mangochi X | | | | |
| Months Enrolled | | | | |
| Inter-household LNS-P&L | | | | |
| Maternal Adherence | | | | |
| Maternal Poor Appetite | | | | |
| Maternal Nausea | | | | |
| Maternal Vomiting | | | | |
| Nausea and Vomiting During Pregnancy | | | | |
| Maternal Diarrhea | | | | |
| Constant | | | | |
| N | | | | |
| Wald $Chi^2$ | | | | |
| Prob > $Chi^2$ | | | | |

Significance codes: *** ($p < .01$), ** ($p < .05$), * ($p < .1$).

Notes: Dependent variables are (1) hWTP for a day's supply of LNS-P&L, (2) difference in hWTP for a day's supply of LNS-P&L and bonya, (3) long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L, and (4) difference in long-term (throughout pregnancy) hWTP for a day's supply of LNS-P&L and bonya. Controls for mother's age, mother's education, food insecurity score, asset index, per capita food expenditures, per capita household income, primary language spoken in household, maternal height, maternal gestational age at enrollment, parity, season and year of maternal enrollment into the trial, enumerator, version of questionnaire, and months from enrollment to survey administration are included in the model (unreported). The

variable ‘Mangochi’ indicates whether the site of enrollment into the study was Mangochi (=1) or another site (=0). Cluster-robust standard errors in parentheses.

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**Impact of Lipid based nutrient supplements (LNS) on child sleep in Rural Malawi (iLiNS-DYAD)**

Statistical Analysis Plan,

Appendix 21: Sleep patterns. Version 01.0 (17.02.2015)

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**Study objective**

To assess whether LNS consumed by the child from 6 to 18 months improves the amount and quality of sleep among infants and young children in rural Malawi.

## 1. Study Materials

The data that will be used in this analysis will come from iLiNS-DYAD-M trial. The targeted population includes all children between 6 and 18 months born to women enrolled in the main iLiNS-DYAD-M trial. Participants from the main study were randomized to receive either LNS or MMN or IFA. Women in IFA group received IFA during pregnancy and a placebo after birth until 6 months. Their children from 6 to 18 months received nothing. Women in the MMN group received MMN during pregnancy and one daily tablet of MMN after birth until 6 months. Their children from 6 to 18 months received nothing. Women in the LNS group received LNS during pregnancy and one daily sachet of LNS-P&L (20g of LNS) after birth until 6 months, and their children received 2 daily sachets of LNS-20gM (20g of LNS) from 6 to 18 months.

In the planned analysis, children born from women randomized into the LNS group will form the intervention group and children born from women randomized into either MMN or IFA group will form the control group.

## 2. Hypotheses to be tested

Children who received complementary foods supplemented with LNS from 6 months to 18 months have more daytime naps than children who did not receive the supplement.

Children who received complementary foods supplemented with LNS from 6 months to 18 months have longer night time sleep duration than children who did not receive the supplement.

Children who received complementary foods supplemented with LNS from 6 months to 18 months wake up less often at night than children who did not receive the supplement.

## 3. Definition of the outcomes

Number of naps during daytime (Daytime Nap index)

For the number of daytime naps, we will construct a variable called daytime nap index. It will be defined as the sum of daytime naps from all collected forms 33 during the follow-up divided by the number of forms collected during this time period. From each form, we will sum the number of times the child took a nap during the previous day after s/he had woken up from the previous night's sleep and before she went to bed the following evening. The data will be extracted from Form 33: Q 3.1.

For the main analysis, the Daytime nap index will cover the whole follow-up period, ie weeks 27 – 78, and the variable will be marked as Daytime nap index$_{27\text{-}78}$.

For secondary analyses, the index will be calculated quarterly (each representing 3 months in follow-up), and marked as Daytime nap index$_{27\text{-}39,}$ Daytime nap index$_{40\text{-}52,}$ Daytime nap index$_{53\text{-}65,}$ Daytime nap index$_{66\text{-}78}$

Duration of night time sleep (Night Time sleep duration index)

For the night time sleep duration, we will construct a variable called night time sleep duration index. It will be defined as the sum of night time sleep duration from all collected forms 33 during the follow-up divided by the number of forms collected during this time period. From each form, the nighttime sleep duration will be defined as the difference in number of hours between the time the child went to bed (for the overnight sleep) and woke up on the following morning. The data will be extracted from Form 33: Q 3.2.

For the main analysis, Night Time sleep duration index will cover the whole follow-up period, ie weeks 27 – 78, and the variable will be marked as Night Time sleep duration index$_{27\text{-}78}$.

For secondary analyses, the index will be calculated quarterly (each representing 3 months in follow-up), and marked as Night Time sleep duration index$_{27\text{-}39,}$ Night Time sleep duration index$_{40\text{-}52,}$ Night Time sleep duration index$_{53\text{-}65,}$ Night Time sleep duration index$_{66\text{-}78}$

Number of wake-ups during the night time (Night waking index)

For the number of night time wake ups, we will construct a variable called night waking index. It will be defined as the sum of night time wake-ups from all collected forms 33 during the follow-up divided by the number of forms collected during this time period. From each form, we will sum the number of times the child woke up during the previous night. The data will be extracted from Form 33: Q 3.3.

For the main analysis, Night waking index will cover the whole follow-up period, ie weeks 27 – 78, and the variable will be marked as Night waking index$_{27\text{-}78}$.

For secondary analyses, the index will be calculated quarterly (each representing 3 months in follow-up), and marked as Night waking index$_{27\text{-}39,}$ Night waking index$_{40\text{-}52,}$ Night waking index$_{53\text{-}65,}$ Night waking index$_{66\text{-}78.}$

## 4. Basis for the analysis: Intention to treat

Primarily, the analysis will be based on the principle of modified intention-to-treat. The modification concerns two participants who were accidentally allocated to another group than actually randomized. For each participant, the randomization code was pre-packed and sealed in an individual envelope that was opened and used for group allocation at enrolment. For these two individuals, the randomizer made a recording error, i.e. s/he noted down in a data collection form an incorrect group code or wrote the code with unclear handwriting. The incorrect code was later transcribed into the computer software that was used to plan participant visits and allocate interventions. These two participants were told to belong to the erroneously recorded intervention group and they received that intervention throughout the trial – hence they will also be analyzed in that group (rather than the one written on the randomization slip). Another modification is that children who have form 33 data collected 2 times or more will be included in the analysis of each outcome variable.

## 5. Time points for the analyses

The analysis for the study will cover five time intervals, Weeks 27-78, Weeks 27-39, Weeks 40-52, Weeks 53-65, and Weeks 66-78.

## 6. Presentation of the study findings and hypothesis testing

Success of enrolment and follow-up

The success of enrolment and follow-up for all registered participants will be described in a flow chart (figure 1).

Baseline Information

Selected participant and mothers' summary characteristics at enrollment will be tabulated by intervention arms as indicated in table 1. Hypotheses testing about the difference between the LNS and control groups will be performed by the testing methods indicated in Table 1. P-values from these tests will be obtained but will not be presented in Table 1 of the eventual write up.

Comparison of sleep pattern between LNS and Control groups

The Histograms of day time naps, nighttime sleep duration and night waking frequency for the LNS and Control groups will be shown in Figure 2, Figure 3 and Figure 4 respectively. The difference in group means and standard deviations for Daytime Nap index, Night Time sleep duration index, and Night waking index frequency will be presented as indicated in Table 2. The table also shows 95% confidence intervals between the LNS and the Control groups. The differences for number of day time naps, Night Time Sleep Duration and Wake up Times by intervention group will also be shown graphically in Figure 5a, Figure 5b and Figure 5c respectively.

**Evaluation**

The differences in child sleep between LNS and Control groups will be evaluated using the superiority test. This is because we hypothesize that infants in the LNS group will have higher mean daytime naps index, longer mean night time sleep duration index and lower mean nighttime waking index than children in the control group. A one sided test of significance will be used, with a P value of 0.05 denoting significant difference in sleep amount and quality. If a statistically significant difference is not found, it will be concluded that the data do not support the hypothesis.

General notes on statistical methods

7.1 Software

Analyses will be done in Stata version 12.

### 7.2 Preparing sleep pattern data for analysis

All the day time naps, nighttime sleep duration and night waking frequency data were completed during the 4-weekly visits. The data were checked for suspicious and missing values using Stata and Microsoft excel and corrections were effected where necessary. Day time naps and night waking frequency were recorded as whole numbers on the data collection form. The sleep duration was calculated as the difference in number of hours between the time the child went to bed (for the overnight sleep) and woke up on the following morning.

### 7.4 Confidence intervals

The confidence intervals (CI) at 95% level will be provided for all the three main outcomes. The general group (at baseline) level comparison will also contain 95% CI.

### 7.5 Covariate adjustment

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

## 7. References

## 8. Legends to the figures

Figure1. Participant flow Chart

Figure2. Histogram of Child Day Time Nap

Figure3. Histogram of Child Sleep Duration

Figure4. Histogram of Child Wake Up Time

Figure5 (a-c) slopes for child daytime naps, Night time sleep duration and Night waking up frequency

**Figure 1: Participant flow**

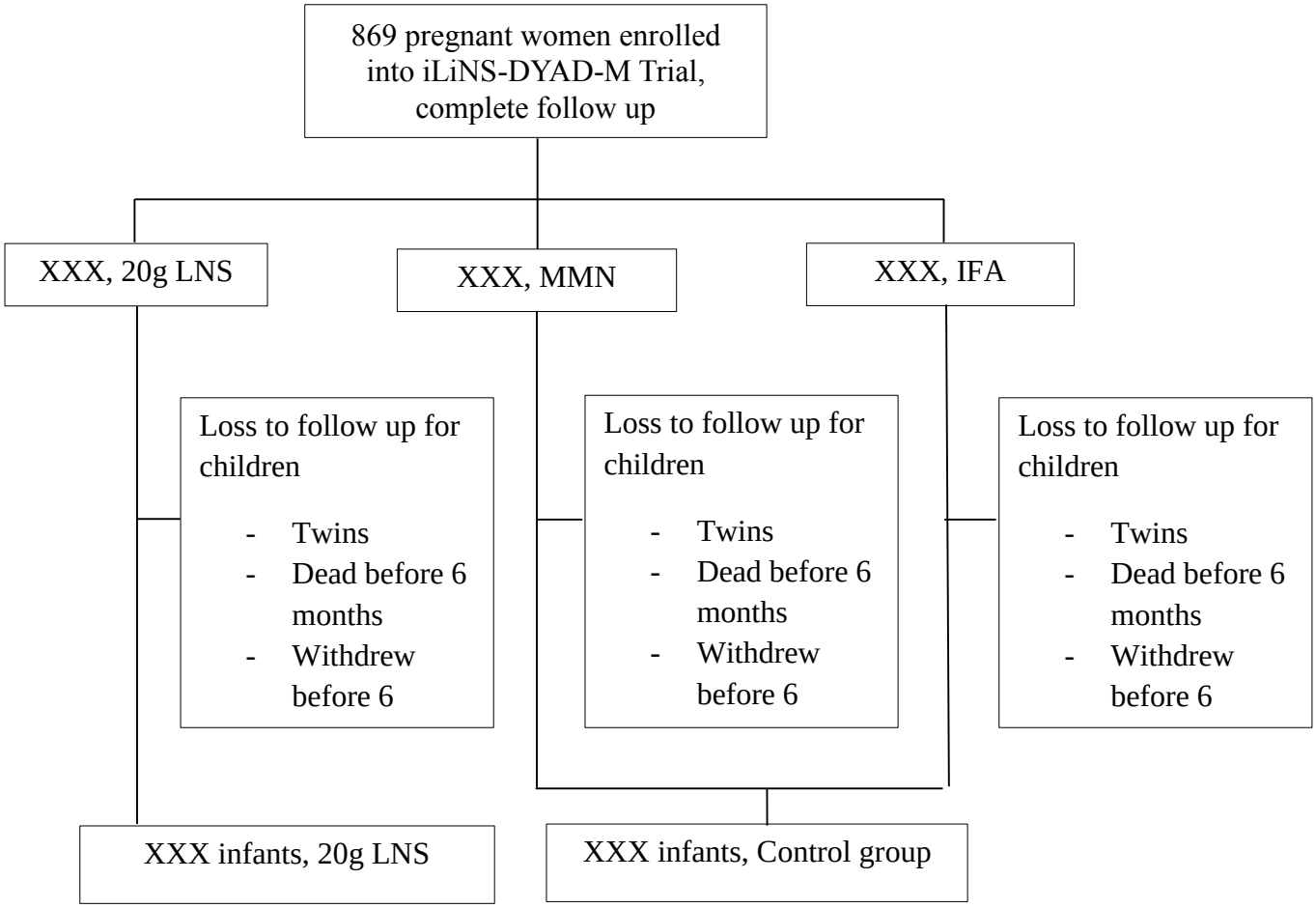


Figure2. Histogram of Child Day Time Naps

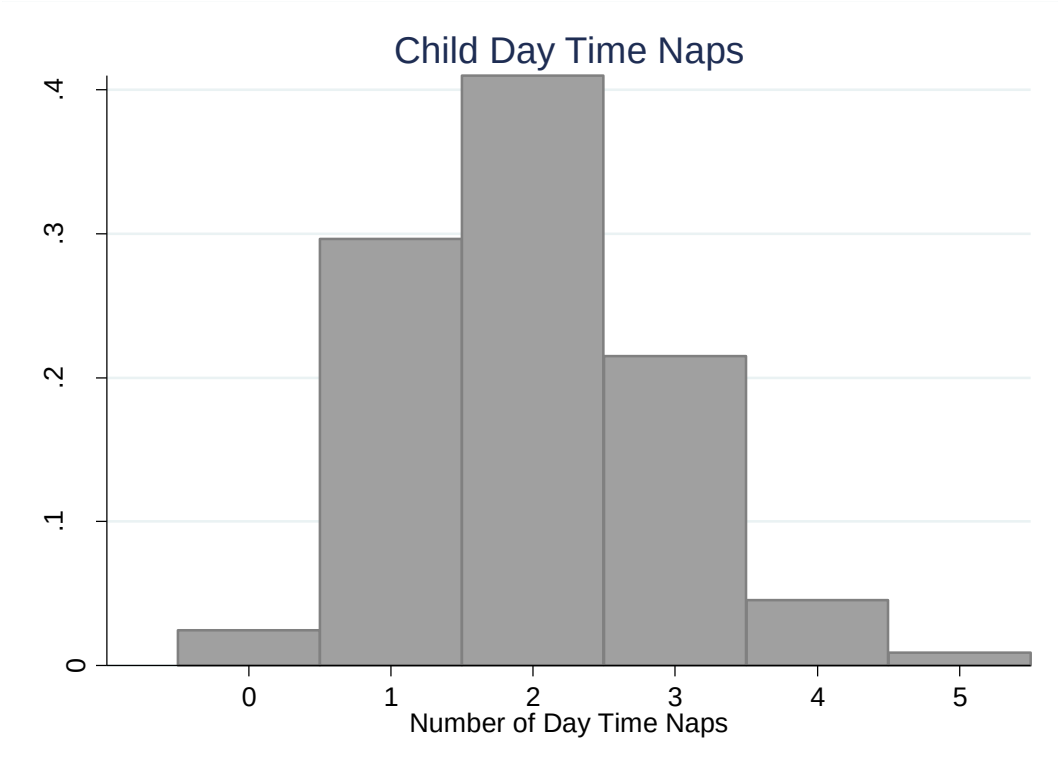


Figure3. Histogram of Child Sleep Duration

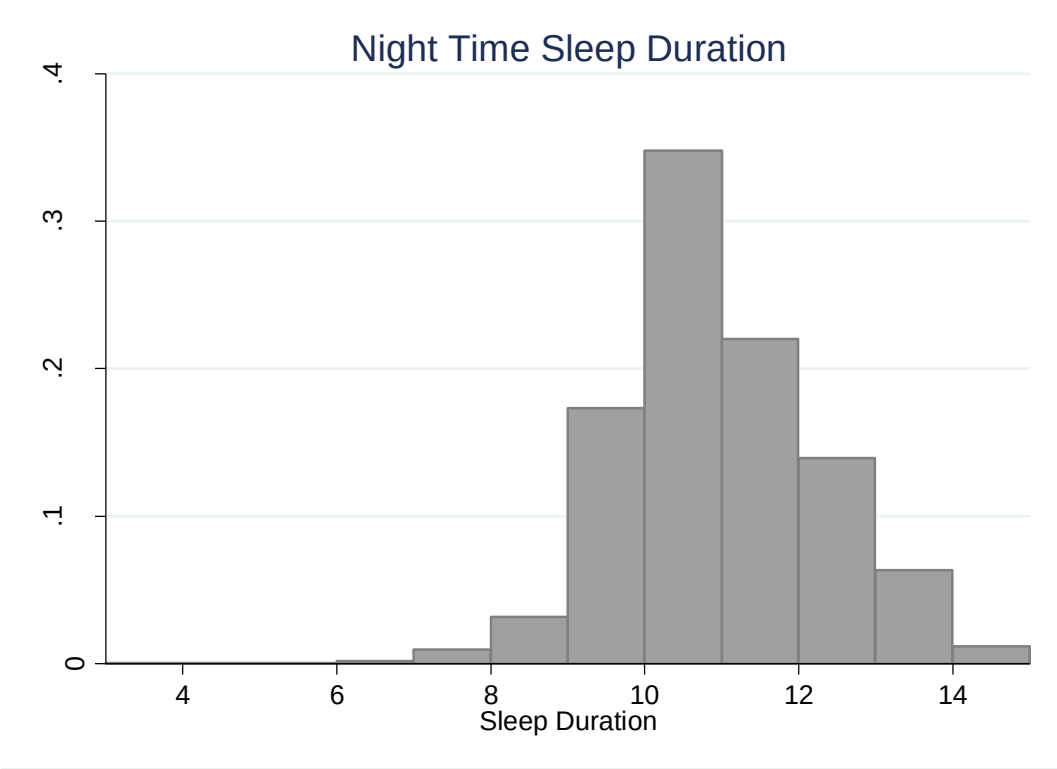
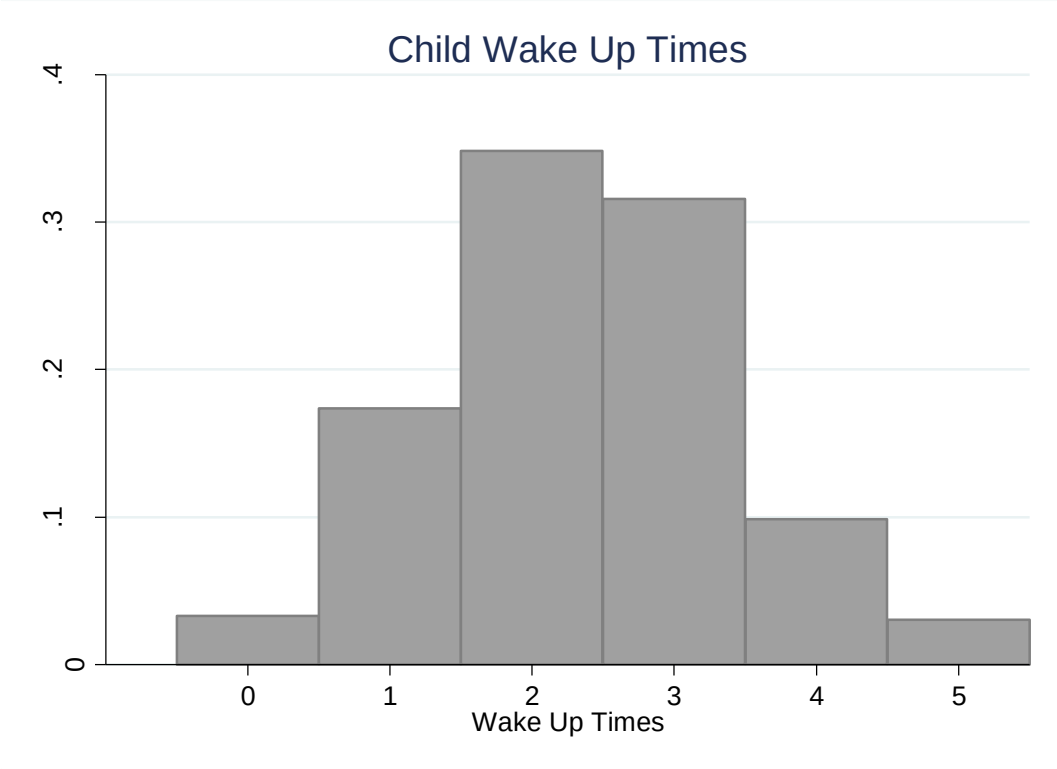


Figure4. Histogram of Child Wake Up Time



## 9. Tables

**Table 1 Baseline characteristics of the participating women at enrolment and Children by study group**

| **Variable** | **LNS Group** | **Control Group (IFA and MMN)** | **Test** |
|---|---|---|---|
| Number of participants | xxx | xxx | |
| **Maternal characteristics** | | | |
| Age, year (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | T Test |
| Mean (SD) maternal education,<br>completed years at school | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Mean (SD) proxy for socioeconomic<br>status | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| BMI, $kg/m^2$ (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| **Infants characteristics** | | | |
| Age, months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Proportion of Males (percentage) | xxx / xxx (xx%) | xxx / xxx (xx%) | Chi-square |
| Child Naps at 5 months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | xxxxx |
| Child Sleep duration at 5 months (mean, SD,N) | xx.x (xx.x) | xx.x (xx.x) | xxxxxx |
| Child wake up time at 5 | xx.x (xx.x) | xx.x (xx.x) | xxxxxx |

| | | | |
|---|---|---|---|
| months (mean, SD, N) | | | |
| Weight, kg at 6 months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |

