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

Statistical Analysis Plan

Version 04.0 (03.11.2013)

Prepared by:

Lotta Alho, MSc, study statistician

Yin Bun Cheung, PhD, responsible study statistician

Jan Peerson, MSc, study statistician

Approved by:

Name, degree	**Position**	**Signature**
Per Ashorn, MD, PhD	Principal investigator	
Kenneth Maleta, MBBS, PhD	Co-Principal investigator	
Kathryn Dewey, PhD	Principal investigator for the iLiNS-Project	
Ulla Ashorn, PhD	Responsible investigator, social studies	
Steve Vosti, PhD	Responsible investigator, economic studies	

Contact details: Ms. Lotta Alho, University of Tampere School of Medicine, Finland. Email: Lotta.alho@uta.fi

Table of Contents

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)

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 28 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 analysed 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 and pairwise comparisons will be tested with logistic regression. 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.

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 Fisher's exact test.

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 logistic 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)

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)

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

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	xxx (xx.x%)	xxx (xx.x%)	xxx (xx.x%)	Chi-squared

positive HIV test				
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.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) 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	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

model[b]										
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 11.11

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]	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[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]	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 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 11.11

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 intervention on child size at 6 months (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 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. Description of the other two objectives is presented in the main analysis plan.

The aim of the secondary analyses described in appendix 1 is to compare infant growth in three different intervention groups by six months of age. The following outcomes will be used to indicate infant growth.

1. Mean weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), mid-upper arm circumference (MUAC)-for-age and head circumference-for-age Z-scores at the age of six months
2. Prevalence of stunting, underweight, wasting at the age of six months
3. The prevalence of small mid-upper arm circumference and small head circumference at the age of six months

This analysis will only include participants in the complete follow-up. This means that women in the iron and folic acid group will have received IFA tablets during pregnancy and placebo tablets during first six months of lactation and participants in multiple micronutrient and LNS groups will have gotten either IFA tablet or LNS supplementation during pregnancy and first six months of lactation. The participating babies will not have received any supplements during this period.

2. Hypotheses to be tested

1. The mean weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WHZ), mid-upper arm circumference (MUAC)-for-age and head circumference-for-age Z-scores of infants born to mothers provided with LNS during pregnancy and up to 6 months after delivery will be greater than that of infants whose mothers received either iron-folate or micronutrient supplementation.

2. The prevalence of moderate-to-severe stunting, wasting, underweight, small mid-upper arm circumference and small head circumference will be lower among infants born to mothers provided with LNS during pregnancy and up to 6 months after delivery than among infants whose mothers 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 secondary growth outcomes

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

Weight-for-age will be calculated from age, sex, and weight information from the measurement taken at the study clinic at six 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; Form 29: Q1.2, Q2.2.*

The prevalence of underweight

Moderate to severe underweight will be defined as a WAZ-score < -2.0 and severe underweight as WAZ-score < -3.0. The prevalence of underweight will be calculated by dividing the number of infants with WAZ < -2 or WAZ < -3 Z-score units by the number of all infants 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 length-for-age Z-score (LAZ)

Length-for-age will be calculated from age, sex, and length information from the measurement taken at the study clinic at six 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; Form 29: Q1.2, Q2.3.*

The prevalence of stunting

Moderate to severe stunting will be defined as a LAZ-score < -2.0 and severe stunting as LAZ-score < -3.0. The prevalence of stunting will be calculated by dividing the number of infants with LAZ < -2 or LAZ < -3 Z-score units by the number of all infants 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 weight-for-length Z-score (WLZ)

Weight-for-length will be calculated from age, sex, weight and length information from the measurement taken at the study clinic at six 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; Form 29: Q1.2, Q2.2, Q2.3.*

The prevalence of wasting

Moderate to severe wasting will be defined as a WLZ-score < -2.0 and severe wasting as WLZ-score < -3.0. The prevalence of wasting will be calculated by dividing the number of infants with WLZ < -2 or WLZ < -3 Z-score units by the number of all infants 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, Q2.3.*

Mean MUAC-for-age Z-score

MUAC-for-age will be calculated from age, sex, and MUAC information from the measurement taken at the study clinic at six 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; Form 29: Q1.2, Q2.4.*

Prevalence of acute small MUAC

A small MUAC will be defined as a MUAC Z-score < -2.0 and a very small MUAC as MUAC Z-score < -3. The prevalence of small or very small MUAC will be calculated by dividing the number of infants with MUAC Z-score < -2 or MUAC Z-score < -3 Z-score units by the number of all infants 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 measurement taken at the study clinic at six 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; Form 29: Q1.2, Q2.5.*

Prevalence of small head circumference

A small head circumference will be defined as a head circumference Z-score < -2.0 and a very small head circumference as head circumference Z-score < -3. The prevalence of small or very small head circumference will be calculated by dividing the number of infants with head circumference Z-score < -2 or head circumference head Z-score < -3 Z-score units by the number of all infants 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.*

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.

