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

Statistical Analysis Plan

Version 1.0 (31.05.2013)

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1 Version history

Version number	Version date	Prepared by	Description of the completed editions
01.0	31.05.2013	Lotta Alho Yin Bun Cheung Jan Peerson	Original document (no appendixes included)

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 modulates 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, 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 intervemtion), 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 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 3: 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 4: 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 5: 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 6: 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 7: 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 8: 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 9: 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 10: 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 scrambled 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 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.*

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

Weight-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, Q2.2.*

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 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, Q2.4.*

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 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, Q2.5.*

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 categorization will be based on the seriousness of the SAE. For example, if a person experienced first something else and then a death, the individual will be categorized in the "death" category. Similarly, if a person experienced both a hospitalization and something else which was not a death, the individual will be categorized in the "hospitalization" 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 categorization will be based on the seriousness of the SAE. For example, if a person experienced first something else and then a death, the individual will be categorized in the "death" category. Similarly, if a person experienced both a hospitalization and something else which was not a death, the individual will be categorized in the "hospitalization" 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 six 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 6 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 six 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, 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 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.

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

The proportions of babies with low birth weight, preterm birth, or various forms of undernutrition in the newborn period will be tabulated by intervention group as shown in Table 3. Global null hypothesis of no differences between groups will be tested with 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.

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

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

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

12.3 Multiple comparisons

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

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

12.4 Confidence intervals

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

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

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. However, the final decision on the use of covariates will be decided based on preliminary analyses on the cleaned dataset that includes information on the clustering of participants in the same group but does not provide information on the actual intervention delivered to each group.

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

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-DOSE 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

None

16 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

17 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) newborn length-for-age (LAZ) 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) newborn length-for-age (LAZ) z-score, 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) duration of the pregnancy,	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

weeks										
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
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