**Table 2 Comparison of sleep patterns between children in the control and intervention groups, intention-to-treat analysis**

| **Variable** | | **Result by study groups** | | |
|---|---|---|---|---|
| | **LNS** | **Control** | **Difference in means (95%CI)** | **P-value** |
| Mean (SD) Daytime naps | | | | |
| Mean (SD) Daytime nap index$_{27\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Daytime nap index$_{27\text{-}39}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Daytime nap index$_{40\text{-}52}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Daytime nap index$_{53\text{-}65}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Daytime nap index$_{66\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) nighttime sleep duration | | | | |
| Mean (SD) nighttime sleep duration index$_{27\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) nighttime sleep duration index$_{27\text{-}39}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) nighttime sleep duration index$_{40\text{-}52}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) nighttime sleep duration index$_{53\text{-}65}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |

| | | | | |
|---|---|---|---|---|
| Mean (SD) nighttime sleep duration index$_{66\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Night Waking frequency | | | | |
| Mean (SD) Night Waking index$_{27\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Night Waking index$_{27\text{-}39}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Night Waking index$_{40\text{-}52}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Night Waking index$_{53\text{-}65}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |
| Mean (SD) Night Waking index$_{66\text{-}78}$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xxx |

**Figure 5a Child Number of Day Times Naps by intervention group**

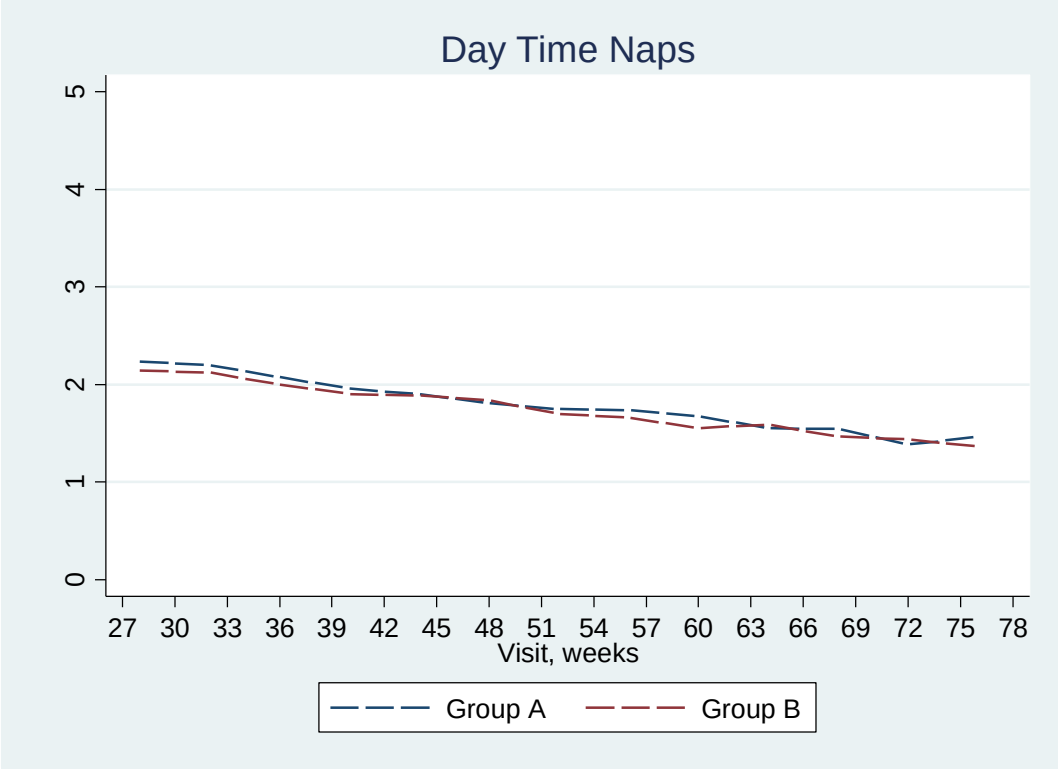


**Figure 5b Child Night Time duration by intervention group**

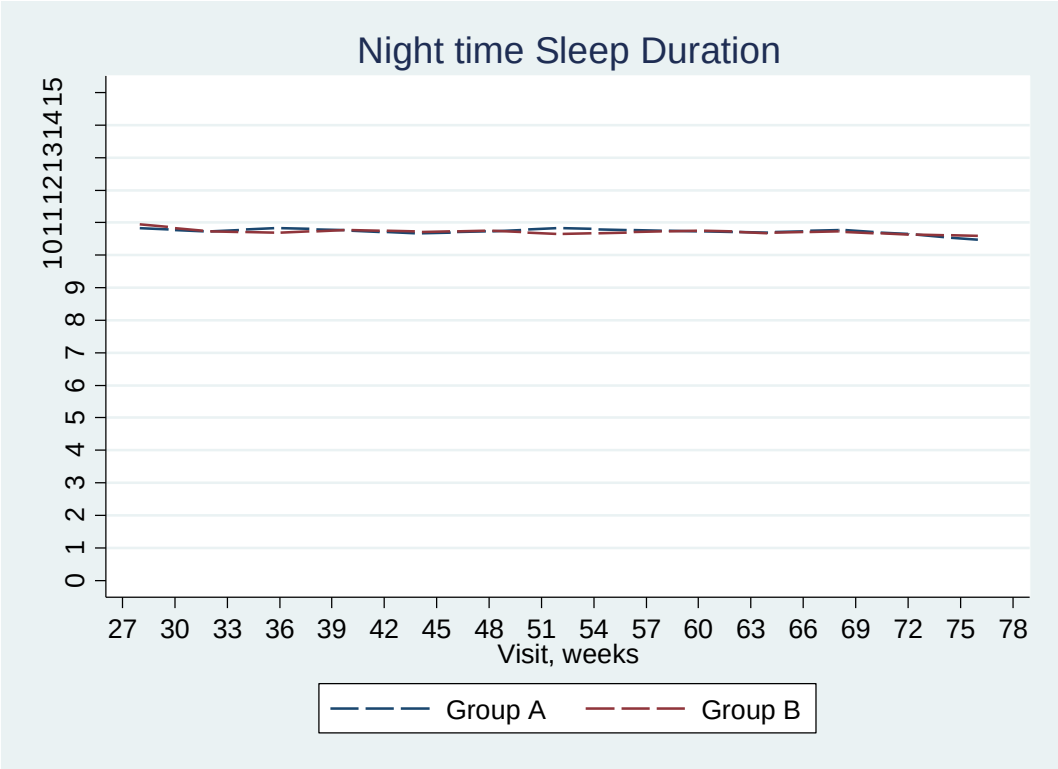
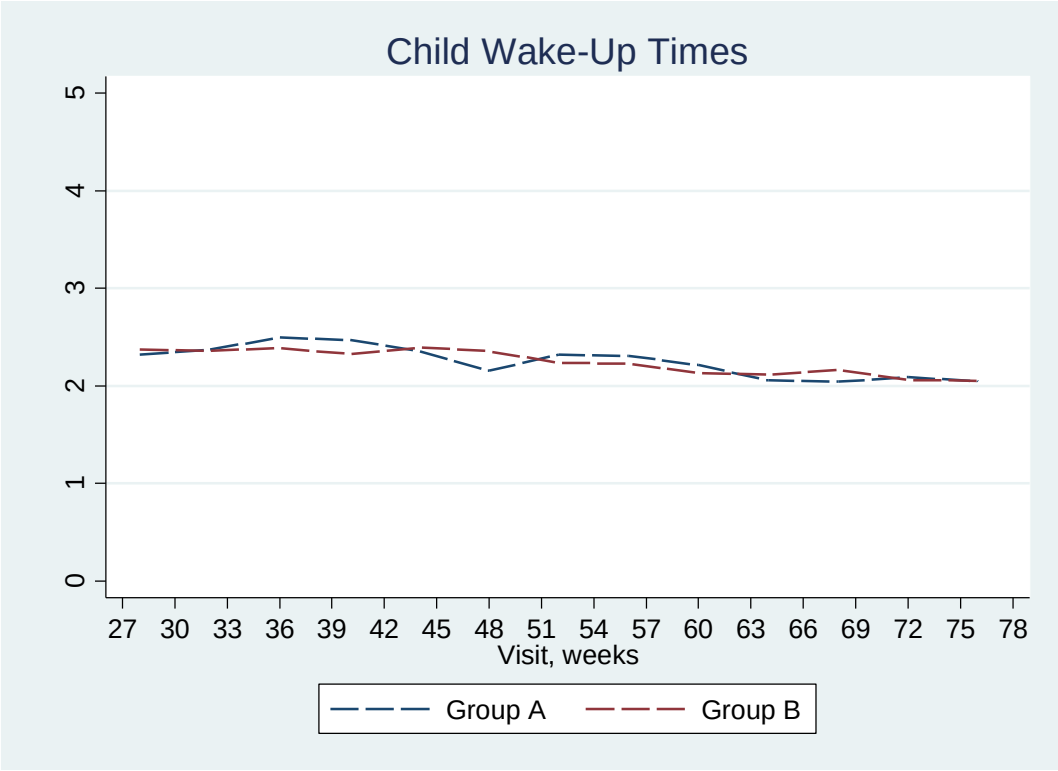


**Figure 5c Child waking up times by intervention group**



**Supplementing Maternal and Infant Diet with Micronutrient Fortified Lipid-based Nutrient Supplements (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 22: Effect of lipid-based nutrient supplements on delivery complications (version 01.0, 2015-05-25)

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Version history

| **Version number** | **Version date** | **Prepared by** | **Description of the completed editions** |
|---|---|---|---|
| 01.0 | 2015-05-25 | Alho, Cheung, Juha Pyykkö | Original document |

## 1. Overview and study objectives

The main aim of the trial was to determine whether lipid-based nutrient supplements (LNS) consumed by the mother during pregnancy and the first 6 months of lactation, and by the child from age 6-18 months, improves foetal and child growth, micronutrient status and neurobehavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

The aim of the secondary analyses described in appendix 22 is to compare delivery complications in three different intervention groups. Details of the objectives are as follows:

- To determine if there are differences in the proportion of delivery complications between the intervention groups.
- If there are differences between the groups, a secondary objective is to identify possible effect modifiers and intermediate outcomes in the pathway between intervention and delivery complications.

## 2. Hypotheses to be tested

The study group has already completed some exploratory analyses on the incidence of delivery complications and observed a higher incidence of caesarean sections in the LNS group than the IFA group. The purpose of the present analysis – which should be considered exploratory rather than confirmatory – is to analyse more carefully the association between the intervention and the delivery complication. The study group has defined the following (post-hoc) hypotheses that will be tested in the analysis. All hypotheses apply to the main defined target group for the iLiNS-DYAD trial, i.e. rural Malawian women who live in Mangochi District.

1. Women who receive LNS supplementation during pregnancy will have a higher incidence of caesarean sections than women who receive a standard supplementation with IFA.
2. Women who receive LNS supplementation during pregnancy will have a higher incidence of obstructed labour than women who receive a standard supplementation with IFA.
3. Women who receive LNS supplementation during pregnancy will have a higher incidence of any delivery complication than women who receive a standard supplementation with IFA.
4. The association between the dietary supplementation scheme and the incidence of the above mentioned delivery complications (caesarean section, obstructed labour, or any delivery complication) is modified by defined maternal characteristics (height, parity, educational level, HIV infection, malaria infection, oral periapical infections, elevated plasma CRP concentration, elevated plasma AGP concentration).
5. The association between the dietary supplementation scheme and the incidence of above mentioned delivery complications is mediated through fetal head size.

## 3. Definition of the outcome variables

- **Delivery by caesarean section.** Planned caesarean section or emergency caesarean section. *The data will be extracted from Form 23: Q2.4.*
- **Any signs of obstructed labour.** Caesarean section (described above), vacuum extraction, prolonged labour, large perineal tear or symphysiotomy. *The data will be extracted from Form 23: Q2.4, Q3.3, Q3.6, Q3.7, Q3.8.*
- **Any delivery complication.** Obstructed labour (described above), or stillbirths or neonatal death. *The data will be extracted from Form 06: Q1.2, Q7.6.1, Q7.6.2; Form 23: Q2.1, Q2.4, Q3.3, Q3.4, Q3.6., Q3.8; Form 24: Q2.1; Form 47: Q2.1.*

## 4. Time points for the analysis

All outcomes are based on delivery and newborn details after birth and during neonatal period. Maternal size is determined at the enrolment.

## 5. Basis for the analysis: Intention to treat and per protocol

Analysis will be by intention-to-treat.

## 6. Approach to analysis and exclusions specific to this analysis

All tests will be two-sided, at 5 % or 10 % level of significance.

Twins will be excluded from analysis. There will be no other exclusions. All available data will be used.

All analyses will primarily be carried out with the existing data set, i.e. missing data points will be considered missing in the analysis. As a sensitivy analysis, we will repeat the analyses with a dataset, where missing values have been imputed with a multiple imputation by chained equations (MICE) method.

## 7. Presentation of the study findings and hypothesis testing

### 7.1 Success of enrolment

All enrolled participants and the success of their follow-up will be described in a flow chart (Figure 1) according to the CONSORT guidelines.

### 7.2 Baseline information

Maternal characteristics at enrolment will be tabulated by study group (Table 1).

### 7.3 Success of follow-up

Comparison of analysis sample to those lost to follow-up will be presented (Table 2). The drop-out rates (proportion of participants who drop out) will be compared between the three intervention groups and the statistical significance of any observed difference will be tested with Fisher's exact test. Additionally, we will compare the baseline characteristics between those who are included in or excluded from the analysis on delivery complications. The statistical significance of any observed difference will be tested with t-test or Fisher's exact test. P-values for these tests will be described in the text.

### 7.4 Comparison of the outcomes between the three intervention groups

The total numbers and proportions of participants experiencing each outcome in the entire sample will be presented in the text. Outcome variables will then be tabulated by intervention group (IFA / MMN / LNS) and risk ratios (95% CIs) will be calculated (Table 3). We will do hypothesis testing with Fisher's exact test and null-hypothesis of no difference between groups will be rejected if $P<0.05$. Pairwise comparisons will be done with log-binomial regression. For pairwise comparisons with $P<0.05$, the hypothesis of no difference in proportions between groups will be rejected only if the global null-hypothesis is also rejected. P-values for both tests will be presented. The following outcomes will be presented:

- proportion of participants who delivered by caesarean section
- proportion of participants with any signs of obstructed labour
- proportion of participants with any delivery complications

### 7.5 Comparison of maternal and child characteristics among participants with or without caesarean section

In order to understand potential mechanisms how LNS intervention might be associated to caesarean section we will compare selected maternal and child characteristics among participants with or without caesarean section.

Variables that show differences in the distribution between the two groups of participants may theoretically act in two different ways in the pathway between the intervention and caesarean section outcome:

a) Variables that can not be affected by the intervention can be effect modifiers – i.e. they may make the participant especially vulnerable for the outcome (e.g. LNS intervention may be a risk factor for caesarean section among short or primiparous women, but not among tall or multiparous women). These kinds of associations are analysed with interaction tests and stratified models (see chapter 7.6).
b) Variables that can be affected by the intervention may act as intermediate outcomes, between the intervention and the ultimate outcome (e.g. LNS intervention may increase the child's head-size, which may lead to caesarean section). These kinds of associations are analysed with attenuation analyses that are built on stepwise multivariate regression models (see chapter 7.7).

The list of variables included in this analysis includes maternal and child size, duration of pregnancy, maternal parity, and variables that have been shown to modify the association between intervention and some birth outcomes in our earlier analyses (such as maternal infections or inflammation).

Table 4 shows how the results from this analysis will be tabulated and compared. The statistical significance of any observed differences will be tested with t-test or Fisher's exact test.

The following characteristics will be compared between those with or without caesarean section:

- proportion of male children
- proportion of primiparous women
- mean (SD) gestational age at birth in weeks
- mean (SD) child length-for age Z-score (LAZ)
- mean (SD) child weight-for-height Z-score (WLZ)
- mean (SD) child head circumference Z-score
- mean (SD) maternal height in centimeters
- mean (SD) maternal BMI
- mean (SD) maternal age in years
- mean (SD) maternal AGP at enrolment
- mean (SD) maternal CRP at enrolment
- proportion of women with high AGP (>1) at enrolment
- proportion of women with high CRP (>5) at enrolment
- proportion of mothers with dental periapical infection
- proportion of women with a positive HIV test
- proportion of women with a positive malaria test (RDT)
- mean (SD) maternal education, competed years at school
- proportion of women with less than 4 years of education
- mean (SD) child head circumference (cm) / maternal height (cm)

For continuous variables, we will also show Kernel density plots describing the complete distributions of variable values (an example is shown in Figure 2).

### 7.6 Effect modification

There will be tests for interaction between the intervention group and selected other variables on their association with caesarean section. As a sensitivity test, we will complete similar analyses using any signs of obstructed labour, or any delivery complications as the outcome variable.

Variables included in this analysis include items that have been observed to modify the effect of a nutritional intervention either in our earlier analyses from the iLiNS-DYAD trial or in some other intervention trials carried out by other research groups.

The interaction will be tested with likelihood ratio testing. If a statistically significant interaction ($P<0.10$) is found, we will proceed to stratified analyses, assessing the association between the intervention and caesarean section separately among those with or without the defined characteristics. For the interaction tests, variables will be treated as continuous variables, where possible. For the stratified analyses, we will dichotomise all the predictor variables, either at median or at another logical point (e.g. primiparous vs other women).

For variables that have shown effect modification in our previous analyses, we will proceed to stratified analyses regardless of the interaction test result. These variables are listed as number 1-9 in the list below.

Variables included in the effect modification analysis include the following:

1. Maternal primiparity
2. Maternal height
3. Maternal BMI at enrolment
4. Maternal education
5. Maternal HIV status at enrolment
6. Maternal peripheral blood malaria parasitaemia at enrolment
7. Maternal AGP at enrolment (>1 vs ≤1)
8. Maternal CRP at enrolment (>5 vs ≤5)
9. Maternal periapical infections
10. Child sex
11. Food insecurity at enrolment
12. Gestational age at enrolment
13. Maternal age
14. Maternal anemia at enrolment
15. Season at enrolment
16. Study site

### 7.7 Association attenuation analyses by cumulative stepwise multivariate regression models

This analysis is designed to identify possible intermediate outcomes in the pathway between intervention and delivery complications. The analyses are primarily carried out with caesarean section as the outcome variable. As a sensitivity test, we will complete similar analyses using any signs of obstructed labour or any delivery complications as the outcome variable.

The analyses will be started by building a regression model that includes only two variables: caesarean section as the outcome and intervention group as the predictor. We will then add one or more additional variables into the model, considered as a potential intermediate outcome. If the addition of any specific variable attenuates the association between the intervention and caesarean section (as indiated by a decrease in log-binomial regression co-efficient or a loss of statistical significance), the added variable will be deemed an intermediate outcome in the pathway between the intervention and caesarean section.

Variables tested as potential intermediate outcomes in this analysis will include the following:

- child's LAZ
- child's WLZ
- child's head circumference Z-score
- duration of pregnancy.

In the first phase, each of the above variables will be independently added into the regression models. In the second phase, we will test different combinations of the above in the model. These models will be presented in Table 5.

The attenuation tests will be completed both for the unstratified analyses (full data set) and also for any of the stratified analyses showing statistically significant association between the intervention and caesarean section.

## 8. Detailed statistical methods

### 8.1 Software

All analyses will be done using R version 3.1.2 or higher (R Foundation for Statistical Computing, Vienna, Austria) and Stata version 13.1 or higher (StataCorp, TX, USA).

### 8.2 Covariate adjustment

Comparison of the outcomes between the three intervention groups is planned to be completed and shown in tables and figures without any covariate adjustment. As sensitivity test, we will complete similar analyses using an adjusted regression model. The covariates to be included in the models will be derived from the list in chapter 7.6.

### 8.3 Multiple comparisons

The study involves multiple objectives and therefore multiple sets of hypothesis. Statistically, the different sets of hypotheses are considered independent families of hypotheses. Statistical adjustment for multiple comparisons in one family of hypotheses does not need to consider the other families.

For analyses presented in this analysis plan, each family consists of three hypotheses, two comparing an intervention group versus the control group and one comparing the two intervention groups to each other. To account for the three comparisons, we will begin the analysis by testing the global null hypothesis of no difference between groups. If the global null hypothesis is rejected, raw P-values are used in the comparisons between intervention and control groups. Pairwise comparisons will be carried out in any case but the interpretation of the results is that pairwise comparisons are rejected only if the global null hypothesis is also rejected. This closed testing procedure is adopted to prevent inflated type I errors caused by multiple testing (Cheung 2014).

## 9. Design of tables and figures

The tables listed below will be examined by the manuscript writing group, and final decisions on how to best consolidate results across sites for presentation in a manuscript will follow later.

Table 1. Baseline characteristics of the participating women at enrolment, by study group1

Table 2. Comparison of analysis sample to those lost to follow-up

Table 3. Comparison of delivery complications by study group1

Table 4. Comparison of maternal and birth characteristics by the outcome variables

Table 5. Association attenuation analyses1

Figure 1. Participant flow

Figure 2. Outcome characteristics

Additional Tables and Figures as needed to describe or illustrate interactions. On the following pages, Tables and Figures show an example format.

## 10. References

Cheung, Y. B., *Statistical Analysis of Human Growth and Development*, CRC Press, 2014

TABLE 1. BASELINE CHARACTERISTICS OF THE PARTICIPATING WOMEN AT ENROLMENT, BY STUDY GROUP[1]

| **Characteristic** | **IFA** | **MMN** | **LNS** | **P-value**[2] |
|---|---|---|---|---|
| Number of participants | N | N | N | N/A |
| Mean (SD) maternal age, years | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Mean (SD) maternal weight, kg | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Mean (SD) maternal height, cm | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Mean (SD) maternal BMI, kg/m$^2$ | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Mean (SD) gestational age at enrolment, weeks | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Mean (SD) maternal education, completed years | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx) | x.xx |
| Proportion of nulliparous women | xx.x% | xx.x% | xx.x% | x.xx |
| Proportion of anaemic women (Hb < 100 g/l) | xx.x% | xx.x% | xx.x% | x.xx |
| Proportion of women with a positive HIV test | xx.x% | xx.x% | xx.x% | x.xx |
| Proportion of women with a positive malaria test (RDT) | xx.x% | xx.x% | xx.x% | x.xx |

[1] IFA, iron and folic acid; LNS, lipid based nutrient supplement; MMN, multiple micronutrients.