For variables targeted to be measured at six months of age, the data are considered missing if the actual measurement date is over +/- 8 weeks from target.

5. Time points for the analyses

All the above analyses will primarily be done when the child is 6 months old.

6. Presentation of the study findings and hypothesis testing

6.1 Comparison of the anthropometric measurements at 6 months of age between the three intervention groups

The group means and standard deviations for WAZ, LAZ, WLZ, MUAC Z-score and head circumference Z-score at six months of age 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 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.

6.2 Comparison of the dichotomous growth outcomes at 6 months of age between the three intervention groups

The proportions of infants with underweight, stunting, wasting, acute undernutrition and small head circumference will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups and pairwise comparisons will be tested with logistic regression. 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 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. Legends to the figures

Figure 1. Box-Whisker plots of WAZ at 6 months of age by group

Figure 2. Box-Whisker plots of LAZ at 6 months of age by group

Figure 3. Box-Whisker plots of WLZ at 6 months of age by group

Figure 4. Box-Whisker plots of MUAC Z-score at 6 months of age by group

Figure 5. Box-Whisker plots of head circumference Z-sore at 6 months of age by group

9. Figures

Figure 1. Box-Whisker plots of WAZ at 6 months of age by group

Figure 2. Box-Whisker plots of LAZ at 6 months of age by group

Figure 3. Box-Whisker plots of WLZ at 6 months of age by group

Figure 4. Box-Whisker plots of MUAC Z-score at 6 months of age by group

Figure 5. Box-Whisker plots of head circumference Z-sore at 6 months of age by group

10. Tables

Table 1. Continuous growth 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) 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) 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) 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) head circumference-	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

for-age z-score	(x.xx)	(x.xx)	(x.xx)		xx)		xx)		xx)	

Table 2. Dichotomous growth 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 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 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 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

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

Beard JL, Hendricks MK, Perez EM, Murray-Kolb LE, Berg A, Vernon-Feagans L, Irlam J, Isaacs W, Sive A, Tomlinson M. 2005. Maternal iron deficiency anemia affects postpartum emotions and cognition. J Nutr. 135:267–272.

Bloch MH, Hannestad J. 2011. Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. Mol Psychiatry. 17:1272–1282.

Frith AL, Naved RT, Ekström E-C, Rasmussen KM, Frongillo EA. 2009. Micronutrient supplementation affects maternal-infant feeding interactions and maternal distress in Bangladesh. American Journal of Clinical Nutrition. 90:141–148.

Leung BMY, Kaplan BJ. 2009. Perinatal Depression: Prevalence, Risks, and the Nutrition Link—A Review of the Literature. Journal of the American Dietetic Association [Internet]. 109:1566–1575.

Prado EL, Ullman MT, Muadz H, Alcock KJ, Shankar AH, SUMMIT Study Group. 2012. The effect of maternal multiple micronutrient supplementation on cognition and mood during pregnancy and postpartum in Indonesia: a randomized trial. PLoS ONE. 7:e32519.

Rahman A, Bunn J, Lovel H, Creed F. 2007. Maternal depression increases infant risk of diarrhoeal illness: -a cohort study. Archives of Disease in Childhood. 92:24–28.

Smith Fawzi MC, Kaaya SF, Mbwambo J, Msamanga GI, Antelman G, Wei R, Hunter DJ, Fawzi WW. 2007. Multivitamin supplementation in HIV-positive pregnant women: impact on depression and quality of life in a resource-poor setting. HIV Med. 8:203–212.

Stewart RC, Umar E, Tomenson B, Creed F. 2013. Validation of screening tools for antenatal depression in Malawi-A comparison of the Edinburgh Postnatal Depression Scale and Self Reporting Questionnaire. J Affect Disord. 150:1041–1047.

Stewart RC. 2007. Maternal depression and infant growth: a review of recent evidence. Matern Child Nutr. 3:94–107.

