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

Statistical Analysis Plan

Version 14.0 (25.11.2014)

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1 Version history

Version number	Version date	Prepared by	Description of the completed editions
01.0	04.06.2013	Lotta Alho Yin Bun Cheung 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). Added Appendix 02: The impact of intervention on maternal fever (prepared by Lotta Alho). 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). Added Appendix 04: Malaria immunity analyses (prepared by Upeksha Chandrasiri & Stephen Rogerson)
04.0	03.11.2013	Alho, Cheung, Peerson	Added Appendix 05: The impact of intervention on maternal periodontal infections (prepared by Ulla Harjunmaa)
05.0	10.04.2014	Alho, Cheung, Peerson	Revised main birth outcome analysis: Edited methods on pairwise comparisons for binary end points and SAE's and corrected the definition of perinatal mortality rate. Revised Appendix 01: Secondary growth outcomes (prepared by Lotta Alho). Added Appendix 06: Willingness-to-pay for lipid-based nutrient supplements during pregnancy (prepared by Emy Reimao).

06.0	12.06.2014	Alho, Cheung, Peerson	Added Appendix 07: The impact of the interventions on iron status and inflammation (prepared by Josh Jorgensen)
07.0	04.07.2014	Alho, Cheung, Peerson	Added Appendix 08: Characterisation of microbial communities in the placenta, chorion, amnion, vagina and oral cavity (prepared by Ronan Doyle) Added Appendix 09: The impact of LNS on maternal salivary cortisol concentration (prepared by Brietta Oaks) Added Appendix 10: The impact of LNS on maternal cholesterol and triglycerides in plasma and fatty acids in plasma and breast milk (prepared by Brietta Oaks)
08.0	25.07.2014	Alho, Cheung, Peerson	Added Appendix 11: Developmental outcomes at age 18 months (prepared by Elizabeth Prado) Added Appendix 12: Maternal cognition and mother-infant interaction at 6 months post-partum (prepared by Elizabeth Prado)
09.0	19.08.2014	Alho, Cheung, Peerson	Added Appendix 13: The impact of intervention on maternal anthropometry and placental weight (prepared by Kenneth Maleta)
10.0	31.08.2014	Alho, Cheung, Peerson	Added Appendix 14: The impact of the interventions on maternal vitamin A status (prepared by Andrew Hall and Marjorie Haskell)
11.0	11.09.2014	Alho, Cheung, Peerson	Added Appendix 15: Comparison of the main effect of treatment group on change in vitamin B12 and folate status during pregnancy, and vitamin B12 in breast milk at 6 months postpartum (prepared by Lindsay Allen)
12.0	13.09.2014	Alho, Cheung, Peerson	Added Appendix 16: The impact of intervention on maternal reproductive tract infections and malaria (prepared by Minyanga Nkhoma)
13.0	16.10.2014	Alho, Cheung, Peerson	Added Appendix 17: Effect on breastfeeding practices from birth to six months (prepared by Mary Arimond, Kathryn Dewey and Jan Peerson)

14.0	25.11.2014	Alho, Cheung, Peerson	Revised Appendix 04: Secondary growth outcomes (prepared by Upeksha Chandrasiri).

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 ≤ 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.

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)

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: Characterisation 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 vitamin B12 and folate status during pregnancy, and vitamin B12 in breast milk at 6 months postpartum (added on 11.09.2014)

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)

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)

Contents

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

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)

Contents

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)

Table of Contents

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 02.0, modified on 25.11.2014)

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

Contents

1. Version history

Version number	Version date	Prepared by	Description of the completed editions
01.0	24.10.2013	Upeksha Chandrasiri Prof. Stephen Rogerson	Original appendix 04
02.0	25.11.2014	Upeksha Chandrasiri Prof. Stephen Rogerson	Updated Appendix 04 to cover malaria immunity at 6 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 ($\leq$ 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 only, prepared by the iLiNS sub-contract investigators at the University of Melbourne. Subsequent statistical plans will be submitted at later stage.

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.

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. Of note this statistical plan will only report the plan of analyses for hypothesis1/ objective 1.

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

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.

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.

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 on 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.