[2] P-value obtained from ANOVA (continuous variables) or Fisher's exact test (proportions). The P-values will be calculated but not shown in eventual publications, unless the journal editors require them.

TABLE 2. COMPARISON OF ANALYSIS SAMPLE TO THOSE LOST TO FOLLOW-UP

| Characteristic | Included | Excluded | P-value[1] |
|---|---|---|---|
| Number of participants | | | |
| Mean (SD) maternal age, years | | | |
| Mean (SD) maternal education, completed years | | | |
| Proportion with severely food insecure households | | | |
| Proportion of nulliparous women | | | |
| Proportion of women with a BMI < 18.5 kg/m² | | | |
| Proportion of anaemic women (Hb < 100 g/l) | | | |
| Proportion of women with a positive HIV test | | | |
| Proportion of women w/ posit. malaria test (RDT) | | | |

[1] P-value obtained from ANOVA (continuous variables) or Fisher's exact test (proportions)

TABLE 3. COMPARISON OF DELIVERY COMPLICATIONS BY STUDY GROUP[1]

| | **Result by study group** | | | | **Comparison between LNS and MMN group** | | **Comparison between LNS and IFA group** | | **Comparison between MMN and IFA group** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristic** | **IFA** | **MMN** | **LNS** | **P-value[2]** | **Risk ratio (95 % CI)** | **P-value[3]** | **Risk ratio (95 % CI)** | **P-value[3]** | **Risk ratio (95 % CI)** | **P-value[3]** |
| Caesarean sections | x.x% (N) | x.x% (N) | x.x% (N) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx |
| Obstructed labour | x.x% (N) | x.x% (N) | x.x% (N) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx |
| Delivery complications | x.x% (N) | x.x% (N) | x.x% (N) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx | x.xx (xx to xx) | x.xx |

[1] IFA, iron and folic acid; LNS, lipid based nutrient supplement; MMN, multiple micronutrients.

[2] P-value obtained from Fisher's exact test.

[3] P-value obtained from log-binomial regression.

TABLE 4. COMPARISON OF MATERNAL AND BIRTH CHARACTERISTICS BY THE OUTCOME VARIABLES

| | Caesarean section | | | |
|---|---|---|---|---|
| | No | Yes | Difference (95% CI) | P-value[1] |
| Proportion of male children | | | | |
| Proportion of primiparous women | | | | |
| Mean (SD) gestational age at birth, weeks | | | | |
| Mean (SD) child length-for-age Z-score | | | | |
| Mean (SD) child weight-for-height Z-score | | | | |
| Mean (SD) child head circumference Z-score | | | | |
| Mean (SD) maternal height, cm | | | | |
| Mean (SD) maternal BMI, $kg/m^2$ | | | | |
| Mean (SD) maternal age, years | | | | |
| Mean (SD) maternal AGP at enrolment | | | | |
| Mean (SD) maternal CRP at enrolment | | | | |
| Proportion of women with dental periapical infection | | | | |
| % of women with a positive HIV test at enrolment | | | | |
| Proportion of women with a positive malaria test (RDT) at enrolment | | | | |
| Proportion of women with high AGP (>1) at enrolment | | | | |
| Proportion of women with high CRP (>5) at enrolment | | | | |
| Mean (SD) maternal education, completed years at school | | | | |
| Proportion of women with less than 4 years of education | | | | |
| Mean (SD) child head circumference / maternal height | | | | |

[1] P-value obtained from t-test (continuous variables) or Fisher's exact test (proportions).

TABLE 5. ASSOCIATION ATTENUATION ANALYSES[1]

| | Model 1 | | Model 2 | | Model 3 | | … | Model N | |
|---|---|---|---|---|---|---|---|---|---|
| Caesarean section | Risk ratio | P-value | Risk ratio | P-value | Risk ratio | P-value | … | Risk ratio | P-value |
| Group | | | | | | | | | |
| MMN | x.xx | x.xx | x.xx | x.xx | x.xx | x.xx | … | x.xx | x.xx |
| LNS | x.xx | x.xx | x.xx | x.xx | x.xx | x.xx | … | x.xx | x.xx |
| Child's head circumference Z-score | | | x.xx | x.xx | | | … | x.xx | x.xx |
| Child's WLZ | | | | | x.xx | x.xx | … | x.xx | x.xx |
| Child's LAZ | | | | | | | … | x.xx | x.xx |
| Duration of pregnancy, weeks | | | | | | | … | x.xx | x.xx |

[1] Risk ratios and P-values obtained from respective log-binomial regression model

FIGURE 1. PARTICIPANT FLOW

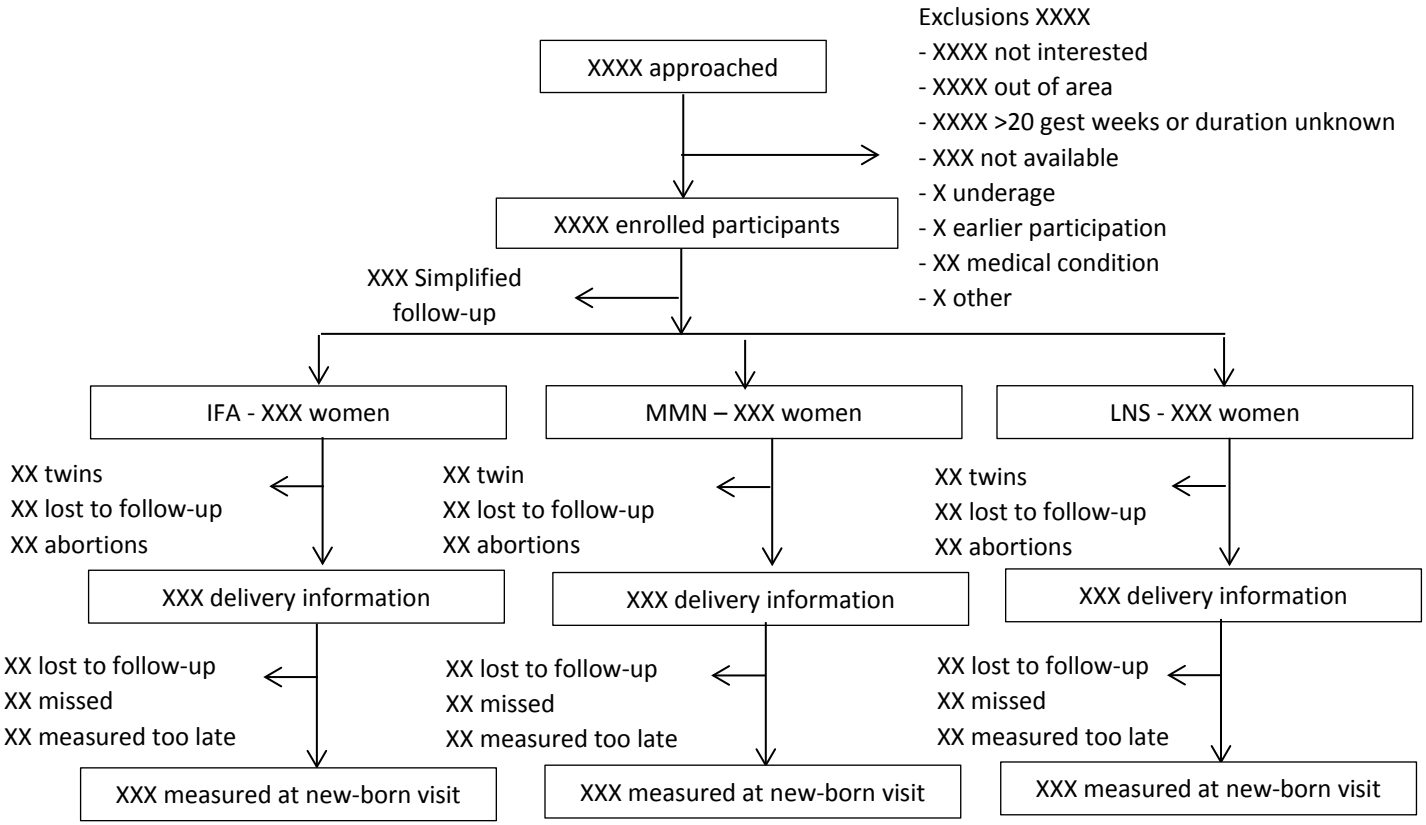
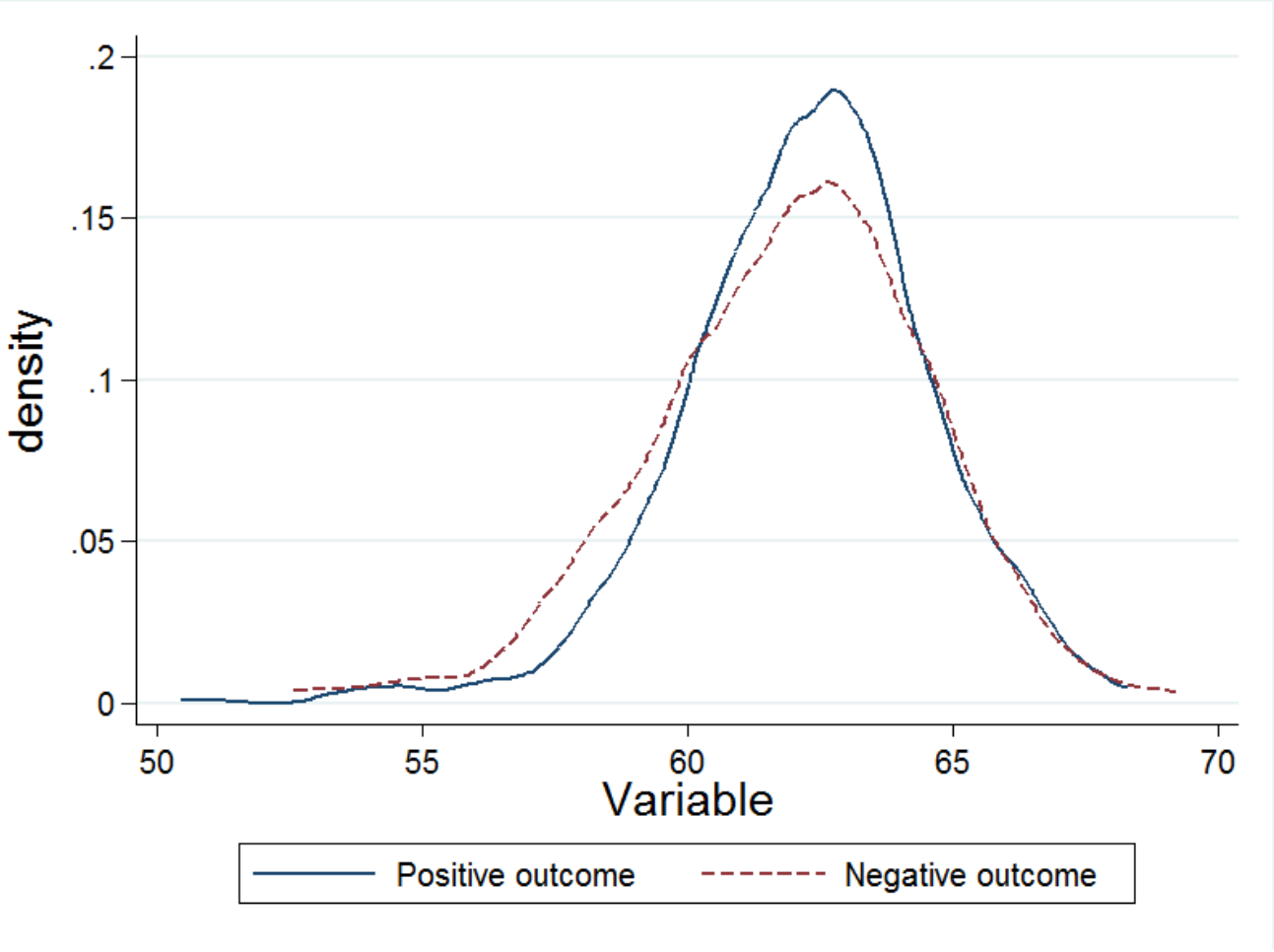


FIGURE 2. OUTCOME CHARACTERISTICS



**Impact of Lipid-Based Nutrient Supplements (LNS) on Child Appetite in Rural Malawi (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 23: Child appetite (Version 02.0, 19.04.2016)

Prepared by Harmony Phiri

Version history:

| | | |
|---|---|---|
| 2015-06-22 | Version 01.0 | Original document |
| 2016-04-19 | Version 02.0 | Finalised some small details in the document |

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## 1. Study objective

To assess whether daily consumption of LNS from 6 to 18 months improves appetite among infants and young children in rural Malawi

## 2. Study Materials

The data for this analysis will come from the iLiNS-DYAD-M trial. The targeted population includes all children between 6 and 18 months born to 869 pregnant women enrolled into the complete follow-up arm of the iLiNS-DYAD-M trial. Participants in this arm of the main study were randomized to receive either LNS or MMN or IFA during pregnancy. Women in the IFA group received IFA during pregnancy and a placebo after birth until 6 months. Their children from 6 to 18 months received no extra supplementation. Women enrolled in the MMN group received MMN during pregnancy and one daily tablet of MMN after birth until 6 months. Their children from 6 to 18 months received no extra supplementation. Women in the LNS group received LNS during pregnancy and one daily sachet of LNS-P&L ( 20g of LNS) after birth until 6 months; their children received 2 daily 10-g sachets of LNS-20gM (20g of LNS).

In the planned analysis, children born to women randomized into the LNS group will form the intervention group and children born to women randomized into either MMN or IFA groups will form the control group.

## 3. Hypothesis to be tested

The prevalence of anorexia in infants and young children who received 20g daily doses of LNS from 6 to 18 months is lower than that of infants and young children who did not receive the supplement.

## 4. Definition of primary outcomes

### 4.1. Anorexia Index

Weekly proportion of days during which anorexia was reported will be determined from daily child appetite status reports expressed as a percentage. Anorexia will be considered to be present when the child's appetite was reported to be either absent or reduced on a particular day. The formula to be used is: number of days in a week (in a visit) when child appetite was reported to be either reduced or absent (answer alternative 2 and 3) / sum of days with valid data on child appetite status during that week (answer alternative 1, 2 or 3)* 100. *The data will be extracted from iLiNS-DYAD-M Form 27: Q 2.6 (HomAppetite*).

Following this, various child anorexia indices will be calculated for all participants as mean proportion of days when anorexia was reported during the specified study follow-up weeks. The child anorexia indices are, in this statistical analysis, named by the words "Anorexia Index" followed by a range of study follow-up week numbers (which also reflect a child's age) marked as subscripts to indicate the time period of interest. For the main analysis, "Anorexia Index$_{27\text{-}78}$" will be calculated. This will cover all time points throughout the entire follow up period e.g. from week 27 to week 78 of follow-up. Thereafter the entire follow-up period will be split in 4 quarters by calculating four more Anorexia indices e.g. Anorexia Index $_{27\text{-}39}$, Anorexia Index $_{40\text{-}52}$, and Anorexia Index $_{53\text{-}65}$ and Anorexia Index $_{66\text{-}78}$. The formula to be used is: Anorexia Index = sum of days with anorexia reports / sum of days with valid data on appetite within period of interest.

## 5. Basis for the analysis

### 5.1. Intention-to-treat

This analysis will be based primarily on the principle of modified intention-to-treat. The first modification concerns two participants who were erroneously allocated to a study group other than the one into which they were actually randomized. For each participant, the randomization code was pre-packed and sealed in an individual envelope that was opened and used for group allocation at enrolment. For these two individuals, the randomizer made a recording error, i.e. s/he noted down in a data collection form an incorrect group code or wrote the code with unclear handwriting. The incorrect code was later transcribed into the computer software that was used to plan both participant

follow-up visits and distribution of interventions. These two participants were told to belong to the erroneously recorded intervention group and they received that intervention throughout the trial – hence they will also be analysed in that group (rather than the one written on the randomization slip). All randomized participants will be eligible to be included in the analyses, with the exception that subjects with missing data on *Homappetite* variable and those for whom Form 27 was administered less than 7 times will be excluded. Furthermore, twins will also be excluded from the analysis.

### 5.2. Per protocol analysis

In addition to the modified intention-to-treat analysis, a per-protocol analysis will be performed as supplemental evidence. This analysis will only include infants and young children in the LNS arm of the study who had adherence percentage >70% during the intervention period and all infants and young children in the control arm of the study.

## 6. Time points for the analyses

Analysis for the planned study will include 52 time points, representing the 52 weeks of follow up of the children in the study starting from week 27 to week 78.

## 7. Presentation of the study findings and hypothesis testing

### 7.1. Success of enrolment and follow-up

All enrolled participants and the success of their follow-up will be described in a flow chart (Figure 1)

### 7.2. Baseline information

We will tabulate selected summary characteristics at enrolment by intervention arms, as indicated in table 1. We will test hypotheses about a difference between the LNS and control groups using chi-square for categorical variables and t-test for continuous variables. P-values from these tests will be obtained but will not be shown in the eventual write up because at baseline, all possible differences should be due to random variation.

### 7.3. Comparison of Anorexia Indices between the intervention and control groups between week 27 and week 78

The 5 Anorexia Indices will be compared among the LNS group children with those in the control group and results will be shown as in Table 2.

The change over time of proportion of days when anorexia was reported will also be compared graphically (Figure 3).

## 8. Evaluation

The differences in effect of LNS on child anorexia will be evaluated using the superiority test. This is because, we hypothesize that infants and young children in the LNS group will have lower prevalence of anorexia than infants and young children in the control group. A one sided test of significance will be used, with a P value of 0.05 denoting significant difference in child anorexia. If a statistically significant difference will not be found, it will be concluded that the data do not support the hypothesis.

## 9. General notes on statistical methods

### 9.1. Software

Analyses will be done in Stata version 12.

### 9.2. Preparing child appetite data for analysis

Child appetite data were collected weekly during the entire follow-up period. During data collection, appetite status was recorded as being "Normal", "Reduced" or "None". In this analysis, the appetite variable will be dichotomized; responses "Reduced" and "None" will be combined and renamed as "Reduced". The dichotomized responses will then be "Normal" and "Reduced".

Prior to analysis, child appetite data were checked for suspicious and missing values using both Stata and Microsoft excel and corrections were effected where necessary.

### 9.3. Confidence intervals

The confidence intervals (CI) at 95% level will be provided for the study outcome. The general group (at baseline) level comparison will also contain 95% CI.

### 9.4. Covariate adjustment

The primary analysis is planned to be completed and shown in tables and figures without any covariate adjustments.

Secondarily, a sensitivity analysis will be carried out in which we will construct and show a regression model for the five appetite indices (Anorexia Index$_{27\text{-}78}$, Anorexia Index $_{27\text{-}39}$, Anorexia Index $_{40\text{-}52}$, and Anorexia Index $_{53\text{-}65}$ and Anorexia Index $_{66\text{-}78}$). The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association with any of the five appetite indices (a $p<0.1$ level), will be included in all the 5 models – i.e. all the models will be adjusted for the same set of covariates.

