Efficacy of lipid-based nutrient supplements (LNS) for pregnant and lactating women and their infants (iLiNS-DYAD-G)

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

September 18, 2015 (version 2)

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Contents

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Version History Log

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

Version	**Date implemented**	**Details of significant changes**
1	February 24, 2015	n/a
2	September 18, 2015	Added the following outcome: Fatty acid status in breast milk at 6 months postpartum Added in data analysis plan: "Data will be analyzed to determine if there are any differences in effect by period. If so, we will limit to period 3. If not, all women will be included in the analyses."

I. Study objectives

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

The objectives for the present analyses are to determine the effect of LNS on maternal cholesterol, triglycerides, and fatty acids in plasma and breast milk. Specifically:

1) To determine if there are differences in mean plasma high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, or triglycerides at 36 wk gestation among women who received either LNS, multiple micronutrient capsules (MMN), or iron and folic acid capsules (IFA) during pregnancy.

2) To determine if there are differences in the following mean plasma fatty acids (as a percentage of total fatty acids) or fatty acid ratios:
    a) arachidonic acid (AA)
    b) docosahexaenoic acid (DHA)
    c) eicosapentaenoic acid (EPA)
    d) α-linolenic acid (ALA)
    e) sum of DHA and EPA
    f) sum of all long chain omega-3 fatty acids: DHA, EPA, and docosapentaenoic acid (DPA)
    g) linoleic acid (LA):AA
    h) ALA:DHA
    i) AA:EPA
    j) omega-6 fatty acids:omega-3 fatty acids

    measured at 36 weeks gestation among women who received either LNS, MMN, or IFA during pregnancy.

3) To determine if there are differences in mean breast milk fatty acid levels (listed above) or ratios (listed above) at 6 mo postpartum between groups of women who were provided either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.

4) To determine if differences exist in the prevalence of high or low cholesterol (LDL-C, HDL-C, or total) and high triglyceride concentration measured in plasma at 36 weeks gestation among women who received either LNS, MMN, or IFA during pregnancy.

II. Hypotheses

1) Women who receive LNS during pregnancy will have higher mean plasma concentrations of HDL-C, total cholesterol, and triglycerides, and higher mean plasma fatty acid proportions ofAA, EPA, DHA, and ALA compared to the IFA and MMN groups.
2) Women who receive LNS during pregnancy will have a lower prevalence of low total cholesterol (< 10^{th} percentile of IFA group) in plasma at 36 wk gestation compared to the IFA and MMN groups.
3) Women who receive LNS during pregnancy and lactation will have higher fatty acid levels (AA, EPA, DHA and DPA) in breast milk at 6 mo postpartum compared to the IFA and MMN groups.

III. Outcome variables

1) Plasma cholesterol
    a. LDL-C, HDL-C and total cholesterol will be analyzed as continuous outcomes.
    b. Low cholesterol (HDL-C, LDL-C, and total) concentration will be defined as < 10^{th} percentile of the IFA group.

    In addition, the following clinical definitions will also be examined:

    c. High total cholesterol, defined as ≥ 6.2 mmol/L (240 mg/dL).(1)
    d. High LDL-C, defined as ≥ 4.13 mmol/L (160 mg/dL).(1)
    e. Low HDL-C, defined as < 1.29 mmol/L (50 mg/dL).(2)

2) Plasma triglycerides
    a. Triglycerides will be analyzed as a continuous outcome.
    b. High triglycerides concentration, clinically defined as ≥ 2.26 mmol/L (150 mg/dL).(2)

3) Plasma fatty acids
    a. Fatty acids to be analyzed as continuous outcomes (percentage of total fatty acids) and ratios include:
        i. AA
        ii. DHA
        iii. EPA
        iv. ALA
        v. sum of DHA and EPA
        vi. sum of all long chain omega-3 fatty acids: DHA, EPA, and DPA
        vii. LA:AA
        viii. ALA:DHA
        ix. AA:EPA
        x. omega-6 fatty acids:omega-3 fatty acids
    b. All of the above fatty acids and ratios will also be analyzed as binary outcomes, with high fatty acids and ratios defined as > 50^{th} percentile of the IFA group.
4) Breast milk fatty acids

a. Same continuous outcomes and ratios as listed above.