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 01.0, added on 24.10.2013)

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

2. Introduction

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

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

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

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

This appendix will report statistical plan for “Hypothesis 1”

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

5. Definition of primary outcomes for objective 1

5.1. 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 1/3rd 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

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 $p<0.05$.

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.

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

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.

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

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

9.6. 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

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

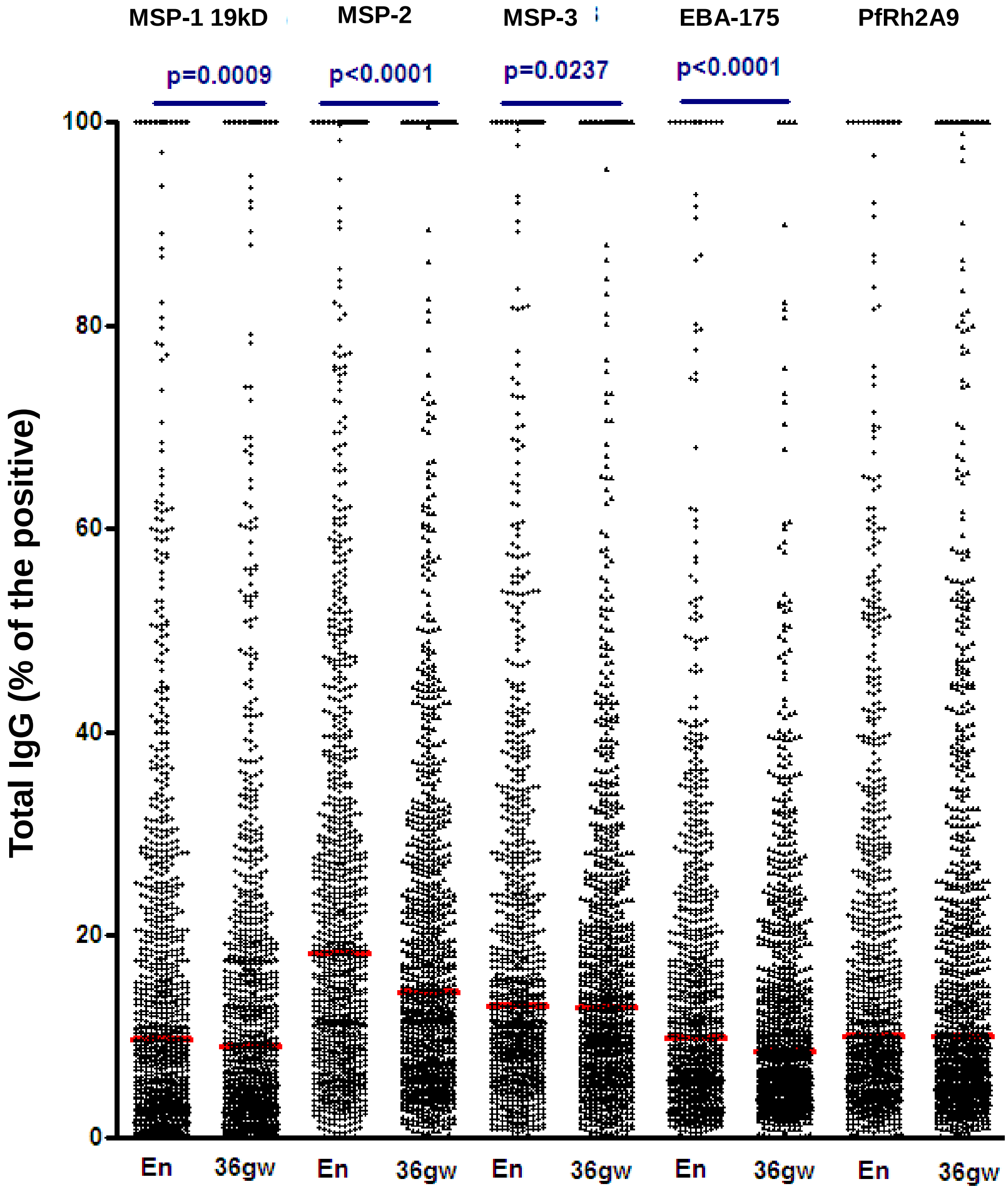
Table 3: Magnitude of antibody level change or rate of antibody level change categorized by supplementation groups

	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.

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.

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