1. Baseline maternal age
2. Primiparity
3. Baseline maternal BMI
4. Baseline maternal education
5. Child sex
6. Child baseline age
7. Asset index
8. Study site

## 10. References

## 11. Design of figures and tables

The below figures and tables will be prepared based on the analysis:

**Figure 1: Participant flow**

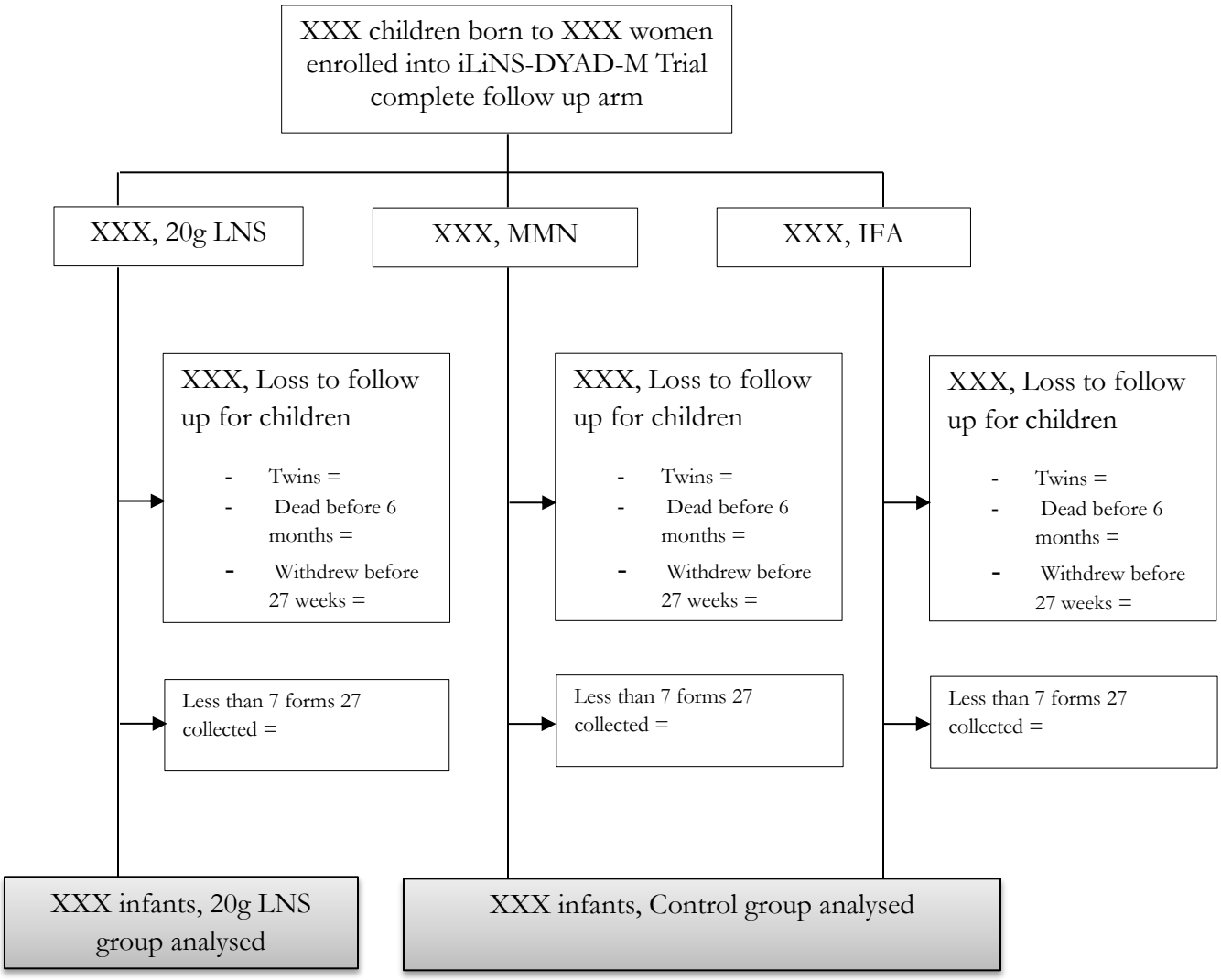

**Figure 2: Distribution of anorexia prevalence for the whole study population**

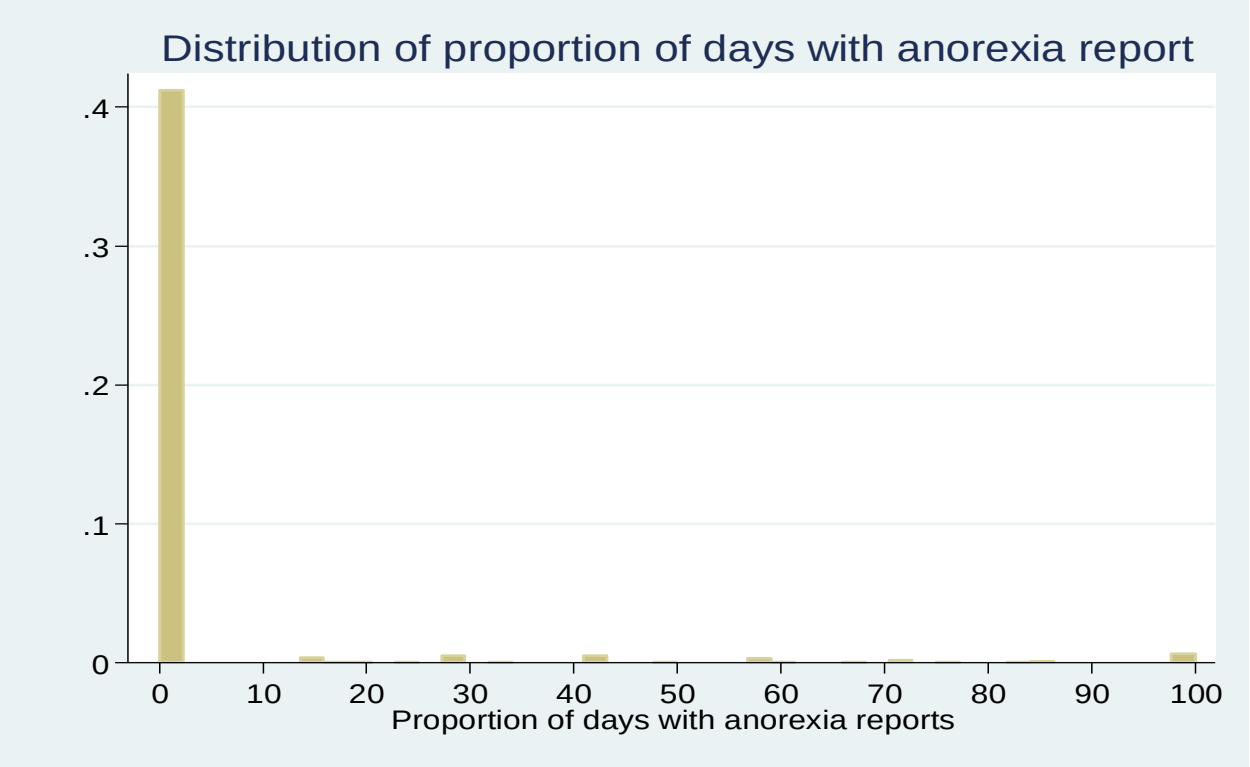

**Figure 3: Mean proportion of days when anorexia was reported for LNS and Control (IFA +MMN) groups**

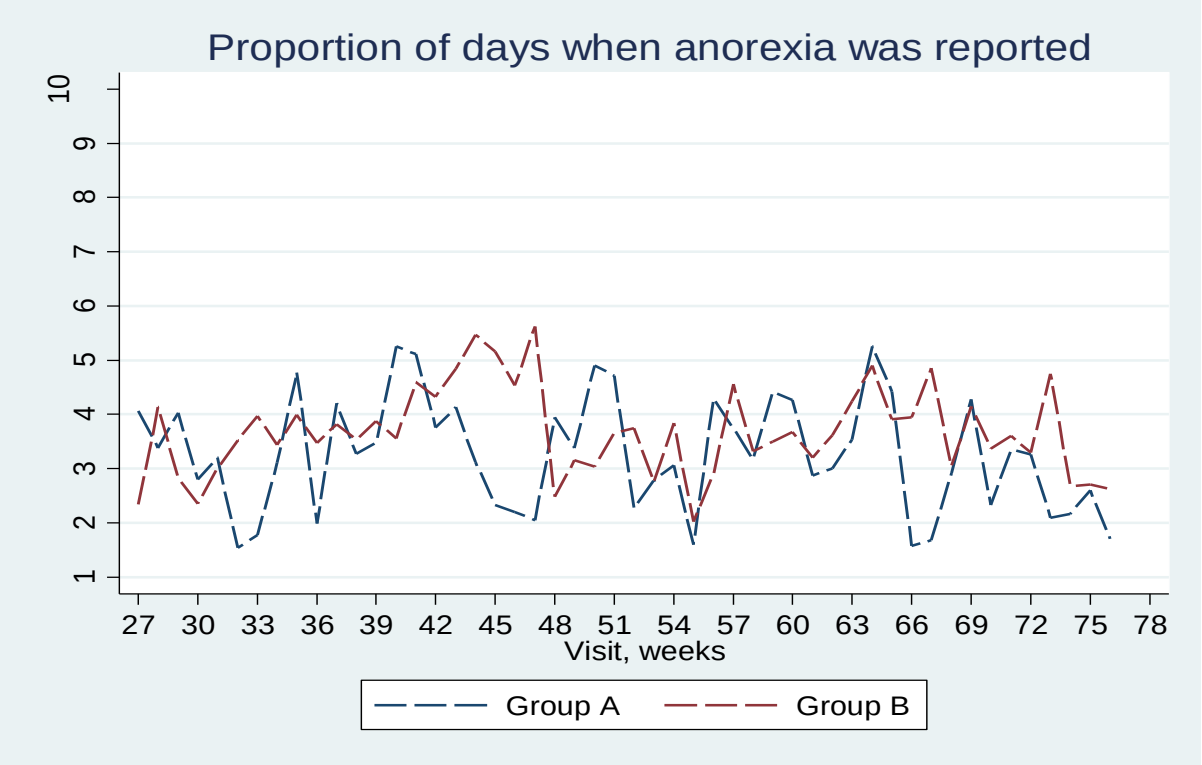

**Table 1 Baseline characteristics by study group**

| **Variable** | **Results by study groups** | | |
|---|---|---|---|
| | LNS | Control (IFA + MMN) | **Test** |
| **Maternal characteristics** | | | |
| Number of participants | xxx | xxx | |
| Age, year (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Completed school years (mean, SD) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Socioeconomic index (mean, SD) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| BMI, $kg/m^2$ (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| **Infants characteristics** | | | |
| Number of participants | xxx | xxx | |
| Age, months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Number of Males (percentage) | xxx/xxx (xx%) | xxx/xxx (xx%) | Chi-squared |
| Child appetite at 5 months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| Weight, kg at 6 months (mean, SD, N) | xx.x (xx.x) | xx.x (xx.x) | ANOVA |
| **Other characteristics** | | | |
| Site | | | |
| Mangochi | xxx | xxx | |
| Malindi | xxx | xxx | |
| Lungwena | xxx | xxx | |

**Table 2 Comparison of prevalence of anorexia for LNS and control (IFA+MMN) groups, modified intention-to-treat analysis**

| **Variable** | **Results by study groups** | | | | **Adjusted results by study groups** | | | |
|---|---|---|---|---|---|---|---|---|
| | LNS n=xxx | Control (IFA + MMN) n=xxx | Difference in means (95% CI) | P-value | LNS n=xxx | Control (IFA + MMN) n=xxx | Difference in means (95% CI) | P-value |
| **Mean (SD) Proportion of days with anorexia reports (%)** | | | | | | | | |
| Mean (SD) Anorexia index $_{27\text{-}78}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{27\text{-}39}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{40\text{-}52}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{53\text{-}65}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{66\text{-}78}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |

**Table 3 Comparison of prevalence of anorexia for LNS and control (IFA+MMN) groups, per protocol analysis**

| **Variable** | **Results by study groups** | | | | **Adjusted results by study groups** | | | |
|---|---|---|---|---|---|---|---|---|
| | LNS n=xxx | Control (IFA + MMN) n=xxx | Difference in means (95% CI) | P-value | LNS n=xxx | Control (IFA + MMN) n=xxx | Difference in means (95% CI) | P-value |
| **Mean (SD) Proportion of days with anorexia reports (%)** | | | | | | | | |
| Mean (SD) Anorexia index $_{27\text{-}78}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{27\text{-}39}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{40\text{-}52}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{53\text{-}65}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |
| | | | | | | | | |
| Mean (SD) Anorexia index $_{66\text{-}78}$ | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx | xxx (xxx) | xxx (xxx) | xxx (xxx,xxx) | xxx |

**Prevention of Linear Growth Faltering in Infants and Young Children With Lipid-based Nutrient Supplements (iLiNS-DYAD)**

Statistical Analysis Plan

Appendix 24: The effect of LNS on physical activity (added on 30.09.2015)

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## 1 Study objectives

The trial has three sets of objectives, defined at various phases of the trial.

The originally defined objective is to determine whether LNS consumed by the woman during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves foetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid (IFA) during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The aim of the secondary analyses described in appendix 25 is to assess the impact of LNS supplementation to the mothers during pregnancy and first 6 months postpartum and to the offspring from 6 to 18 months of age on physical activity of children at the age of 18 months. This will be done by comparing physical activity of all measured children in the "complete follow-up" group who received one of the three interventions:

a) lipid-based nutrient supplements (LNS group)

b) multiple micronutrient supplementation (MMN group)

c) iron and folic acid supplementation (IFA group)

The two latter groups (IFA and MMN) will be collapsed into a single control group.

A secondary aim is to explore possible effect modifiers of the impact of LNS supplementation on physical activity.

## 2 Hypotheses to be tested

Infants' mean physical activity and % of active infants will be greater in the group provided with LNS from 6 to 18 months of age, and to their mothers during pregnancy and first 6 months postpartum, than in the control group (i.e. who received either iron-folic acid or multiple micronutrient supplementation).

## 3 Definition of the physical activity outcomes

Primary outcome: mean accelerometer counts

Physical activity counts used in the analysis are vector magnitude counts, calculated by taking the square root of the sum of squared activity counts of each three axis. The mean counts/15 s of each day will be averaged over all valid days (i.e. days with minimum of 6 hours of data, see section 7.2. for details) to produce mean of means for each participant.

Data for physical activity will be considered missing if the actual measurement date was over 30 days after the target date.

Secondary outcomes

Mean vertical axis counts: For mean vertical axis accelerometer counts/15 s, mean counts of each day are averaged over all valid days and the average value is used in the analyses.

% time in moderate-to-vigorous physical activity (MVPA): Percentage of time spend in MVPA is averaged over all valid days and the averaged value (per participant) is used in the analysis. MVPA is defined as vertical axis activity counts ≥ 419 counts/15 s (Trost et al. 2011). Trost cut point for vertical axis is used to allow comparison with previous studies using that cut point and older models of accelerometers with only vertical axis readings.

% time being sedentary: Percentage of time spent being sedentary is averaged over all valid days and the averaged value (per participant) is used in the analysis. Sedentary time is defined as vertical axis activity counts ≤48 counts/15 s (Trost et al. 2011).

% of active children: Children, whose mean time in MVPA over all valid days is ≥90 minutes are considered active. Ninety minutes is based on the guidelines of U.S. National Association for Sports and Physical Education (NASPE 2009).

## 4 Basis for the analysis: Intention to treat and per protocol

The primary analysis will be by intention-to-treat, i.e. analysis according to original group assignment regardless of protocol violations**.** For assessing the success of the enrolment, all available data from participants lost to follow-up will be included.

## 5 Time points for the analyses

All the above analyses will be done at the end of the intervention when the child is 18 months old.

## 6 Presentation of the study findings and hypothesis testing

### 6.1 Success of enrolment, follow-up and physical activity measurement

All children in the "complete follow-up" who were not dropped out before 18 months of age were invited to participate in this sub-study. All enrolled participants and the success of their follow-up, including physical activity measurement, will be described in a flow chart (Figure 1). For additional information, drop-out rate (including participants for whom enough accelerometer data was not available) between groups will be tested with Fisher's exact test and baseline characteristics of drop-outs compared to those who completed the study will be tested with t-test or Fisher's exact test. P-values for these tests will be shown in the text.

### 6.2 Baseline information

Participant characteristics at birth and at physical activity measurement (at 18 months) will be tabulated by treatment arms as indicated in Table 1. Baseline information will be tested for differences between groups to give additional information but p-values will not be presented

in Table 1 of the eventual manuscript. Methods used for hypothesis testing are indicated in Table 1.

### 6.3 Comparison of physical activity between the intervention and the control groups

Figure 2 will show the kernel density plots for the main outcome, mean vector magnitude accelerometer counts by groups. Supplemental figures will show the kernel density plots for the secondary outcomes. The group means and standard deviations for the main outcome, mean vector magnitude counts, and for the secondary outcomes: mean vertical axis counts, % of time spent in MVPA and % of time spent being sedentary as well as the number (%) of active children, will be presented as indicated in Table 2. The table will also tabulate the difference in activity outcomes and their 95% confidence intervals between the intervention groups.

The difference between the two groups will be tested with Student's t-test (model without covariates) and regression model (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$.

As a sensitivity analysis, we will compare the three original groups (LNS, IFA and MMN). The differences will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$.

## 7 General notes on statistical methods

### 7.1 Software

All analyses will be done in Stata/SE version 12. The WHO 2006 multi-centre growth standard will be used for age-and-sex standardization of weight, length (height), weight-for-height, MUAC and head circumference.

### 7.2 Preparing physical activity data for analysis

Data that was originally compiled by ActiLife software from ActiGraph GT3X+ devices, will be extracted and combined using the following procedure:

- .gt3x files will be converted to .agd files (with 3 axes and 15s epoch length) and exported into a .csv file consisting data from several participants in ActiLife software
- .csv files will be brought to Stata/SE software and transferred to .dat file.
- Strings of consecutive zeroes of 20 minutes or more as well as night time (between 8:00 p.m. and 5:00 a.m.) will be deleted.
- First and last day of measurement will also be deleted as incomplete days

The data is used for the analyses if the participant has minimum of 4 valid days of data, i.e. days with minimum of 6 hours of data after the above mentioned data reduction.

### 7.3 Preparing anthropometric data for analysis

The same as for the primary outcome analysis

### 7.4 Multiple comparisons

The same as for the primary outcome analysis

### 7.5 Confidence intervals

The same as for the primary outcome analysis

### 7.6 Interaction and effect modification

The following variables will be tested for interaction between the intervention group and the primary outcome (mean vector magnitude accelerometer counts). All tests will be done using the likelihood ratio test. The variables tested could logically modify the effect of the nutritional intervention on infancy and physical activity. Variables included (as continuous variables where possible) in this analysis include:

1. The participant's length-for-age (below / above sample median) at 6 months
2. The participant's weight-for-length (below/above sample median) at 6 months
3. The participant's sex
4. Season of activity measurement
5. Birth order (first-born or not)
6. Maternal education
7. Maternal age
8. Household food security (HFIAS)

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as stratified by the respective predictor variable.

### 7.7 Covariate adjustment

The main analysis, the results of which will be shown in tables and figures, will be completed without any covariate adjustments.

As a secondary analysis we will construct a regression model for physical activity, adjusting for the participant's sex, season of activity measurement, birth order, maternal education, maternal age, and household food insecurity.

As a sensitivity test for the latter analysis, we will use two alternative methods to build the regression model:

1. Stratifying the model by the effect modifiers.
2. Inclusion in the model of only those variables that are associated with physical activity (mean vector magnitude accelerometer counts) at $p<0.1$ level.

## 8 References

National Association for Sport and Physical Education (NASPE). Active start: A statement of physical activity guidelines for children from birth to age 5. 2nd Edition ed. Sewickley, PA, USA: American Alliance for Health, Physical Education, Recreation and Dance; 2009.

Trost SG, Fees SF, Haar SJ, Murray AD, Crowe LK. Identification and Validity of Accelerometer Cut-Points for Toddlers. *Obesity* 2012; 20(11): 2317-2319

## 9 Legends to the figures

Figure 1. Participant flow

Figure 2. Box-Whisker plots of time in moderate-to-vigorous physical activity by groups

## 10 Figures

Figure 1. Participant flow

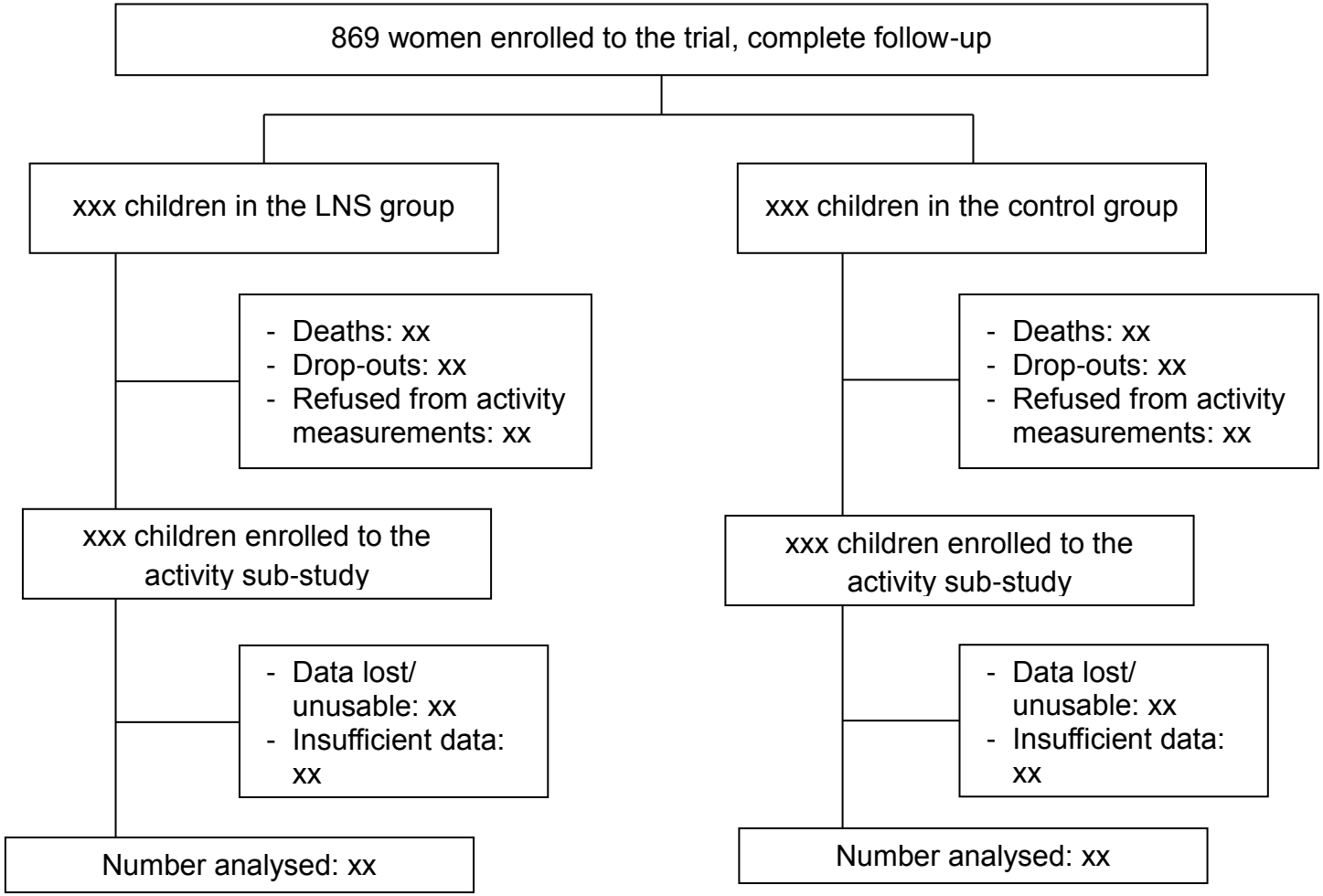
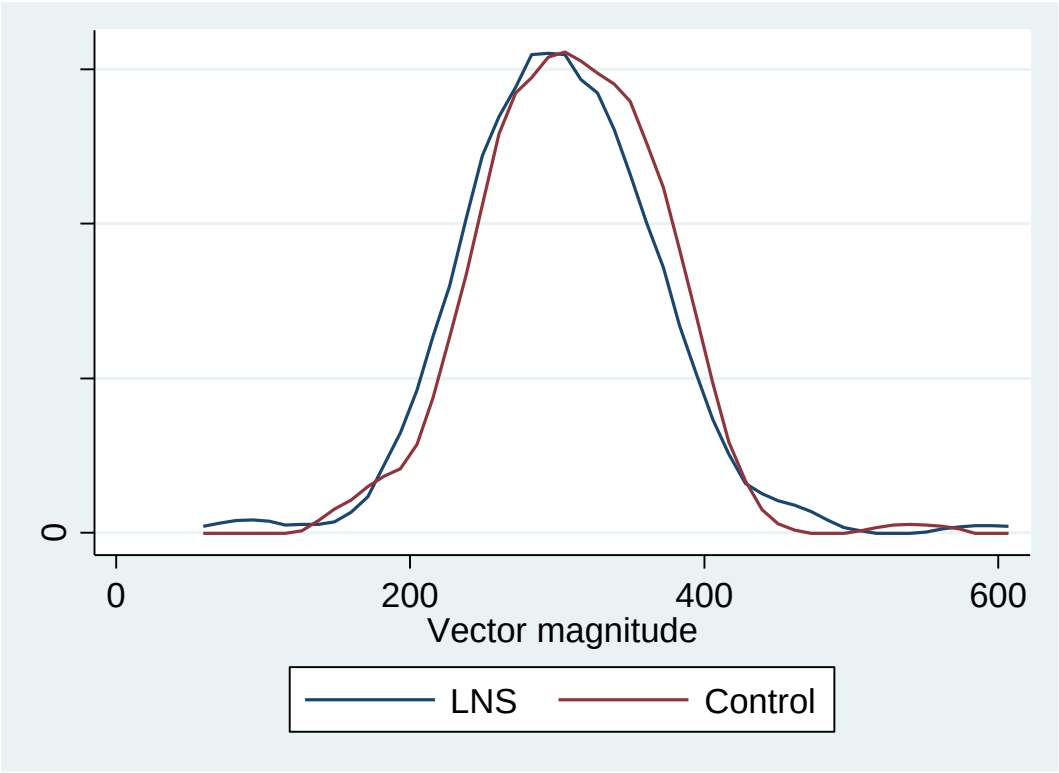