IV. Basis for the analysis: Intention to treat

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

In addition to the intention to treat analysis, a per protocol analysis will be performed including subjects meeting minimum criteria for adherence to study protocol. Adherence is recorded biweekly by interview of study subject and verified by collection and count of remaining intervention supplements. Good adherence will be defined as consumption on $\geq$ 70% of supplement days and minimum adherence will be defined as consumption on > 50% of supplement days.

V. Time points

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

VI. Statistics software

Analyses will be performed using SAS version 9.4.

VII. Outliers

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

VIII. Data transformation

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

IX. Covariates and effect modifiers

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

Interactions will be examined between the intervention group and baseline outcome variable, maternal age, parity, and baseline BMI. If a statistically significant interaction ($p<0.1$) is found, group means will be examined at different levels of the predictor variable, either by category for categorical effect modifiers, or at selected percentile cutoffs for continuous variables. Variables to be examined as covariates include:

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

Data will be analyzed to determine if there are any differences in effect by period. If so, we will limit to period 3. If not, all women will be included in the analyses.

X. Presentation of study findings

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

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

The proportion of women with cholesterol < 10^{th} percentile and with fatty acids > 50^{th} percentile will be tabulated by intervention group as shown in Table 2. Global null hypothesis of no differences between groups will be tested with chi-square test or Fisher's exact test. Pairwise comparisons between groups will be done in the context of logistic regression if global null-hypothesis is rejected with $P<0.05$. Risk ratios between intervention groups are also presented in Table 2.

Group means and standard deviations for breast milk fatty acid levels (AA, ALA, DHA) and ratios will be tabulated by intervention group and presented in Table 3. The table will also indicate the differences in means and their 95% confidence intervals between the intervention groups. The difference between the three groups will be tested with ANOVA (model without covariates) and ANCOVA (model with covariates) and null-hypothesis of no difference between groups will be rejected if $P<0.05$. If the null-hypothesis is rejected, post-hoc pairwise comparisons of the three intervention groups will be Tukey-Kramer test for ANOVA. For all pairwise comparisons with $P<0.05$, the null-hypothesis of no difference in means between groups will be rejected.

XI. Tables

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

	Result by study group				**Comparison between LNS and IFA group**		**Comparison between LNS and MMN group**		**Comparison between MMN and IFA group**	
Outcome	**IFA (n=xxx)**	**MMN (n=xxx)**	**LNS (n=xxx)**	**p-value**	**Difference in means (95% CI)**	**p-value**	**Difference in means (95% CI)**	**p-value**	**Difference in means (95% CI)**	**p-value**
Mean (SD) total cholesterol, mg/dL	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) HDL-C, mg/dL	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) LDL-C, mg/dL	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) triglycerides, mg/dL	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) AA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) ALA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx

Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) DHA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) omega-6:omega-3	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx

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

	Number of outcomes / women with outcome data				**Comparison between LNS and IFA group**		**Comparison between LNS and MMN group**		**Comparison between MMN and IFA group**	
Outcome	**IFA (n=xxx)**	**MMN (n=xxx)**	**LNS (n=xxx)**	**p-value**	**Risk ratio (95 % CI)**	**p-value**	**Risk ratio (95 % CI)**	**p-value**	**Risk ratio (95 % CI)**	**p-value**
Total cholesterol <10th percentile	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
HDL-C < 10th percentile	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
LDL-C < 10th percentile	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
High AA	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
High DHA	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
High ALA	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx

RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
High omega-6:omega-3 ratio	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx
RR, adjusted model					x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx	x.xx (xx.x to xx.x)	0.xxx

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

	Result by study group				**Comparison between LNS and IFA group**		**Comparison between LNS and MMN group**		**Comparison between MMN and IFA group**	
Outcome	**IFA (n=xxx)**	**MMN (n=xxx)**	**LNS (n=xxx)**	**p-value**	**Difference in means (95% CI)**	**p-value**	**Difference in means (95% CI)**	**p-value**	**Difference in means (95% CI)**	**p-value**
Mean (SD) AA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) ALA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) DHA, % total fatty acids	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Mean (SD) omega-6:omega-3	xxx.x (x.x)	xxx.x (x.x)	xxx.x (x.x)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx
Difference in mean, adjusted model					x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx	x.xx (x.xx, x.xx)	0.xxx

References

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