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 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

10. 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) <20 years No. (%) 20-25 26-30 >30	XX	XX	XX	24 (20-28) 310 (30.83%) 298 (29.62%) 238 (23.66%) 160 (15.90%)	X.XX (XX to XX)
Gravidity: Number (%) Primigravidae Secundigravidae Multigravidae (3-5 pregnancies) Grand multigravidae (>5 pregnancies)	XX	XX	XX	199 (19.76%) 202 (20.06%) 381 (37.84%) 225 (22.34%)	X.XX (XX to XX)
Malaria prevalence: Number (%) at enrolment, PCR 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 (%) Haemoglobin levels 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	Median	Median	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

	(IQR)	(IQR)	(IQR)							
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

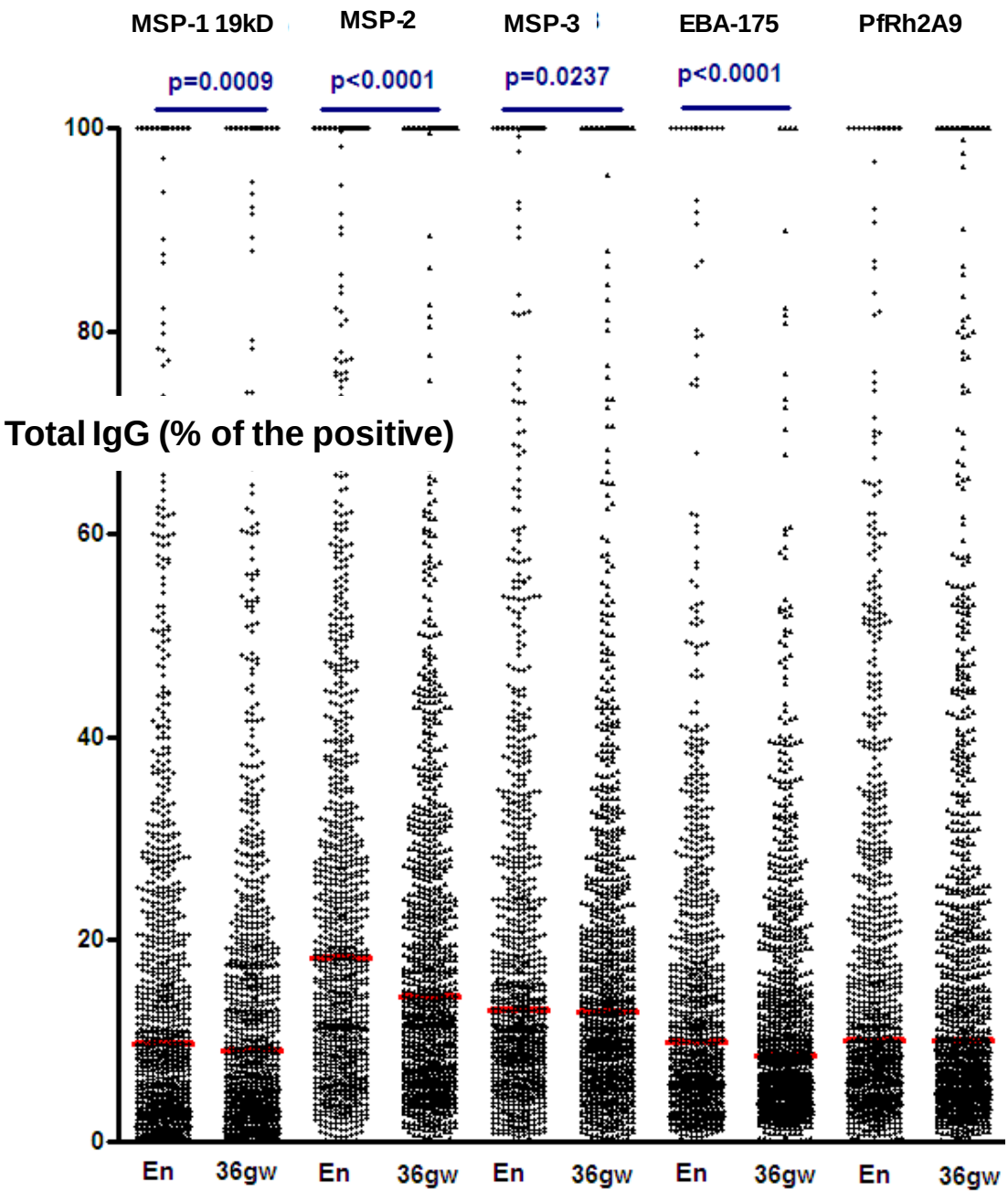
a. P-value calculated using the Chi2 test
b. P-value calculated using logistic regression reporting Relative Risk Ratios (RR)
d. P-value calculated using multivariate logistic regression reporting odds ratios (OR) while adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia 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