Figure 2. Kernel density plots of mean vertical axis counts/15 s by groups



## 11 Tables

Table 1 Background characteristics of participants and their mothers at baseline and at physical activity measurement

| **Variable** | **LNS n=xxx** | **CONTROL n=xxx** | **Not enrolled n= xxx** | **Test** |
|---|---|---|---|---|
| **Situation at baseline (maternal enrolment)** | | | | |
| Mean (SD) maternal age, y | Xx (xx) | Xx (xx) | Xx (xx) | ANOVA |
| Mean (SD) maternal education, completed years of schooling | Xx (xx) | Xx (xx) | Xx (xx) | ANOVA |
| Mean (SD) maternal BMI ($kg/m^2$) | Xx (xx) | Xx (xx) | Xx (xx) | ANOVA |
| % of severely food insecure households | Xx | Xx | Xx | Fisher's exact test |
| **Situation at physical activity measurement** | | | | |
| Percentage of males | xx% | xx% | | Fisher's exact test |
| Mean (SD) age months | xx.x (xx.x) | xx.x (xx.x) | | Student's t-test |
| Season of activity measurement | I: xx.x%<br>II: xx.x%<br>III: xx.x%<br>IV: xx.x% | I: xx.x%<br>II: xx.x%<br>III: xx.x%<br>IV: xx.x% | | Fisher's exact test |
| Mean (SD) length-for-age z-score | xx.xx (xx.xx) | xx.xx (xx.xx) | | Student's t-test |
| Mean (SD) weight-for-length z-score | xx.xx (xx.xx) | xx.xx (xx.xx) | | Student's t-test |
| Walking unassisted | xx% | xx% | | Fisher's exact test |
| Mean (SD) minutes being carried/day | xx (xx) | xx (xx) | | Student's t-test |

Table 2 Physical activity at the trial groups

| | | | | **Comparison between the groups** | |
|---|---|---|---|---|---|
| **Variable** | LNS | Control | P-value | Difference in means (95% CI) | P-value |
| Mean (SD) vector magnitude accelerometer counts/ 15 s | xxx (xx) | xxx (xx) | x.xxx | xx.x (x.x to x.x) | x.xxx |
| Mean (SD) vertical axis accelerometer counts/15 s | xxx (xx) | xxx (xx) | x.xxx | xx.x (x.x to x.x) | x.xxx |
| % of time in MVPA, by vertical axis | xx.x (x.x) | xx.x (x.x) | x.xxx | xx.x (x.x to x.x) | x.xxx |
| % of time sedentary, by vertical axis | xx.x (x.x) | xx.x (x.x) | x.xxx | xx.x (x.x to x.x) | x.xxx |
| % of children reaching recommendation of 90 min of MVPA/day | xx.x (x.x) | xx.x (x.x) | x.xxx | xx.x (x.x to x.x) | x.xxx |

LNS, lipid-based nutrient supplement; MVPA, moderate-to-vigorous physical activity; SD, standard deviation

**Prevention of Linear Growth Faltering in Infants and Young Children With Lipid-based Nutrient Supplements (iLiNS-DYAD)**

Statistical Analysis Plan

Appendix 25: The impact of LNS or MMN on child salivary cortisol concentration (added on 24.01.2016)

Prepared by Christine Stewart, Brietta Oaks, and Kevin Laugero

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## 1. Study objective

The primary objective for the main trial is to determine whether LNS consumed by women during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation.

This statistical analysis plan addresses the following secondary objective: to assess the effect of antenatal supplementation with LNS or multiple micronutrients on HPA function, as indicated by basal and stress-related cortisol, in the infant at 6, 12 and 18 months of age.

The three intervention groups are as follows:

- **Control group:** Women during pregnancy: 1 tablet of iron+ folate daily until delivery (60 mg iron + 400 ug folic acid); Women during lactation (from delivery to 6 months post-partum): 1 daily tablet of calcium (200 mg), akin to placebo; Children from 6 to 18 months of age: None

- **MMN group:** Women during pregnancy: 1 tablet of multiple micronutrients daily until delivery; Women during lactation (from delivery to 6 months post-partum): 1 daily tablet of multiple micronutrients; Children from 6 to 18 months of age: None

- **LNS group:** Women during pregnancy: 1 sachet of LNS-P&L (20 g of LNS) daily until delivery Women during lactation (from delivery to 6 months post-partum): 1 daily sachet of LNS-P&L (20 g of LNS) Children from 6 to 18 months of age: 2 daily sachet of LNS-20gM (20 g of LNS)

***Objective of this analysis:*** There are three potential pathways that will be evaluated in this analysis and illustrated in the figure below (Figure 1):

1) A direct effect of maternal LNS or MMN on infant 6 mo cortisol (Hypothesis 1)
2) A direct effect of maternal + child LNS on infant cortisol measured at 12 and 18 months of age. (Hypothesis 2)
3) An interactive effect of maternal LNS or MMN with maternal prenatal cortisol concentrations measured at baseline or 36 weeks on infant 6 mo cortisol, possibly through the unmeasured activity of placental 11βHSD2. This would be apparent if the association between intervention group and infant cortisol differed based on maternal cortisol concentrations at baseline or 36 weeks. (Exploratory hypothesis 3)

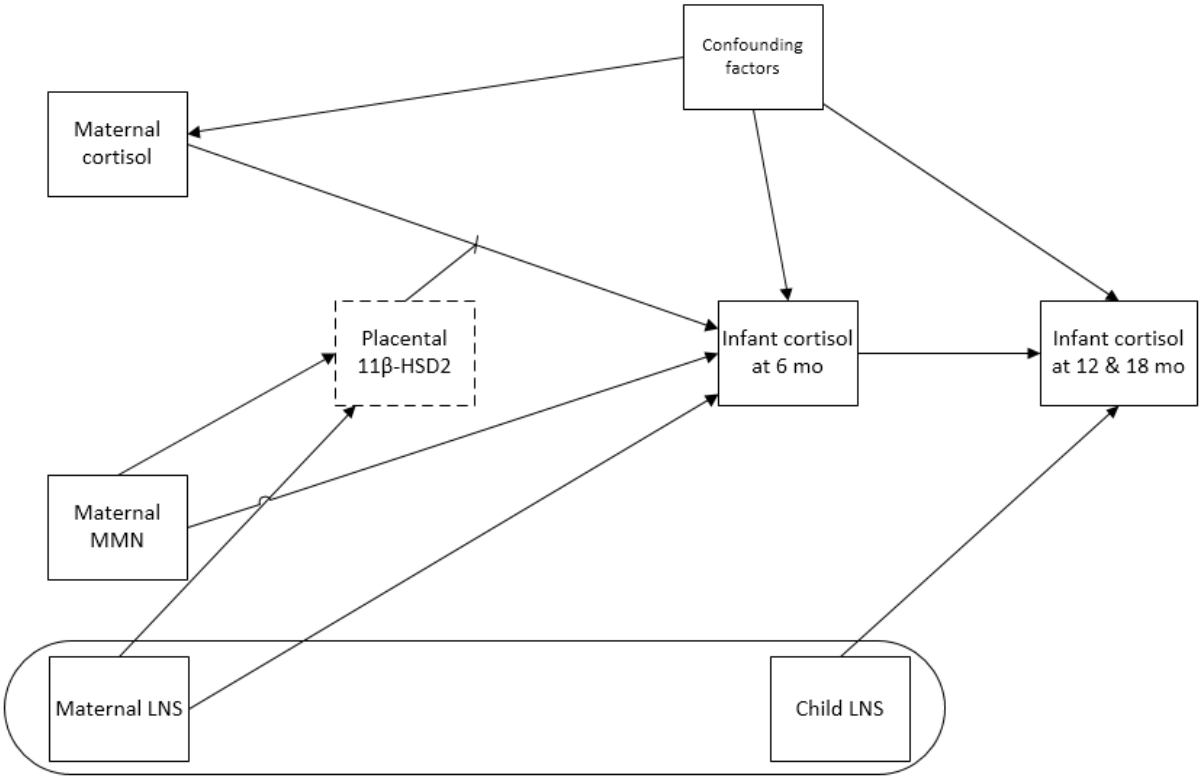


**Figure 1:** Potential pathways through which LNS or MMN will be associated with infant cortisol measured at 6, 12, and 18 mo of age

## 2. Hypotheses

SA1: To evaluate whether maternal supplementation is associated with infant cortisol at 6 months of age

> H1. Infants whose mothers received LNS or MMN during pregnancy through 6 mo of lactation will have altered basal salivary cortisol concentration and stress-related change in cortisol at 6 mo of age compared to infants in the IFA group.

SA2: To evaluate whether maternal and infant LNS supplementation is associated with infant cortisol at 12 and 18 months of age.

> H2. Infants whose mothers received LNS through pregnancy and 6 mo of lactation and who received LNS themselves from 6-18 mo of age will have altered basal salivary cortisol concentration at 12 and 18 mo of age and altered stress-related change in cortisol at 18 mo of age compared to infants in the IFA group.

SA3 (Exploratory): To assess whether the association between intervention group and infant cortisol at 6, 12, and 18 months of age is modified by maternal cortisol concentrations at baseline or 36 weeks.

H3 (Exploratory). There will be a significant interaction between intervention group and maternal cortisol concentrations during pregnancy on infant cortisol. Among those with high cortisol during pregnancy, there will be a significant effect of LNS or MMN on child cortisol. This effect will be attenuated among those with low cortisol during pregnancy.

**3. Outcome variables**

Salivary cortisol concentration at 6 mo of age

Saliva samples were collected at two time points: when the children arrived at the clinic, considered the 'basal' timepoint, and again 15 minutes after the blood draw, considered the 'stress-related' value. Cortisol was analyzed using Salimetrics high-sensitivity salivary cortisol enzyme immunoassay, which can detect cortisol levels ranging from 0.193 to 82.77 nmol/L (0.007-3.0 µg/dL).

Cortisol at 12 mo of age

Saliva samples were collected when the children arrived at the clinic and cortisol assayed as described above.

Cortisol at 18 mo of age

Saliva samples were collected as during the 6 mo visit, when they arrived at the clinic and again 15 minutes after the blood draw, and cortisol assayed as described above.

**4. Basis for the analysis: Intention to treat**

The primary analysis will be by intention-to-treat. That is, results for all children will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on participants who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available. Multiple imputation will be used to estimate missing values and used as a sensitivity test on the primary analysis. If the results differ by more than 10%, both sets of results will be presented.

**5. Time points**

Saliva samples for infant cortisol analyses were collected at 6, 12 and 18 mo of age. The primary timepoint of interest is the 18 month assessment, which reflects the cumulative exposure of LNS over pregnancy and infancy and the MMN exposure during pregnancy.

**6. Statistics software**

Analyses will be performed using SAS version 9.4 or above.

**7. Outliers**

Outliers will be visually inspected by creating box and whisker plots and scatterplots. Outliers which are clearly implausible will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible will be kept.

**8. Data transformation**

Distribution of cortisol will be log transformed and key baseline variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created. Hypothesis testing will be performed on transformed values, but data will be presented in tables and figures with medians and interquartile ranges.

Stress-related change in cortisol will be calculated by subtracting the basal cortisol measure from the post-stressor measure (the blood draw). Cortisol will also be categorized into high vs. low values using the $75^{th}$ percentile of the control group (IFA) as a cutoff.

**9. Covariates and effect modifiers**

The covariates to be included will be derived from the list below. Each variable that shows a statistically significant association with each outcome ($P<0.1$), will be included in the model. Time since waking and time since last meal/breastfeeding will be included in all models regardless of their association with the outcome variables.

Interactions will be examined between the intervention group and the variables listed below on their association with cortisol concentration. If a statistically significant interaction ($p<0.1$) is found, group means will be examined at different levels of the predictor variable, either by category for categorical predictors, or at selected percentile cutoffs for continuous variables. Variables that show no interaction with the intervention group can be used as covariates in the main analysis. Variables to be examined as covariates include:

Baseline values:

1. Maternal cortisol at baseline
2. Maternal perceived stress at baseline
3. Maternal BMI at baseline
4. Maternal height
5. Gestational age at enrolment
6. Parity (primiparous vs. multiparous)
7. Maternal education
8. Maternal age
9. Site of enrollment
10. Season at baseline
11. Maternal malaria at baseline
12. Maternal HIV status at baseline
13. Maternal Hb at baseline
14. Maternal iron status (ZPP and sTfR) at baseline
15. Maternal inflammatory markers (CRP and AGP) at baseline
16. Infant gender
17. Household food insecurity score at baseline, adjusted for month of enrolment
18. Asset index at baseline

Variables to be examined as effect modifiers include:

1. Maternal cortisol at baseline and 36 weeks

2. Maternal age
3. Parity
4. Infant gender

All analysis will adjust for time of day of infant cortisol sampling and time since last meal at the point the sample was collected.

Linear regression will be used to test the difference in means between the IFA group and the MMN or LNS groups. To compare the difference in proportions with high or low cortisol by group, either log binomial regression or robust Poisson models will be used. Final selection of the model will depend on model fit.

## 10. Presentation of study findings

General characteristics of the study group will be presented in Table 1 and a CONSORT-style diagram will be presented to illustrate the study flow and participation rates at each visit.

Group means and standard deviations for salivary cortisol concentration will be tabulated by intervention group and presented in Table 2. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups. Dichotomous outcomes will be reported as prevalences (Table 3). Comparisons will be made between groups using prevalence ratios (95% CIs). For all pairwise comparisons with $p<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

If any tests of interaction are found to be statistically significant ($p<0.1$), results will be stratified and reported in additional tables.

Results may also be presented in graphical format illustrating the mean basal cortisol at each timepoint or the cortisol response to stress (Figures 2 and 3).

Tables and Figures

Table 1. Characteristics of study participants

| Characteristic | IFA | MMN | LNS |
|---|---|---|---|
| | N= | N= | N= |
| | | | |

Table 2. Mean (SD) salivary cortisol concentration by supplement group at 6, 12 and 18 mo of age

| | IFA | MMN | LNS | Comparison of LNS vs. IFA | | Comparison of MMN vs. IFA | |
|---|---|---|---|---|---|---|---|
| | n= | n= | n= | Difference in means β (95% CI) | p-value | Difference in means β (95% CI) | p-value |
| 6 mo basal cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | |
| 6 mo stress-related change in cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | |
| 12 mo basal cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | |
| 18 mo basal cortisol (nmol/L) | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | |
| 18 mo stress-related change in cortisol | (mean ± SD) | (mean ± SD) | (mean ± SD) | | | | |

Table 3a. Differences between groups in the proportions of children with high basal cortisol at 6, 12 and 18 mo of age

| | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of LNS vs. IFA** | | **Comparison of MMN vs. IFA** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| 6 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| 12 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| 18 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |

Table 3b. Differences between groups in the proportions of children with low basal cortisol at 6, 12 and 18 mo of age

| | **IFA n (%)** | **MMN n (%)** | **LNS n (%)** | **P-value** | **Comparison of LNS vs. IFA** | | **Comparison of MMN vs. IFA** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **Risk ratio (95 % CI)** | **P-value** | **Risk ratio (95 % CI)** | **P-value** |
| 6 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| 12 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |
| 18 mo | x (x.x) | x (x.x) | x (x.x) | 0.xx | x.xx (x.xx - x. xx) | 0.xx | x.xx (x.xx - x. xx) | 0.xx |

Figure 1: Consort diagram

Figure 2: Mean (SE) salivary cortisol in infants at 6, 12, and 18 mo of age by intervention group

Figure 3: Mean (SE) basal and post-stressor salivary cortisol in infants at 6 and 18 mo of age by intervention group

**Prevention of Linear Growth Faltering in Infants and Young Children With Lipid-based Nutrient Supplements (iLiNS-DYAD)**

Statistical Analysis Plan

Appendix 26: Associations between environmental exposures, infant morbidity, and gut microbiota in iLiNS-DYAD-M (added on 2016-04-25)

Prepared by Emma Kortekangas

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Version 1, 2016-05-05

Prepared by Emma Kortekangas

Approved by .Per Ashorn, Ken Maleta, and Kathryn Dewey

Version History Log

| **Version** | **Date implemented** | **Details of significant changes** |
|---|---|---|
| 1 | 2016-05-05 | This is the first version. |

## Introduction

Child health is determined by various factors including nutrition and living environment.[1] [2] Childhood undernutrition is an important public health concern and often manifests itself as stunted linear growth which is linked to decreased health and economic productivity later in life.[3] According to previous studies, nutrient deficiencies and poor hygiene are among the most important risk factors for stunting.[4] In several studies nutrient supplements have been used to target this issue. However, results from these studies are inconclusive with some suggesting a significant effect on fetal or child growth while others have shown rather small or no effects.[5] [6] [7] [8] It is clear that nutritional intake alone can not explain why some individuals experience growth faltering or other morbidities and others do not. Instead, several nutritional and environmental factors interact and can lead to children not reaching their full developmental potential. For instance, poor hygiene increases the risk of diarrhea and other recurrent infections which hinder growth while undernutrition makes children more prone to contract diseases.[9] [10]

Recent studies support an association between poor childhood growth and gut microbiota composition. Children affected by stunting seem to have a less mature gut microbiota than healthy children, and in experimental studies growth phenotypes of healthy and undernourished infants have been reproduced in mice through transplantation of feces.[11] [12] [13] The gut microbiota also interacts with the immune system in various ways and has been linked to the development of several autoimmune diseases.[14] [15] [16]There is little knowledge on the association with common childhood illnesses like respiratory tract and gastrointestinal infections, though in some studies diarrhea has been shown to affect microbiota composition.[17] Furthermore, microbiota composition has been associated with malaria prevalence.[18] Common childhood infections like diarrhea, respiratory tract infections and malaria might interact with the microbiota through inflammatory processes or modify the associations between nutrition and microbiota, but these questions have not been thoroughly studied to date. It is also not known whether the gut microbiota mediates associations between infections and child growth.

A nutrition intervention trial conducted by the International Lipid-Based Nutrient Supplement Study Group in Malawi (iLiNS-DYAD-M; NCT01239693) studied the effect of a lipid-based nutrient supplement (LNS) on child growth. In addition, stool and other biological samples were collected at various time points. Administration of LNS to mothers during pregnancy and lactation and children from 6 to 18 months did not result in improved linear growth at 18 months.[8] However, analyses of stool samples and experimental mice studies revealed a causal link between gut microbiota composition and growth.[13] This raises the question how gut microbiota composition and development are determined. Nutrition is known to influence microbiota[19] [20], but a recent study did not show differences in gut microbiota of children who received different types of nutrient supplements. [21] Therefore, interactions of the gut

microbiota with environmental and nutritional factors, infections and immunity need to be further studied.

## Study objectives

In this study we will assess associations between environmental exposures, general morbidity, and gut microbiota composition in infants. Since both the gut microbiota and morbidity have been associated with child growth, our objective is to study which factors determine the composition of gut microbiota and whether morbidity leads to microbiota alterations that in turn could affect child growth. In particular, we will assess how the prevalence of common childhood morbidity symptoms and environmental exposures are associated with microbiota composition and maturity. We will use clinical and laboratory data obtained during the iLiNS-DYAD-M study from infants between birth and 18 months.

Study questions:

1. Which environmental exposures predict gut microbiota composition and maturation at 18 and 30 months?
2. Do infections in infancy predict subsequent gut microbiota composition and maturation at 18 and 30 months? If yes, is the association between infections and microbiota independent of environmental factors?

## Hypotheses

To study whether environmental exposures predict microbiota composition and maturation, we will test the following hypothesis.

- Children with high levels of adverse environmental exposures have less diverse and mature gut microbiota at 18 and 30 months than children with low levels of environmental exposures.

Additionally, we will conduct descriptive analyses on differences in microbiota composition between children with high and low levels of adverse environmental exposures.

To study the question of whether infections predict microbiota composition and maturation, we will test the following hypotheses on long-term and short-term effects on the microbiota.

- Children with high prevalence of infections between birth and 18 months of age have less diverse and mature gut microbiota at 18 and 30 months than children with low prevalence of infections in the same time period.
- Children with diarrhea in the two weeks preceding stool sample collection have less diverse and mature gut microbiota than children with no diarrhea.

Additionally, we will conduct descriptive analyses on differences in microbiota composition between children with high and low prevalence of infections.

## Description of predictor and outcome variables

### Microbiota

| **Table 1: Microbiota Variables** | | |
|---|---|---|
| Microbiota Maturity | Microbiota-for-Age Z-Score | A measure to determine the relative microbiota age of children and compare it to their chronological age. |
| Alpha Diversity | Number of OTUs | Number of distinct OTUs, general measure of species richness. |
| | Shannon Index | Estimates species richness and evenness, measures uncertainty in predicting an OUT in a sample. |
| | Balance Weighted Phylogenetic Diversity (BWPD) | An abundance weighted phylogenetic diversity measure, calculated using a phylogenetic tree and thereby taking into account phylogenetic similarity. |
| Beta Diversity | OTU counts on species and genus level | Relative abundances of species and genera for each participant. |
| | Core Microbiome | This measure will be calculated on group level. The core microbiome is defined as a set of taxa shared across microbiomes and are thought to perform critical ecosystem function within their host. Using QIIME, we will define the taxa present in at-least 50% of the samples in each group based on a presence/absence data set. |
| | Generalized UniFrac Distance | This measure will be calculated on group level. UniFrac measures the difference between samples by calculating distances between species on a phylogenetic tree. The output of UniFrac calculations is a distance matrix with distances of all samples to each other. |

## Environmental exposures

| **Table 7: Environmental exposure variables** | |
|---|---|
| Low household assets Z-score (continuous) | Measure of socioeconomic status, includes information on building materials of the house, sources of water, electricity and cooking fuel and sanitary facility |
| High dependency ratio (continuous) | Number of household members age 15 and younger and 65 and older divided by the number of household members between ages 16 and 64 |
| High household crowding (continuous) | Total number of people living in the household |
| High number of domestic animals (continuous) | Number of chickens, goats and cows in the household, each type of animal analyzed separately |
| Source of drinking water (dichotomous) | Well, river or lake as primary source of drinking water (vs. borehole or pipe) |
| Sanitary facility (dichotomous) | No sanitary facility in household (vs. any sanitary facility) |
| Season (categorical) | Dry and cold, dry and hot, rainy |
| Residential location | GPS coordinates of households |

## Morbidity

| **Table 8: Morbidity Variables** | |
|---|---|
| Longitudinal prevalence of diarrhea between birth and 18 months | Proportion of days with caregiver-reported diarrhea in the study period. This will be calculated as the number of days with three or more liquid/semi-liquid stools divided by the total number of days of follow up for each participant in the specified time period. |
| Longitudinal prevalence of any infectious disease symptoms between birth and 18 months | Proportion of days with any caregiver-reported infectious disease symptoms in the study period. Any infectious disease symptoms will include diarrhea, vomiting, fever, difficult breathing, cough and nasal discharge. This will be calculated as the number of days with any of the above symptoms divided by the total days of follow up per child for the specified time period. |
| High gastrointestinal morbidity | Prevalence of diarrheal symptoms above the 75th percentile |
| Low gastrointestinal morbidity | Prevalence of diarrheal symptoms below the 25th percentile |

| | |
|---|---|
| High overall infectious morbidity | Prevalence of any symptoms above the 75th percentile |
| Low overall infectious morbidity | Prevalence of any symptoms below the 25th percentile |
| Prevalence of fever in weekly temperature measurements between birth and 18 months | Proportion of weekly visits on which the data collector measured a high temperature calculated as number of visits with a tympanic temperature of 37.5$^{o}$ C or above divided by total number of visits on which temperature was measured. |
| High prevalence of fever | Prevalence above the 90th percentile |
| Low prevalence of fever | Prevalence below the median |

## Presentation of the results and analytical approach

The participant flow will be detailed as in Figure 1. The study enrolled 1391 pregnant women of whom 869 were part of the complete follow-up scheme from which data will be included in these analyses. There were 806 live-born infants in the complete follow-up scheme including 10 pairs of twins. By 18 months a total of 118 children were lost to follow-up and 61 had died. Clinical morbidity data is available from 781 participants and data from stool samples is available from 631 participants.

Baseline characteristics of included and excluded participants will be shown in Table 1. The hypothesis of no differences in baseline characteristics between the groups will be tested with t-test for continuous variables and chi-square test for proportions.

Regression models will be used to assess linear associations between environmental exposure variables and MAZ-scores and alpha diversity measures. Exposures which show an association with the outcome at significance level $p<0.1$ in the bivariable regression will be included in the multivariable models. Standardized regression coefficients and p-values from bivariable and multivariable models will be presented as in Tables 2 and 3.

Regression models will be used to assess linear associations between morbidity variables and MAZ-scores and alpha diversity measures. Standardized regression coefficients and p-values from unadjusted models and models with covariate adjustment will be presented as in Tables 4 and 5.

To assess the effect size of morbidity on microbiota outcomes, means of MAZ-scores and alpha diversity measures in participants with high and low morbidity will be presented as in Tables 6 and 7. Differences in means with 95% confidence intervals will also be included in the tables. The null hypothesis of no differences in participants with high and low morbidity will be tested with t-test for normally distributed variables and Mann-Whitney test for not normally distributed variables. The null hypothesis will be rejected if $p<0.05$.

Exploratory analyses on differences of gut microbiota between children with high and low levels of environmental exposures and morbidity and will be performed using random forests models to determine the most discriminatory species and genera. The differences in the abundance of these species and genera derived from the random forests models will further be assessed with difference in means tests. Additionally, generalized UniFrac distances will be calculated analyze beta diversity between participants with high and low levels of environmental exposures and morbidity.

P-values will be adjusted using the Benjamin-Hochberg correction were applicable and both raw and adjusted p-values will be reported.

## Effect modification and covariates

For each analysis, possible effect modification will be assessed for variables which could logically interact between the independent variables and the outcome. Statistically significant interactions ($p<0.1$) will be further examined with stratified analysis. Variables that are not found to be effect modifiers will be used as covariates if they have a significant association with an outcome ($p<0.1$) or if they are found to be confounders to the association between the primary exposure and the outcome. The following lists possible effect modifiers and covariates to be considered by analysis.

- duration of breast feeding
- hunger index
- site, resident location
- education level of the mother
- age of the mother
- marital status of the mother
- HIV status of the mother
- sex of the child
- delivery mode
- season
- intervention group

## General notes on data processing and analysis

### Statistical software

The analyses will be done in R version 3.2.1, Stata version 13 and qiime version 1.9.1.

### Outliers

We will start by inspecting the data for outliers, which will involve visual inspection of box plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers that are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers that are plausible or possible will be

kept. In an extreme situation, a sensitivity analysis (excluding all outliers) will be done to determine if such outliers have undue influence on the results.

## Data transformation

If normality of the outcome variable is a model assumption then transformations will proceed as follows. Continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately. If no suitable transformation can be found then analysis will be done on ranked data or categories will be created.

## Missing data

If outcome data are missing for a participant at a certain time point, the missing data will not be imputed and the participant will be excluded from the respective analyses. If clinical morbidity data are available for only a few weeks, the participant will be excluded from the respective analyses. Missing data for independent variables, covariates, and effect modifiers will be reported and if necessary imputed using chained equation methods. Imputation will be deemed necessary for a group of independent variables if their combination results in more than 10% dropped observations. If a single independent variable is missing more than 20% then it will instead be dropped from the analysis. A sensitivity analysis will be performed and the results with and without imputed data will be presented.

## Tables and Figures

Figure 1. Participant flow

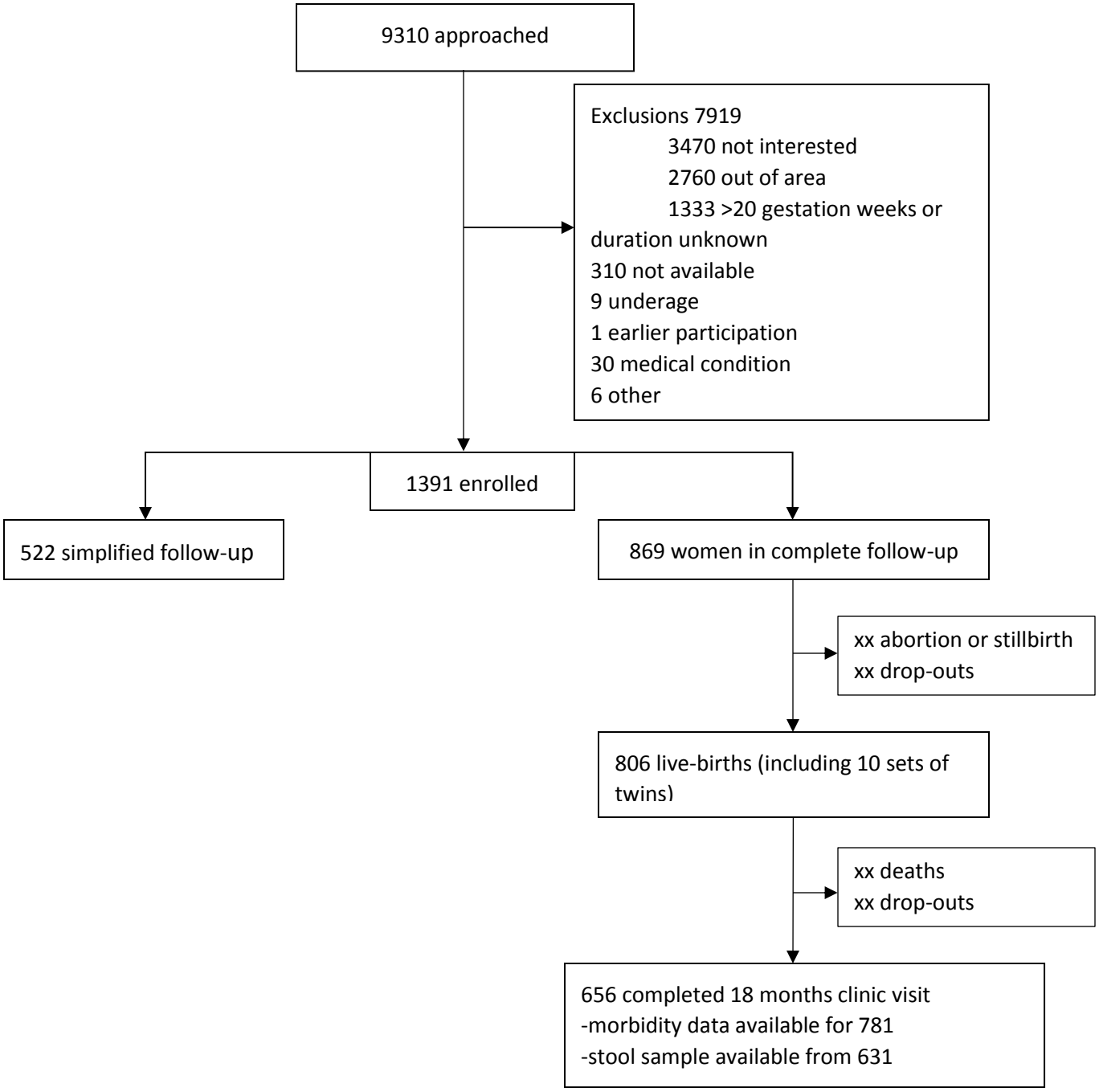

Table 1. Baseline characteristics of included and excluded participants

| Characteristic | Included | Excluded | P-value |
|---|---|---|---|
| Participants, n | xxx | xxx | 0.xx |
| Maternal age at enrollment, years | xx.x (x.x) | xx.x (x.x) | 0.xx |
| Maternal education completed, years | x.x (x.x) | x.x (x.x) | 0.xx |
| Severely food insecure households | xx.x | xx.x | 0.xx |
| Positive malaria RDT of the mother at enrollment | xx.x | xx.x | 0.xx |
| Gestational age at birth, weeks | xx.x (x.x) | xx.x (x.x) | 0.xx |
| LAZ at birth | x.xx (x.xx) | x.xx (x.xx) | 0.xx |
| Length at birth | xx.x (x.x) | xx.x (x.x) | 0.xx |
| LAZ at 18 months | x.xx (x.xx) | x.xx (x.xx) | 0.xx |
| Length at 18 months | xx.x (x.x) | xx.x (x.x) | 0.xx |

Values are in mean (standard deviation) or percentages. P-values are obtained from t-test (continuous variables) or chi-square test (proportions)
LAZ, length-for-age Z score; RDT, rapid diagnostic test

Table 2. The association between environmental exposure variables and the study participants' microbiota diversity and maturity at 18 months. Results from bivariable analyses.

| Predictor variable | | MAZ-score | | Observed species | | Shannon Index | | BWPD | |
|---|---|---|---|---|---|---|---|---|---|
| | | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value |
| Household assets Z-score | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Dependecy ratio | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of people in the household | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of children under five in the household | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Total number of domestic animals | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of chickens | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of goats | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of cows | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Source of drinking water is well, river or lake | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Type of sanitary facility is none or regular pit latrine | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Season when stool sample was collected | Dry, hot | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| | Rainy | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |

MAZ-score, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity

Table 3. The association between environmental exposure variables and the study participants' microbiota diversity and maturity at 18 months. Results from multivariable analysis.

| Predictor variable | | MAZ-score | | Observed species | | Shannon Index | | BWPD | |
|---|---|---|---|---|---|---|---|---|---|
| | | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value |
| Household assets Z-score | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Dependency ratio | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of people in the household | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of children under five in the household | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Total number of domestic animals | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of chickens | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of goats | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of cows | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Source of drinking water is well, river or lake | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Type of sanitary facility is none or regular pit latrine | | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Season when stool sample was collected | Dry, hot | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| | Rainy | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |

MAZ-score, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity

Table 4. The association between morbidity variables and the study participants' microbiota diversity and maturity at 18 months.

| Predictor variable | MAZ-score (unadjusted) | | MAZ-score (with covariate adjustment) | | Observed species (unadjusted) | | Observed species (with covariate adjustment) | |
|---|---|---|---|---|---|---|---|---|
| | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value |
| Number of episodes of gastroenteritis per year between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Longitudinal prevalence of diarrhea between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Number of episodes of any disease per year between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Longitudinal prevalence of any symptoms between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Prevalence of fever between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Prevalence of malaria between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |

MAZ-score, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity

Table 5. The association between morbidity variables and the study participants' microbiota diversity at 18 months.

| Predictor variable | Shannon Index (unadjusted) | | Shannon Index (with covariate adjustment) | | BWPD (unadjusted) | | BWPD (with covariate adjustment) | |
|---|---|---|---|---|---|---|---|---|
| | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value | Regression coefficient | P-value |
| Longitudinal prevalence of diarrhea between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Longitudinal prevalence of any symptoms between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |
| Prevalence of fever between birth and 18 months | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx | x.xx | 0.xx |

MAZ-score, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity

Table 6. MAZ-scores and alpha-diversity measures at 18 months in participants with high and low morbidity.

| Outcome | Stratification by overall morbidity prevalence | | | P-value | | Stratification by gastrointestinal morbidity prevalence | | | P-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Participants with high prevalence | Participants with low prevalence | Difference in means (95%CI) | raw | adjusted | Participants with high prevalence | Participants with low prevalence | Difference in means (95%CI) | raw | adjusted |
| MAZ-score at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| Observed species at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| Shannon Index at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| BWPD at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |

MAZ, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity, CI, confidence interval; SD, standard deviation

Table 7. MAZ-scores and alpha-diversity measures at 18 months in participants with high and low fever prevalence.

| Outcome | Stratification by prevalence of fever | | | P-value | |
|---|---|---|---|---|---|
| | Participants with high prevalence | Participants with low prevalence | Difference in means (95%CI) | raw | adjusted |
| MAZ-score at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| Observed species at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| Shannon Index at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |
| BWPD at 18 months, mean (SD) | x.xx (x.xx) | x.xx (x.xx) | x.xx (x.xx-x.xx) | 0.xx | 0.xx |

MAZ, microbiota-for-age Z-score; BWPD, balance-weighted phylogenetic diversity, CI, confidence interval; SD, standard deviation

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**Prevention of Linear Growth Faltering in Infants and Young Children With Lipid-based Nutrient Supplements (iLiNS-DYAD)**

Statistical Analysis Plan

Appendix 27: The impact of the interventions on human milk oligosaccharides (HMOs) and proteins (added on 27.07.2016)

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | 27.07.2016 | Josh Jorgensen | Original document (appendix 27) |
| | | | |

## 2. Study objectives

The trial has three sets of objectives, defined at various phases of the trial. The originally defined objective is to determine whether LNS consumed by women during pregnancy and the first 6 mo of lactation, and by the child from 6-18 mo, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only, or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. Description of the other two objectives is presented in the main analysis plan.

The objectives of the secondary analyses are to determine the main effect of intervention on breast milk glycans (human milk oligosaccharides, HMO) and proteins. Details of this objective are as follows:

2.1. Main effect of intervention on human milk oligosaccharides and proteins

a. To determine if there are differences in mean abundance of total human milk oligosaccharides (HMOs) and sialylated and fucosylated HMOs at 6 mo postpartum between groups of women who are provided either LNS or multiple micronutrient (MMN) capsules during pregnancy and up to 6 mo postpartum, or iron-folic acid (IFA) capsules during pregnancy and a placebo capsule up to 6 mo postpartum.
b. To determine if there are differences in the breast milk concentration of lactoferrin, lactalbumin, lysozyme, antitrypsin, IgA, and osteopontin at 6 mo postpartum between groups of women who are provided either LNS or MMN during pregnancy and up to 6 mo postpartum, or IFA during pregnancy and placebo up to 6 mo postpartum.

2.2. Exploratory analysis

a. To determine if there are differences in the quantity of specific HMOs at 6 mo postpartum between groups of women who are provided either LNS or MMN during pregnancy and up to 6 mo postpartum, or IFA during pregnancy and placebo up to 6 mo postpartum.

## 3. Hypotheses

To ascertain the effect of LNS supplementation on HMOs and milk proteins, we will test the following hypotheses:

1) Supplementation with LNS during pregnancy and lactation will lead to higher mean breast milk concentrations of sialylated and fucosylated HMOs at 6 months postpartum than supplementation with MMN or IFA, although total concentration of HMOs will not be different between groups (n= 654).
2) Supplementation with LNS during pregnancy and lactation will lead to higher concentrations of certain milk proteins (lactoferrin, lactalbumin, lysozyme, IgA, and osteopontin) at 6 months postpartum than supplementation with MMN or IFA (n=643).

## 4. Definition of outcome variables

Human milk oligosaccharides (HMOs)

HMOs were analyzed by nano-liquid chromatography chip time-of-flight mass spectrometry (nano-LC chip-TOF MS) and reported as ion counts. The lower cutoff used to define low total HMOs will be below the 25$^{th}$ percentile of mean total HMOs.

Lactoferrin

Lactoferrin was analyzed by ultra-performance LC (UPLC) and reported as g/L. The lower cutoff used to define low lactoferrin will be below the 25$^{th}$ percentile of mean lactoferrin.

Lactalbumin

Lactalbumin was analyzed by UPLC and reported as g/L. The lower cutoff used to define low lactalbumin will be below the 25$^{th}$ percentile of mean lactalbumin.

Lysozyme

Lysozyme was analyzed by UPLC and reported as g/L. The lower cutoff used to define low lysozyme will be below the 25$^{th}$ percentile of mean lysozyme.

Antitrypsin

Antitrypsin was analyzed by UPLC and reported as g/L. The lower cutoff used to define low antitrypsin will be below the 25$^{th}$ percentile of mean antitrypsin.

Immunoglobulin A (IgA)

IgA was analyzed by UPLC and reported as g/L. The lower cutoff used to define low IgA will be below the 25$^{th}$ percentile of mean IgA.

Osteopontin

Osteopontin was analyzed by UPLC and reported as ion counts. The lower cutoff used to define low osteopontin will be below the 25$^{th}$ percentile of mean osteopontin.

## 5. Statistics software

Analyses will be performed using SAS version 9.3.

## 6. Basis for the analysis: Intention to treat

The primary analysis will be by intention-to-treat. That is, results for all women enrolled will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on participants who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available. A per-protocol sensitivity analysis will be performed to examine whether there are significant differences in outcome variables between those who adhered to protocol (>/= 80% of supplements consumed) and those who did not adhere to protocol (<80% of supplements consumed). If significant differences exist ($p<0.05$), instrumental variable analysis will be used to examine differences between intervention groups in the outcome variables.

## 7. Missing Data

If outcome data are missing for a participant, the missing data will not be imputed and the participant will be excluded from the respective analyses. Missing data for independent variables, covariates, and effect modifiers will be reported and if necessary imputed using chained equation methods. Imputation will be deemed necessary for a group of independent variables if their combination results in more than 10% dropped observations. If a single independent variable is missing more than 20% then it will instead be dropped from the analysis. A sensitivity analysis will be performed and the results with and without imputed data will be presented.

## 8. Outliers

We will start by inspecting the data for outliers, which will involve visual inspection of box plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers that are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers that are plausible or possible will be kept. In an extreme situation, a sensitivity analysis (excluding all outliers) will be done to determine if such outliers have undue influence on the results.

## 9. Data transformation

If normality of the outcome variable is a model assumption then transformations will proceed as follows. Continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately. If no suitable transformation can be found then analysis will be done on ranked data or categories will be created.

## 10. Comparisons of group means

We will perform unadjusted and covariate adjusted testing of differences between intervention groups. The analyses will each begin with testing the null hypothesis of no difference between the intervention groups using ANCOVA for continuous outcomes, logistic regression for dichotomous outcomes, and negative binomial regression for count outcomes. For all analyses, if the global null hypothesis is rejected at 0.05 level, then we will perform post-hoc pairwise comparisons of groups with a Tukey-Kramer adjustment for continuous outcomes. If there is no difference between the IFA and MMN groups (which is generally expected), those two groups will be combined and the analyses will be repeated to evaluate the hypotheses stated above, i.e. to test for differences between the LNS group and the other two groups combined.