11. 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

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)

Contents

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)

Table of Contents

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

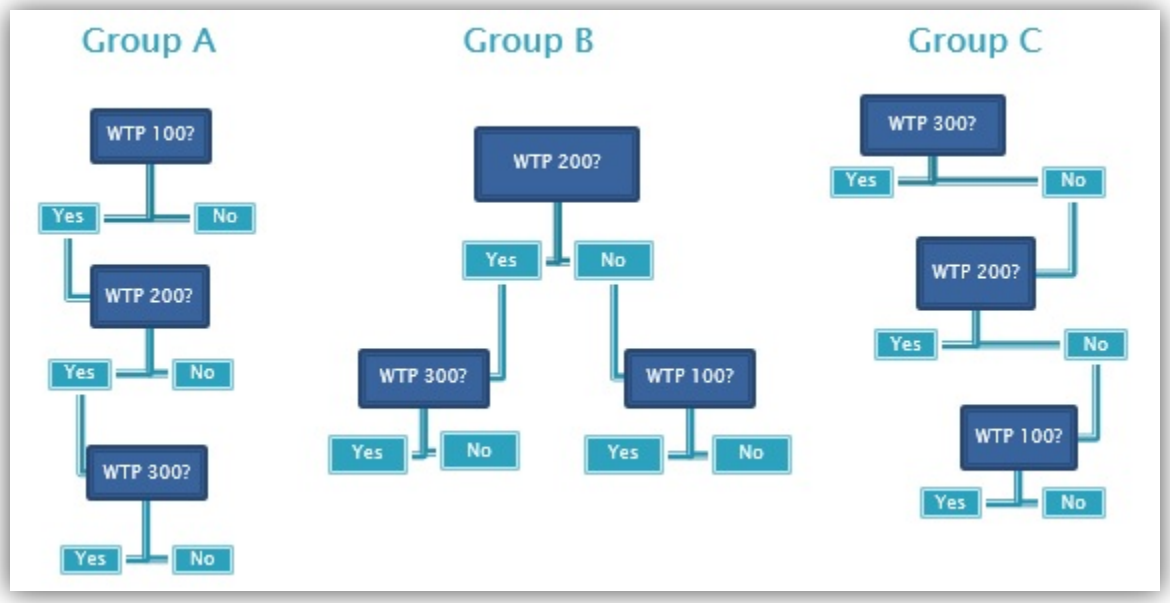
2. Introduction and Context

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 4th 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 (β_4) and to how the iLiNS mother's characteristics affect WTP (β_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)

Contents

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 1st and 3rd 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)

Contents

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 μl^{-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 μl^{-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

Contents

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 >75th percentile and low cortisol as cortisol concentration < 25th percentile at 28 wk of the IFA group.

Cortisol at 36 wk gestation

High cortisol will be defined as cortisol concentrations >75th percentile and low cortisol as cortisol concentration < 25th 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 > 75th 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 < 25th 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

Contents

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 < 10th 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

Contents

Version number	1
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**

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

Contents

Version number	1
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**

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)

Contents

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 10th 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)

Contents

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: Comparison of the main effect of treatment group on change in vitamin B12 and folate status during pregnancy, and vitamin B12 in breast milk at 6 months postpartum (version 01.0, 11 September 2014, prepared by Lindsay Allen)

1) **Study Objectives.**

This analysis falls under the iLiNS-DYAD-M trial, the primary aim of which is to evaluate the efficacy of lipid-based nutrient supplements (LNS) for pregnant women. A secondary aim is to study the impact of LNS on breast milk B12 concentrations at 6 mo postpartum. This substudy analysis will compare the change in plasma B12, folate and homocysteine from enrolment (before 20 wk gestation) through 36 wk gestation 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 400 µg 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).