### 10.1. Covariates

For adjusted analyses, only covariates that are significantly associated with an outcome at 10% level of significance in a bivariate analysis will be included in the final adjusted analysis. Potential covariates will include baseline maternal BMI, HIV status, parity, maternal age, SES, and season at 6 months postpartum.

### 10.2. Effect Modification (Interactions)

The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA, logistic regression, or negative binomial model. Significant interactions ($p < 0.10$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification. We will examine as potential effect modifiers the following variables: baseline maternal BMI, HIV status, parity, maternal age, and SES, and season at 6 months postpartum.

## 11. Multiple hypothesis testing

We are concerned about the possibility of false positive findings but are aware that there is not a standard accepted method for handling this problem, particularly when outcomes are correlated (Streiner, 2015). There are pros and cons to such an adjustment and we will proceed with caution and further discussion. One approach we'll consider is the Benjamini-Hochberg correction and the presentation of both raw and adjusted p-values.

## 12. Baseline differences between those included and excluded from analysis

Because we are evaluating differences among intervention groups within a sub-sample of those from whom HMO and breast milk proteins were analyzed, we will compare socio-demographic and maternal variables between those included (n=654) and excluded (n=737) from the analysis (Table 1). P-values will be obtained from t-test for comparison of means or Fisher's exact test for comparison of proportions.

## 13. Presentation of study findings

Group means and standard deviations for HMOs and proteins at 6 mo postpartum will be tabulated by intervention group and presented as illustrated in Tables 2 & 3. The tables will also indicate the differences in means and their 95% confidence intervals between the intervention groups.

The proportion of women with HMOs and proteins above or below specified cutoffs will be tabulated by intervention group as shown in Tables 4 & 5. Risk ratios between intervention groups are also presented in those tables.

## 14. Reference

Streiner DL: Best (but oft-forgotten) practices: the multiple problems of multiplicity-whether and how to correct for many statistical tests. The American journal of clinical nutrition 2015, 102(4):721-728.

## 15. Tables

**Table 1. Baseline characteristics of the participants included and excluded from the groupwise comparisons at 6 mo postpartum.**

| Characteristic | Included (n=654) | Excluded (n=737) | p-value[1] |
|---|---|---|---|
| Mean (SD) maternal age, years | xx (x.x) | xx (x.x) | 0.xxx |
| Mean (SD) proxy for socioeconomic status | xx (x.x) | xx (x.x) | 0.xxx |
| Proportion of primiparous women | xx.x% (x.x) | xx.x% (x.x) | 0.xxx |
| Mean (SD) BMI, kg/m$^2$ | xx (x.x) | xx (x.x) | 0.xxx |
| Proportion of women with a low BMI (< 18.5 kg/m$^2$) | xx.x% (x.x) | xx.x% (x.x) | 0.xxx |
| Proportion of women with a positive HIV test | xx.x% (x.x) | xx.x% (x.x) | 0.xxx |

[1]P-values obtained from T-test (comparison of means) or Fisher's exact test (comparison of proportions)

**Table 2. Differences between groups in mean (SD) total HMO, sialylated HMO, and fucosylated HMO at 6 mo postpartum.**

| Variable | IFA [n] | MMN [n] | LNS [n] | P-value | Comparison of IFA and MMN | | Comparison of IFA and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P-value | Difference in means or medians (95 % CI) | P-value | Difference in means or medians (95 % CI) | P-value | Difference in means or medians (95 % CI) |
| Total HMO ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| Sialylated HMO ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| Fucosylated HMO ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |

Differences between groups in ion counts of total HMOs will be analyzed. Differences between groups in ion counts of sialylated and fucosylated HMOs, as well as proportion of total HMOs that sialylated and fucosylated comprise will be analyzed.

**Table 3. Differences between groups in mean (SD) lactoferrin, lactalbumin, lysozyme, antitrypsin, IgA, and osteopontin at 6 mo postpartum.**

| Variable | IFA [n] | MMN [n] | LNS [n] | P-value | Comparison of IFA and MMN | | Comparison of IFA and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P-value | Difference in means or medians (95 % CI) | P-value | Difference in means or medians (95 % CI) | P-value | Difference in means or medians (95 % CI) |
| Lactoferrin ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| Lactalbumin ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| Lysozyme ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| Antitrypsin ($\bar{x}$ ± SD) [n] | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |
| | x.x ± x.x [x] | x.x ± x.x [x] | x.x ± x.x [x] | 0.xxx | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) | 0.xxx | x.xx (xx to xx) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| IgA ($\bar{x}$ ± SD) [n] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | 0.xxx<br>0.xxx<br>0.xxx | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) |
| Osteopontin ($\bar{x}$ ± SD) [n] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | x.x ± x.x [x]<br>x.x ± x.x [x]<br>x.x ± x.x [x] | 0.xxx<br>0.xxx<br>0.xxx | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) | 0.xxx<br>0.xxx<br>0.xxx | x.xx (xx to xx)<br>x.xx (xx to xx)<br>x.xx (xx to xx) |

**Table 4. Differences between groups in the proportions of women with total, sialylated, and fucosylated HMOs below specified cutoffs at 6 mo postpartum.**

| | IFA n (%) | MMN n (%) | LNS n (%) | P-value | Comparison of IFA and MMN | | Comparison of IFA and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value |
| Total HMOs < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Sialylated HMOs < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Fucosylated HMOs < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |

**Table 5. Differences between groups in proportion of women with breast milk proteins below specified cutoffs at 6 mo postpartum.**

| | IFA n (%) | MMN n (%) | LNS n (%) | P-value | Comparison of IFA and MMN | | Comparison of IFA and LNS | | Comparison of MMN and LNS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value | Risk ratio (95 % CI) | P-value |
| Lactoferrin < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Lactalbumin < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Lysozyme < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Antitrypsin < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| IgA < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| Osteopontin < x.xx | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |
| | x (x.x) | x (x.x) | x (x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx | x.x (x.x to x.x) | 0.xxx |

**Prevention of Linear Growth Faltering in Infants and Young Children With Lipid-based Nutrient Supplements (iLiNS-DYAD)**

Statistical Analysis Plan

Appendix 28: The effect of the Dyad interventions on Malawian infant microbiota at 1, 6 12, 18 and 30 months (added on 30.09.2016)

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## 1. Version history

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|---|---|---|---|
| 01.0 | 30.09.2016 | Arox Kamng'ona | Original document (Appendix 28). |

## 2. Introduction

The iLiNS-DYAD intervention trial was designed to determine whether provision of lipid-based nutrient supplements (LNS) to women during pregnancy and the first 6 months of lactation, and to the child from 6-30 months of age, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only (IFA), or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. The trial enrolled 1391 pregnant mothers in a rural area in Mangochi district, Malawi, and randomized to receive iron and folic acid supplementation (IFA group), multiple micronutrient supplementation (MMN group) or lipid-based nutrient supplements (LNS group). For a subgroup of 869 participants ("complete follow-up"), the intervention and a detailed follow-up was continued for 30 months after delivery. For the remaining participants (n=522, "simplified follow-up"), there were no further interventions, but the children were clinically examined at 6 and 18 months of age to assess their growth. Key details of the trial have been recorded at the clinical trial registry at the National Institute of Health (USA) (http://www.clinicaltrials.gov/), under the registration number NCT01239693. A full trial protocol is available upon request from the PIs, Per Ashorn or Kenneth Maleta. . This document (called "the statistical analysis plan" or SAP) describes the plan for data analysis, management, and storage.

## 3. Study objectives and justification

**Objectives**: The objective of the iLiNS-DYAD-M trial was to determine whether (i) LNS consumed by women during pregnancy and the first 6 months of lactation, and by the child from 6-30 months, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of (ii) iron and folic acid (IFA) during pregnancy only, or (iii) a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. In the present analysis, we propose to determine the effects of the iLiNS-DYAD intervention in Malawi on infant microbiota at 1, 6, 12, 18 and 30 months.

**Study question**: Does provision of small quantity lipid-based nutrient supplements to women during pregnancy and the first six months of lactation, and to their infants from 6 to 18 months,

affect infant gut microbiota composition and maturation?

**Justification**: Diet is thought to be a key factor in shaping gut microbiota assembly and development [1]. A recent study in mice showed that maternal diet significantly affected maternal gut microbiota [2]. In the same study, a correlation analysis showed a strong relationship between each maternal gut microbial group during the perinatal period and the corresponding offspring microbial profiles at weaning [2]. This suggests that maternal diet affects maternal microbiota, which subsequently shapes the offspring microbiota in mice, however no such data are available for humans. In addition, there is limited evidence regarding specific dietary elements that promote or suppress optimal microbiota development and maturity. A review by Zhang et al. highlights the potential beneficial effects of feeding patterns (breast milk vs. formula feeding), proteins, fats, carbohydrates, fiber and polyphenols in determining the composition of gut microbiota and suppressing populations of pathogenic bacteria as well as affecting metabolic pathways for the benefit of the host [3]. However some dietary constituents such as iron have also been implicated in dysbiosis leading to proliferation of virulent organisms [4]. LNS products include components that may be beneficial for the gut microbiota (n-3 fats, milk powder, fiber, carbohydrates such as lactose) as well as potentially deleterious components (iron fortificant), hence the impact of long term supplementation with small-quantity LNS on gut microbial composition and maturity is worth investigating.

## 4. Hypotheses to be tested

To study whether LNS supplementation influences microbiota composition or maturation, we will test the hypothesis that supplementation of mothers with LNS during pregnancy and first 6 months of lactation and their infants between 6 and 18 months of age will lead to a more diverse and mature gut microbiota between 1 month and 30 months of age compared to supplementation with multiple micronutrients (MMN) during pregnancy and first 6 months of lactation only or iron and folic acid (IFA) during pregnancy only (n=358 at 1 month, 506 at 6 months, 606 at 12 months, 617 at 18 months and 596 at 30 months).

## 5. Definition of outcome variables

To study whether LNS supplementation influences microbiota composition or maturation, we will define the following variables:

**a). Alpha diversity**: Alpha diversity measures the mean diversity of OTUs within a sample. Shannon diversity index (H), which takes into account both richness and evenness, will be used as a measure of alpha diversity. The Shannon diversity index is calculated as follows:

$$\mathrm{H} = -\sum_{i}^{s} P_i \ln P_i$$

Where *s* = total number of species in each infant, and *Pi* is the relative abundance for species *i*. In a given intervention group, the mean H for all the infants will be calculated and then used to compare across groups. We will employ ANOVA to compare the mean differences. If the null hypothesis of no difference across the three intervention groups is rejected, then we will perform pairwise comparison of all the three groups using Tukey's adjustment.

**(c). Microbiome maturity**: Microbiome maturity determines the relative microbiota age of children compared to their chronological age. We will assess the maturity of the gut microbiota in infants in each intervention group using a model that will generate the relative microbiota maturity and microbiota for age Z score (MAZ), as described previously [5] and also as previously calculated by the Gordon lab (https://gordonlab.wustl.edu/).

**b). Beta diversity**: Beta Diversity compares the diversities between ecosystems or samples. Beta diversity is aimed at assessing whether samples within a group are more similar to each other than to samples from other groups. We will employ the Unifrac distance metric to produce a distance matrix that describes the pairwise phylogenetic distances between sets of sequences from different intervention groups [6].

## 6. Basis for the analysis

The analysis will be based on intention to treat. However, because the pattern of microbiome data may be difficult to predict over time, it would be difficult to take into account subjects with missing data points. If outcome data are missing for a participant at a certain time point, the missing data will not be imputed and the participant will be excluded from the respective analyses. Missing data for independent variables, covariates, and effect modifiers will be reported and if necessary imputed using chained equation methods. Imputation will be deemed necessary for a group of independent variables if their combination results in more than 10% dropped observations. If a single independent variable is missing more than 20% then it will instead be dropped from the analysis. A sensitivity analysis will be performed and the results with and without imputed data will be presented.

## 7. Time points

The microbiota outcomes will be examined at 1, 6, 12, 18, and 30 months. The relevant outcome variables can be seen in Table 1.

## 8. Statistics software

Statistical analyses will be performed in Excel, STATA and/or R.

## 9. Outliers

We will start by inspecting the data for outliers, which will involve visual inspection using box plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers that are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers that are plausible or possible will be kept. In an extreme situation, a sensitivity analysis (excluding all outliers) will be done to determine if such outliers have undue influence on the results

## 10. Data transformation

If normality of the outcome variable is a model assumption then transformations will proceed as follows. Continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately. If no suitable transformation can be found then analysis will be done on ranked data or categories will be created

## 11. Analytical approach

See Tables 2 for relevant outcome variables. For this study aim, microbiota outcomes will be examined at 1, 6, 12, 18, and 30 months

For microbiota maturity and alpha diversity we will perform unadjusted and covariate adjusted testing of differences between intervention groups. The analyses will each begin with testing the null hypothesis of no difference between the intervention groups using ANCOVA for all the outcomes. For adjusted analyses, only covariates that are significantly associated with an outcome at 10% level of significance in a bivariate analysis will be included in the final adjusted analysis. This means we may have different sets of covariates for each outcome. Covariates to be considered include maternal HIV status at enrollment, season at enrollment, baseline socio-economic status and food security and infant age. For all analyses, if the global null hypothesis is rejected at 0.05 level, then we will perform post-hoc pairwise comparisons of groups with a Tukey-Kramer adjustment for continuous outcomes. If there is no difference between the IFA and MMN groups (which is generally expected), those two groups will be combined and the analyses will be repeated to evaluate the hypotheses stated above, i.e. to test for differences between the LNS group and the other two groups combined. The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA, logistic regression, or negative binomial model. Significant interactions ($p < 0.10$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification. For microbiota maturity and alpha diversity, potential effect modifiers to be examined will include HIV status at enrollment, season at enrollment, baseline socio-economic status and food security. For beta diversity analyses, we will employ Unweighted UniFrac ordination (presence/absence) and Weighted Unifrac ordination (presence/absence and relative abundance) based on taxa clustered at 97% 16S rRNA gene sequence similarity. The difference in beta diversity between

groups will be assessed by permutational multivariate analysis of variance (PERMANOVA): analysis of variance using distance matrices and permutation tests. We will then use a species indicator analysis to determine differentially abundant taxa.

## 12. Baseline differences between those included and excluded from analysis

The baseline differences will be compared based on socio-demographic, maternal and infant variables between those included and excluded from the analysis (Table 1). P-values will be obtained from t-test for comparison of means or Fisher's exact test for comparison of proportions.

## 13. Presentation of findings

### 13.1. Characteristics of Study Infants

The characteristics of study infants will be presented as shown in Table 1.

### 13.2. Study profile and follow-up outcome

The study profile and follow-up outcome will be presented as shown in Figure 1.

### 13.3. The impact of intervention on microbiota maturity and alpha diversity

To determine how nutritional supplementation influences alpha diversity and microbiota maturity, we will report Shannon diversity index (H) (Table 3) and microbiota for age Z score (MAZ) (Table 4).

### 13.4. The impact of intervention on beta diversity

To determine how nutritional supplementation influences microbiota beta diversity, the Unifrac outcomes will be presented as principal coordinate analysis plots and/or Hierarchical clustering resulting in dendrograms (not shown).

14. Tables

**Table 1: Characteristics of included and excluded participants**

| Characteristic | Included | Excluded | P-value |
|---|---|---|---|
| Participants, n | xxx | xxx | 0.xx |
| Maternal age at enrollment, years | xx.x (x.x) | xx.x (x.x) | 0.xx |
| Maternal education completed, years | x.x (x.x) | x.x (x.x) | 0.xx |
| Severely food insecure households | xx.x | xx.x | 0.xx |
| Gestational age at birth, weeks | xx.x (x.x) | xx.x (x.x) | 0.xx |
| Positive malaria RDT of the mother at enrollment | xx.x | xx.x | 0.xx |
| HIV status | xx.x | xx.x | 0.xx |
| Household crowding | xx.x | xx.x | 0.xx |
| # of domestic animals | xx.x (x.x) | xx.x (x.x) | 0.xx |
| Access to sanitary facility | xx.x | xx.x | 0.xx |
| Mode of delivery | xx.x | xx.x | 0.xx |
| Site of delivery | xx.x | xx.x | 0.xx |
| Household assets | xx.x | xx.x | 0.xx |
| Weight | x.xx (x.xx) | x.xx (x.xx) | 0.xx |
| Length | xx.x (x.x) | xx.x (x.x) | 0.xx |

Values are in mean (standard deviation) or percentages. P-values are obtained from t-test (continuous variables) or chi-square test (proportions), RDT, rapid diagnostic test

**Table 2. Microbiota Variables**

| | | |
|---|---|---|
| Microbiota Maturity | Microbiota-for-Age Z-Score | A measure to determine the relative microbiotoa age of children and compare it to their chronological age. |
| Alpha Diversity | Shannon Index | Estimates species richness and evenness, measures uncertainty in predicting an OTU in a sample. |
| Beta Diversity | UniFrac Distance | This measure will be calculated on group level. UniFrac measures the difference between samples by calculating distances between species on a phylogenetic tree. The output of UniFrac calculations is a distance matrix with distances of all samples to each other. |

OTU data were filtered using a threshold of at least 0.1% of reads in at least two samples. Microbiota maturity and diversity variables will be calculated with OTU data that have been rarefied to 5000 reads. Other microbiota variables will be calculated with OTU data that have been normalized using cumulative sum-scaling [7].

Table 3: Differences between groups in the Shannon diversity index at 1mo, 6mo, 12mo, 18mo and 30mo

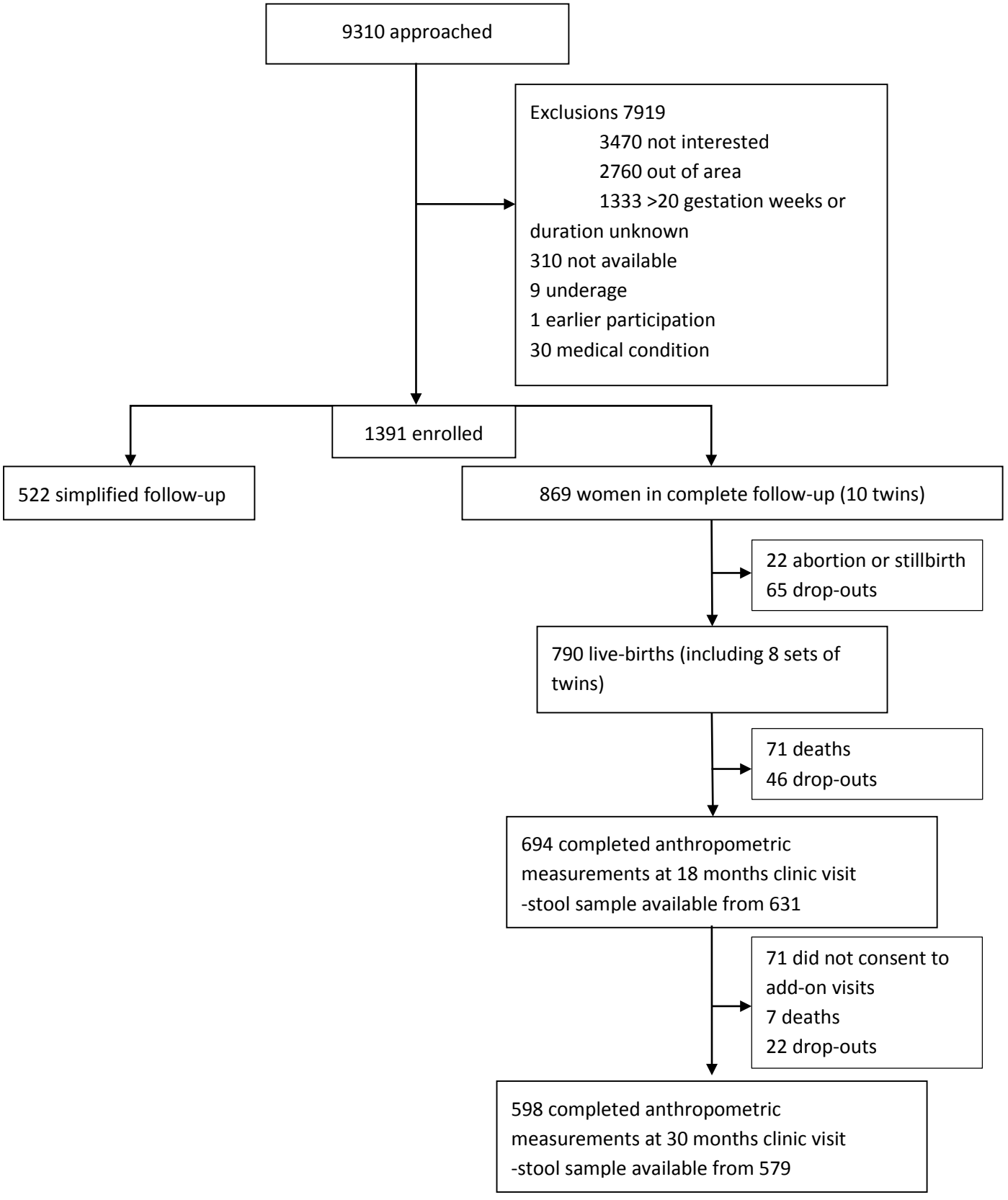
| **Variable** | **Sampling point** | **IFA [n]** | **MMN [n]** | **LNS [n]** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** |
| Shannon Diversity Index (H) - unadjusted ($\bar{x}$ ± SD) [n] | 1mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 6mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 12mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 18mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 30mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| Shannon Diversity Index (H) – with covariate adjustment ($\bar{x}$ ± SD) [n] | 1mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 6mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 12mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 18mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 30mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |

Table 4: Differences between groups in MAZ at 1mo, 6mo, 12mo, 18mo and 30mo

| **Variable** | **Sampling point** | **IFA [n]** | **MMN [n]** | **LNS [n]** | **P-value** | **Comparison of IFA and MMN** | | **Comparison of IFA and LNS** | | **Comparison of MMN and LNS** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** | **Difference in means or medians (95 % CI)** | **P-value** |
| MAZ - unadjusted ($\bar{x}$ ± SD) [n] | 1mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 6mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 12mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 18mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 30mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| MAZ– with covariate adjustment ($\bar{x}$ ± SD) [n] | 1mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 6mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 12mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 18mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |
| | 30mo | x.xx ± x.xx [x] | x.xx ± x.xx [x] | x.xx ± x.xx [x] | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | 0.xx | x.xx (xx to xx) | x.xx (xx to xx) |

## 15. Figures

Figure 1: Study Profile and follow-up outcome



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**Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)**

Statistical Analysis Plan

Appendix 29: Assessment of whether the infant microbiota at 6 months modifies effects of the DYAD interventions on growth or inflammation

Version 01.0, 2018-05-15

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## 1. Version history

| Version number | Version date | Prepared by | Description of the completed editions |
|---|---|---|---|
| 01.0 | | Riley Hughes | Original document Appendix added |

## 2. Introduction

The iLiNS-DYAD intervention trial was designed to determine whether provision of lipid-based nutrient supplements (LNS) to women during pregnancy and the first 6 months of lactation, and to the child from 6-30 months of age, improves fetal and child growth, micronutrient status and neuro-behavioral development to a greater extent than consumption of iron and folic acid during pregnancy only (IFA), or a multiple micronutrient (MMN) tablet during pregnancy and the first six months of lactation. 869 pregnant women were randomized to receive LNS (N=288), MMN (N=291), or IFA (N=290) supplementation. Children born to women in the LNS group received LNS from 6 to 18 months of age, while those born to women provided with MMN or IFA received no supplementation. All children were followed for 30 months, with fecal samples collected at 1, 6, 12, 18, and 30 months. Microbiota composition was characterized using 16S rRNA gene sequencing of the V4 region. Key details of the trial have been recorded at the clinical trial registry at the National Institutes of Health (USA) (http://www.clinicaltrials.gov/), under the registration number NCT01239693. A full trial protocol is available upon request from the PIs, Per Ashorn or Kenneth Maleta. This document (called "the statistical analysis plan" or SAP) describes the plan for data analysis and presentation of findings.