In addition, we will evaluate status in early pregnancy and change in B12 at 36 wk gestation and its association with B12 breast milk concentrations at 6 mo in each treatment group.

2. Study Description. Pregnant women were randomly assigned to receive one of three daily supplements throughout pregnancy. Baseline blood samples were collected at the time of enrollment (<20 wk gestation) and final time point samples were collected at 36 wk gestation, as determined by ultrasonography, as well as breast milk samples at 6 mo 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 36 wk will be compared between groups using an ANCOVA statistical model.

3. Hypotheses to be tested

a) Primary hypothesis A: Plasma B12 concentrations will be higher at 36 wk gestation in pregnant women in Malawi receiving LNS and MMN compared to the group receiving IFA, but there will be no differences in plasma folate among groups.
b) Primary hypothesis B: Plasma homocysteine concentrations will be lower at 36 wk gestation in the group of pregnant women in Malawi receiving MMN or LNS compared to the group receiving IFA.
c) Primary hypothesis C: Breast milk B12 concentrations in the LNS and MMN treatment groups at 6 mo postpartum will be higher than in the placebo group.
d) Secondary/exploratory hypothesis D: The effect of treatment group on breast milk B12 will be modified by maternal plasma B12 in early pregnancy.

4. Definition of the substudy outcomes

Outcomes

a. Concentrations of plasma B12, folate and homocysteine at 36 wk gestation controlling for values at < 20 wk gestation.
b. Percent abnormal values of B12, folate and homocysteine at baseline and 36 wk gestation.
c. Breast milk B12 concentrations at 6 mo postpartum.

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. A biological sample will be collected at 36 wk gestation and 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 minimum criteria for 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 $\geq$ 70% of supplement days and minimum adherence will be defined as consumption on > 50% of supplement days.

6. Time points for the analyses

Biological samples will be collected at baseline (<20 wk gestation) and at term, before delivery (36 wk gestation). Breast milk samples will be collected at 6 mo postpartum.

7.Statistical software

All statistical analyses will be performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

8. Presentation of the study findings and hypothesis testing

Hypotheses A and B will be addressed as follows: The group means and standard deviations for B12, folate and homocysteine at each of the two time points will be presented as indicated in Table 1. An overall ANOVA p-value will be provided and pairwise differences will be denoted by superscript. In addition, the % of abnormal values at baseline and at 36 weeks will be presented as indicated in Table 2 and the risk ratios calculated and compared. Table 3 will show stepwise regression results for difference between treatment groups in breast milk B12 concentrations controlling for baseline B12 status, assessing the effect of parity and maternal age as covariates (Table pending).

Outcome variables will be assessed for conformance to the normal distribution and transformed if needed. Mean change in biomarkers in each of the intervention groups will be compared using ANCOVA (SAS GLM procedure). Post-hoc comparisons will be analyzed by Tukey's HSD test. Correlation analysis will be performed to determine if potential covariates are linearly related to the outcome variable at a 10% level of significance. All variables which are related to the outcome variable will be included as covariates.

9. Description of covariates and potential modifying effects

a) initial B12, folate and homocysteine
b) initial and 36 wk C-reactive protein (CRP)
c) initial and 36 wk alpha-1-glycoprotein (AGP)
d) initial body mass index (BMI)

e) malaria at baseline
f) HIV status at baseline
g) parity
h) age
i) timing of last use of supplement (for effect on breast milk B12)

Table 1. Maternal plasma B12, folate and homocysteine and breast milk vitamin B12.

Variable	Timepoint	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 (95 % CI)	P-value	Difference in means (95 % CI)	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) [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)
Plasma folate (nmol/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)
Plasma homocysteine (umol/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)
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.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 abnormal biochemical values

Cutoff	Timepoint	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
Plasma B12 <150 pmol/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
Plasma B12 150-225 pmol/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
Plasma B12 <100 pmol/L	36 wk	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
Plasma folate <10 nmol/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
Plasma folate <10 nmol/L	36 wk	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
Plasma tHcy >10 umol/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
Plasma tHcy >10 umol/L	36 wk	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

Table 3. Stepwise regression for difference between treatment groups in 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).

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)

Contents

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** **N (%)**	**MMN** **N (%)**	**LNS** **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)

Contents

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…..