## 3. Study objectives and justification

**Objectives**: In the present analysis, we propose to determine whether the infant's microbiota composition at 6 months modifies effects of the LNS versus control (i.e. IFA and MMN) interventions on child growth or inflammation.

**Study Question:** Do certain features (i.e. taxa, patterns of composition) of infant microbiota at 6 months modify the effects of lipid-based nutrient supplements on growth and inflammation?
**Justification:** It has been shown that the gut microbiome responds to diet [1-3]. However, recent research suggests that the gut microbiome also influences host response to diet (i.e. acts as an effect modifier) [4-9]. For instance, in a study conducted by Korpela et al. [6], three different cohorts undergoing different types of fiber-based interventions were analyzed to determine whether differences in participants' microbiomes before the interventions influenced their subsequent response to the interventions. This analysis found that baseline differences in numerous bacterial taxa were associated with differences in participants' responses in outcomes such as serum cholesterol, HOMA (Homeostatic Model Assessment, indicator of insulin sensitivity), and C-reactive protein (CRP). This role of the gut microbiome as a potential effect modifier has yet to be fully explored.

While several studies such as those cited above have documented these effects, to our knowledge, none have explored this role of the gut microbiome in an infant population. Additionally, while some studies have investigated the impact of the microbiome on response in terms of inflammation or weight gain/loss or to interventions involving a high fat diet, none have measured growth in response to lipid-based nutrient supplements containing essential fatty acids (EFA) and numerous micronutrients. The gut microbiome is an important factor to be taken into account when investigating the effects of lipid supplementation, as it has been shown that the gut microbiome, and particularly the human infant gut microbiome, plays a role in lipid metabolism [10].

Furthermore, early gut microbiota composition in children has been shown to predict later growth and health outcomes [11, 12], underscoring the importance of early microbiota composition in development. Of particular importance in the infant gut microbiota are *Bifidobacteria* and *Lactobacilli*. These taxa are among the earliest and most dominant [13-16] colonizers of the infant gut and are also implicated in barrier function and protection against pathogenic bacteria [17-20]. Low abundance of these taxa has also been found to be associated with increased risk of developing allergies or atopy [21-23]. These taxa are also involved in metabolism of α-linolenic acid (ALA) [24-30] and micronutrients, including absorption of Vitamin D and calcium [31-33]. These functions make them relevant not only with respect to the lipid-based nutrient supplement (LNS) provided in the current study, but also with respect to the outcomes of interest (i.e. growth and inflammation). Adequate vitamin D and calcium are crucial for bone

growth and formation [34] while conjugated microbial metabolites of ALA have been shown to exhibit anti-inflammatory and immunomodulatory effects [24, 25].

While certain patterns of microbiota composition such as the Prevotella:Bacteroides ratio have been shown to be relevant to dietary response or health outcomes in adults [35-37], the effect of these patterns may not translate to an infant gut microbiota [38]. The ratio of Firmicutes to Bacteroidetes (F/B ratio) has been found to be of clinical significance in adults [39, 40], a higher F/B ratio being shown to have obesogenic effects. In infants, this ratio may still have clinical significance as it has been found that infants with food allergies have an increased proportion of Firmicutes and decreased proportion of Bacteroidetes [41]. Later development of food allergy has also been found to be associated with an elevated Enterobacteriaceae to Bacteroidaiceae (E/B) ratio in infants [12], suggesting that this pattern of microbiota composition in the infant may affect immune function and inflammation. Furthermore, while healthy infants have lower microbial diversity relative to adults, with the microbiota being dominated by a few species [14], it has been found that, within infants, lower microbiota diversity is associated with increased risk of food allergy and atopic eczema [42-44] and may predispose infants to development of inflammatory diseases such as necrotizing enterocolitis (NEC) [45-47]. Similarly, lower microbiota richness has also been associated with increased risk of developing food allergies [12]. Therefore, these broader patterns of microbiota composition may be applicable in the prediction of immune and inflammatory outcomes in an infant population.

The outcome of this study may help guide approaches to increase the efficacy of interventions by targeting the gut microbiome. Some studies have shown that supplementation with Bifidobacteria or Lactobaccili decreases the risk of allergy development [48-50] and influences fatty acid composition in response to ALA supplementation [30, 51], though it must be recognized that effects may be species-specific [52]. In a similar approach, one could hypothesize that if LNS were formulated with the addition of probiotic bacterial taxa found in this analysis to facilitate a more beneficial outcome of LNS supplementation, this could increase the efficacy of the intervention. Similar to the concept of an adjuvant, which may be added to a vaccine to boost the immune response, this may be thought of as a "probiotic adjuvant."

## 4. Hypotheses

To ascertain whether the infant microbiota at 6 months modifies subsequent effects of LNS supplementation, we will test the following hypotheses:

1) The abundance of Bifidobacteria and Lactobacilli will modify the effect of LNS on growth and inflammation. We hypothesize that among infants with higher abundances of Bifidobacteria and Lactobacilli at 6 months, LNS will have a positive effect on growth and will be associated with reduced inflammation, whereas among infants with lower abundances of Bifidobacteria and Lactobacilli, these effects will not be observed.
2) The ratios of Enterobacteriaceae to Bacteroidaceae (E/B ratio) and Firmicutes to Bacteroidetes (F/B ratio) as well as MAZ score will modify the effect of LNS on growth and inflammation. We hypothesize that among infants with lower E/B and F/B ratios and lower MAZ scores at 6 months, LNS will have a positive effect on growth and will be associated with reduced inflammation, whereas among infants with higher ratios and MAZ scores, these effects will not be observed.
3) Microbial diversity and richness will modify the effect of LNS on growth and inflammation. We hypothesize that among infants with higher diversity and richness at 6 months, LNS will have a positive effect on growth and will be associated with reduced inflammation, whereas among infants with lower diversity and richness, these effects will not be observed.
4) The abundance of Bifidobacteria and Lactobacilli will modify the effect of LNS on microbiota maturity (MAZ). We hypothesize that among children with higher abundances of Bifidobacteria and Lactobacilli at 6 months, LNS will have a positive effect on MAZ (increase in MAZ), whereas among infants with lower abundances of Bifidobacteria and Lactobacilli, this effect will not be observed.

## 5. Definition of variables

*Outcomes*

Growth: We will assess the growth of infants using changes in weight-for-age-z-score (WAZ), length-for-age-z-score (LAZ), weight-for-length-z-score (WLZ), mid upper arm circumference (MUACZ), and head circumference z-score (HCZ). The WHO 2006 Child Growth Standards will be used for age-and-sex standardization of child weight, length, head circumference, arm

circumference and weight-for-length. The outcomes will be defined as the change in growth z-score from 6 months to 12 months.

Inflammation: Inflammation will be assessed at 18 months using measures of C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP). These will be analyzed as continuous variables.

*Effect Modifiers*

MAZ score: The MAZ score determines the relative microbiota age of children compared to their chronological age. We will assess the maturity of the gut microbiota in infants using a model that will generate the relative microbiota maturity and microbiota for age Z score (MAZ), as described previously [5] and also as previously calculated by the Gordon lab (https://gordonlab.wustl.edu/).

E/B ratio: The E/B ratio is another metric that has been used previously as a measure of gut microbiota maturity as Proteobacteria (primarily Enterobacteriaceae) are prevalent in the early gut microbiota, while Bacteroidetes (primarily Bacteroidaceae) become more dominant as the gut community matures [53]. We will assess this ratio using relative abundances of these families.

F/B ratio: The F/B ratio has been found to differ between infants and adults [38] and no benchmark of clinical significance has been established for infants. Therefore, we will not assess differences in groups based on stratifying using a discrete cutoff, but will use linear regression to assess whether the F/B ratio modifies the magnitude and direction of the effect of intervention group on growth and inflammation.

Richness: Richness will be assessed using the Chao1 and Abundance-based Coverage Estimator (ACE) methods. These non-parametric approaches make no assumptions about the underlying distributions of species abundance or detection rates, making them more robust in applications [54].

Diversity: Alpha diversity will be assessed using the Shannon diversity index.

Bifidobacteria and Lactobacilli abundance: Abundance of taxa will be assessed using relative abundance of 16S rRNA.

## 6. Statistics software

Analyses will be performed in Excel, STATA, SAS and/or R.

## 7. Basis for the analysis

The analysis will be based on modified intention to treat. If outcome data are missing for a participant at a certain time point, the missing data will not be imputed and the participant will be excluded from the respective analyses. Missing data for independent variables, covariates, and effect modifiers will be reported and if necessary imputed using chained equation methods. Imputation will be deemed necessary for a group of independent variables if their combination results in more than 10% dropped observations. If a single independent variable is missing more than 20% then it will instead be dropped from the analysis. A sensitivity analysis will be performed and the results with and without imputed data will be presented.

## 8. Time points

As LNS supplementation directly to the infant began at 6 months and there were no significant differences in microbiota composition among intervention groups at that age, the infant microbiota at 6 months will be used as “baseline”. Growth outcomes will be assessed at 12 months and inflammation at 18 months. The relevant outcome variables can be seen in Table 4.

## 9. Outliers

We will start by inspecting the outcomes for outliers, which will involve visual inspection using box plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers that are clearly impossible or implausible values will be corrected if possible, or truncated to the $97.5^{th}$ or $2.5^{th}$ percentile. Outliers that are plausible or possible will be kept in their original

form. In an extreme situation, a sensitivity analysis (excluding all outliers) will be done to determine if such outliers have undue influence on the results

**10. Data transformation**

Normality of the outcome variable is a model assumption. Therefore, continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately, with a preference towards the log transformation. If no suitable transformation can be found, then analysis will be done on ranked data or categories will be created.

**11. Analytical Approach**

The effects of potential microbiome effect modifiers will be assessed with an interaction term with the treatment variable in the ANCOVA or logistic regression. Significant interactions ($p < 0.10$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the effect modifier, in order to understand the nature of the effect modification. We will examine as potential effect modifiers the following variables: baseline Lactobacillus abundance, Bifidobacteria abundance, E/B ratio, F/B ratio, diversity, and richness.

11.1 Intervention Variable

The MNN and IFA intervention groups will be combined into one control group, as neither supplement contains fats or was supplied to the child.

11.1 Covariates

For adjusted analyses, only covariates that are significantly associated with an outcome at 10% level of significance in a bivariate analysis will be included in the final adjusted analysis. Potential covariates will include site of enrollment, pre-pregnancy adjusted baseline maternal BMI, HIV status, parity, maternal age, SES, and season at 6 months postpartum. We will explore potential interactions with other characteristics such as category of breast feeding and antibiotic use. The potential effect modifier and the treatment effect will always be included in the model as single-terms.

11.2 Testing relationship between intervention and effect modifiers

In order to avoid multicollinearity of the intervention variable and the 6-month variable that relates to the outcome (ie. CRP at 6 months when the outcome is CRP at 18 months, or LAZ at 6 months when the outcome is change in LAZ from 6 to 12 months), before an ANCOVA is run, the relationship between the intervention and the 6-month effect modifier will be tested in the subset. If there is a significant difference in mean 6-month effect modifier by intervention group, the primary effect modification model will not be done. If there is no treatment effect, the 6 month effect modifier will be included in the model, regardless of its association with the outcome.

**12. Multiple hypothesis testing**

We are concerned about the possibility of false positive findings but are aware that there is not a standard accepted method for handling this problem, particularly when outcomes are correlated[55]. There are pros and cons to such an adjustment and we will proceed with caution and further discussion. One approach we'll consider is the Benjamini-Hochberg correction ($q = 0.15$) and the presentation of both raw and adjusted p-values.

**13. Baseline differences between those included and excluded from analysis**

The baseline differences will be compared based on socio-demographic, maternal and infant variables between those included and excluded from the analysis (Table 1). P-values will be obtained from t-test for comparison of means or Fisher's exact test for comparison of proportions.

**14. Presentation of study findings**

Table 1: Characteristics of included and excluded participants
Table 2: Characteristics of LNS and non-LNS participants
Table 3. Environmental exposure variables
Table 4: Effect modifier metrics
Table 5: Effect Modification by microbiota at 6 mo on outcome of LNS supplementation

Figure 1: Study Profile and follow-up outcome

## 15. Tables

**Table 1. Characteristics of included and excluded participants**

| Characteristic | Included | Excluded |
|---|---|---|
| Participants, n | xxx | xxx |
| Maternal age at enrollment, years | xx.x (x.x) | xx.x (x.x) |
| Maternal education completed, years | xx.x (x.x) | xx.x (x.x) |
| Severely food insecure households | xx.x | xx.x |
| Gestational age at birth, weeks | xx.x (x.x) | xx.x (x.x) |
| Positive malaria RDT of the mother at enrollment | xx.x | xx.x |
| Maternal HIV status | xx.x | xx.x |
| Household crowding | xx.x | xx.x |
| # of domestic animals | xx.x (x.x) | xx.x (x.x) |
| Access to sanitary facility | xx.x | xx.x |
| Mode of delivery | xx.x | xx.x |
| Site of delivery | xx.x | xx.x |
| Household assets | xx.x | xx.x |
| WAZ | xx.xx (x.x) | xx.xx (x.x) |
| LAZ | xx.x (x.x) | xx.x (x.x) |

**Table 2. Characteristics of LNS and non-LNS participants**

| Characteristic | LNS | Non-LNS |
|---|---|---|
| Participants, n | xxx | xxx |
| Maternal age at enrollment, years | xx.x (x.x) | xx.x (x.x) |
| Maternal education completed, years | xx.x (x.x) | xx.x (x.x) |
| Severely food insecure households | xx.x | xx.x |
| Gestational age at birth, weeks | xx.x (x.x) | xx.x (x.x) |
| Positive malaria RDT of the mother at enrollment | xx.x | xx.x |
| HIV status | xx.x | xx.x |
| Household crowding | xx.x | xx.x |
| # of domestic animals | xx.x (x.x) | xx.x (x.x) |
| Access to sanitary facility | xx.x | xx.x |
| Mode of delivery | xx.x | xx.x |
| Site of delivery | xx.x | xx.x |
| Household assets | xx.x | xx.x |
| WAZ | xx.xx (x.x) | xx.xx (x.x) |
| LAZ | xx.x (x.x) | xx.x (x.x) |

**Table 3: Environmental exposure variables**

| | |
|---|---|
| Household assets Z-score (continuous) | Measure of socioeconomic status, includes information on building materials of the house, sources of water, electricity and cooking fuel and sanitary facility |
| Household crowding (continuous) | Total number of people living in the household |
| # of domestic animals (continuous) | Number of chickens, goats and cows in the household, each type of animal analyzed separately |
| Sanitary facility (dichotomous) | No sanitary facility in household (vs. any sanitary facility) |

**Table 4. Effect modifier metrics**

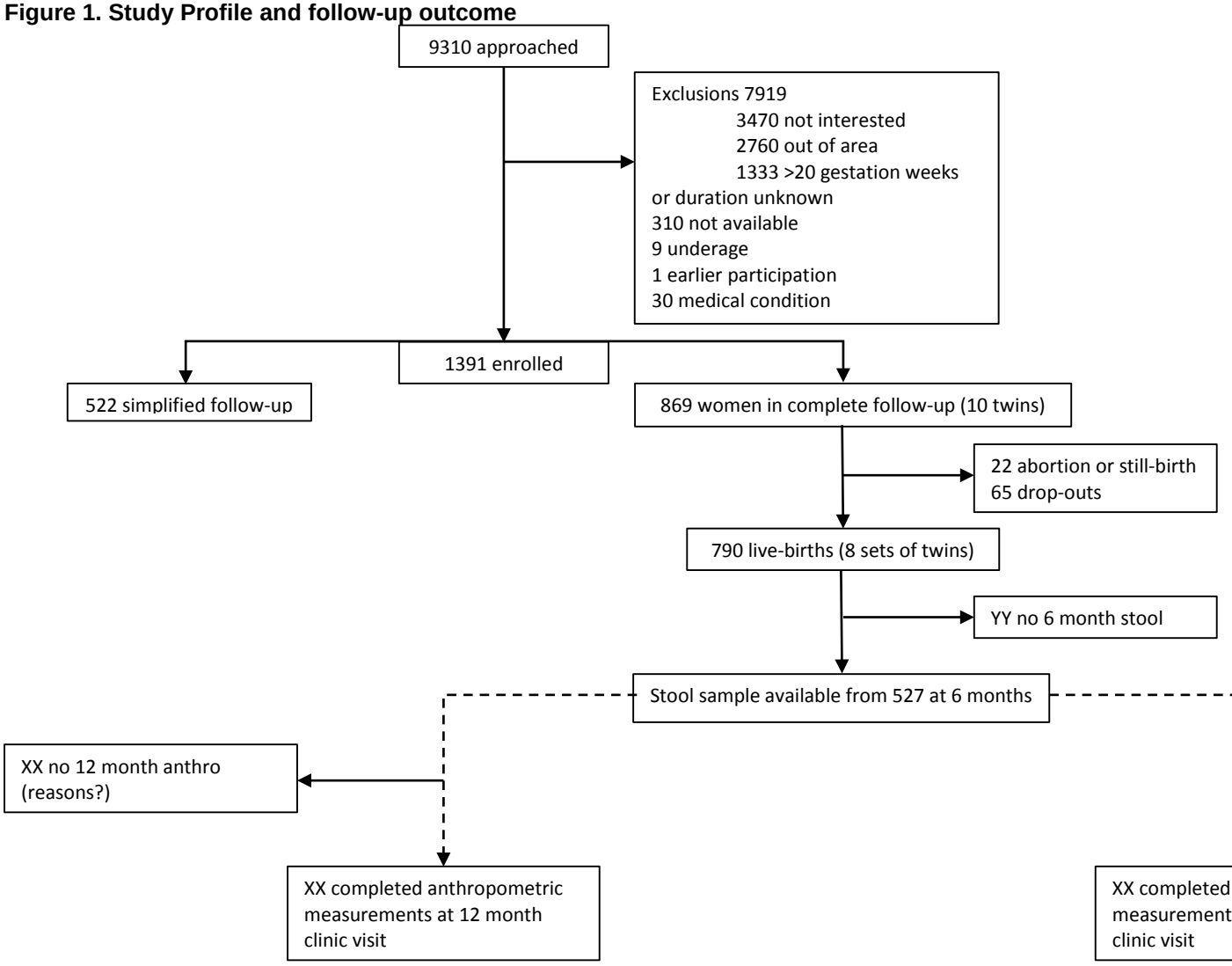
| Effect Modifiers | | |
|---|---|---|
| Metric | Variable | Associations in the literature |
| Bifidobacteria abundance | Relative abundance of Bif. (%) | Barrier function [17, 19, 20]<br>ALA metabolism [24-29]<br>Micronutrient metabolism [31-33]<br>Future weight gain in children [11]<br>Risk of allergies/atopy [22, 23] |
| Lactobacilli abundance | Relative abundance of Lactobacilli (%) | Barrier function [18, 19]<br>ALA metabolism [25, 26]<br>Micronutrient metabolism [32, 33]<br>Risk of allergies [21] |
| E/B ratio | Ratio of relative abundances | Risk of food allergies [12] |
| F/B ratio | Ratio of relative abundances | Risk of food allergies [41] |
| Alpha Diversity | Shannon Index | Risk of food allergies and atopic eczema [42-44] |
| Richness | Chao1, ACE | Risk of food allergies [12] |

**Table 5. Effect Modification of Microbiota at 6mo on outcome of LNS supplementation.**

| **Effect Modifier** | **WAZ** | **LAZ** | **WLZ** | **HCZ** | **CRP** | **AGP** |
|---|---|---|---|---|---|---|
| Bifidobacteria (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |
| Lactobacilli (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |
| E/B ratio (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |
| F/B ratio (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |
| Diversity (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |
| Richness (p-value of interx) | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx | 0.xxx |

## 16. Figures

**Figure 1. Study Profile and follow-up outcome**

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