Effect of LNS supplementation on children's energy and nutrient intakes from complementary foods at 9 and 15 months

Statistical analysis plan

Prepared for:

The International Lipid-based Nutrient Supplements (iLiNS) Project, Ghana

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Version History Log

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

Version	Date implemented	Details of significant changes
1	June 4, 2014	This is the first version

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1.0 Introduction

Part of the iLiNS Dyad-study, which is referred to as the Dietary Sub-study, was to assess the energy and nutrient intakes from non-breast milk sources (foods and beverages) by infants in the three groups (IFA, MMN and LNS groups) at 9 and 15 months of age.

This Statistical Analysis Plan (SAP) has been developed specifically for the Dietary sub-study. It is intended as an addendum to the main SAP, Version 3. As a result, there are various aspects of the analysis that have been described in the main SAP Version 3 and will not be repeated here. Both the main SAP Version 3 and this current SAP will guide the analysis described in this document.

2.0 Description

2.1 Study participants

The study participants were infants born to the pregnant women who were randomized into the Multiple Micronutrient Supplementation (MMN), Lipid-based Nutrient Supplementation (LNS), and Iron and Folic Acid (IFA) groups of the iLiNS intervention trial.

2.2 Sample Size

The sample size of 483 (161/group) was based on being able to detect a difference of 63 kcal between groups with 80% power.

2.3 Data Collection Procedures

The data collection procedures have been described in the iLiNS-Dyad Ghana Dietary standard operation procedure.

3.0 Objectives

1. To compare mean non-breast milk energy among the three intervention groups at 9 and 15 months.
2. To compare the nutrient densities (section 4.2) of complementary diets consumed by children in the three intervention groups at 9 and 15 months.
3. To compare the mean micronutrient density adequacy (MMDA) of complementary diets in the three intervention groups at 9 and 15 months.
4. To compare the percentage of children whose mean micronutrient density adequacy (MMDA) of complementary diets fell below 50% or at or above 75% in the three intervention groups at 9 and 15 months.

4.0 Outcome measures

4.1 Primary outcome

The primary outcome is mean energy intake (kcal) from non-breast milk sources at 9 and 15 months

4.2 Secondary outcomes

Mean nutrient densities from non-breast milk sources at 9 and 15 months for the following nutrients:

- Protein (g/100kcal)
- Iron (mg/100kcal)
- Zinc (mg/100kcal)
- Calcium (mg/100kcal)
- Vitamin A (µg/100kcal)
- Vitamin C (mg/100kcal)
- Vitamin B6 (mg/100kcal)
- Vitamin B12(mg/100kcal)
- Thiamine (mg/100kcal)
- Niacin (mg/100kcal)
- Riboflavin (mg/100kcal)
- Folate (mg/100kcal)

Mean micronutrient density adequacy (MMDA) of complementary diets at 9 and 15 months.

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Percentage of children whose mean micronutrient density adequacy (MMDA) of complementary diets is <50%. Note: This will be used as an indicator for sub-optimal complementary feeding [1]

Percentage of children whose mean micronutrient density adequacy (MMDA) of complementary diets ≥75%. Note: This will be used as an indicator for "near optimal" complementary feeding [1]

4.3 Definition

- **Nutrient density**: This is the amount of nutrient per 100 kcal of complementary food.

- **Minimum target nutrient densities:** Recommended values for the quantity of nutrients per 100 kcal of complementary food that would achieve the recommended nutrient intake after accounting for the daily nutrient delivery from human milk. In this paper, we will use the recommended values reported by [2] and shown below**.**

- **Nutrient Density Adequacy (NDA):** This is the percentage of the minimum target nutrient density fulfilled by the complementary foods (average of two recall days).

- **Mean micronutrient density adequacy (MMDA):** This is the mean of the nutrient density adequacy (NDA) scores for 10 key nutrients (vitamins A, B6, B12 , C, riboflavin, thiamin, folate, iron, zinc, calcium, B12) after each is capped at 100%.

Minimum target nutrient densities at 6-8, 9-11, and 12-23 mo.[1,2]

Nutrient	Minimum Target Nutrient Densities.[3,4]		
	6-8 mo.	**9-11 mo.**	**12-23 mo.**
Protein, *g/100 kcal*	1.4[5]	1.2[5]	0.9[5]
Calcium, *mg/100 kcal*	37[6]	29[6]	61
Iron, *mg/100 kcal*	4.5[7]	3.0[7]	1.0[7]
Zinc, *mg/100 kcal*	1.14[8]	0.81[8]	0.46[8]
Folate, *µg/100 kcal*	10	8	18
Niacin, *mg/100 kcal*	1.5	1.0	0.9
Riboflavin, *mg/100 kcal*	0.08	0.06	0.05
Thiamine, *mg/100 kcal*	0.08	0.05	0.07
Vitamin A, *µg RE/100 kcal*	25	26	19
Vitamin B6, *mg/100 kcal*	0.12	0.08	0.08
Vitamin B12, *µg/100 kcal*	0.01	0.03	0.06
Vitamin C, *mg/100 kcal*	1	1	1

[1] Total energy needs were assumed to be: 6-8 mo.: 615 kcal/d, 9-11 mo.: 686 kcal/d, and 12-23 mo.: 894 kcal/d [3]

[2] Breast milk energy intake was assumed to be: 6-8 mo.: 413 kcal/d, 9-11 mo.: 379 kcal/d, and 12-23 mo.: 346 kcal/d [3]

[3] Nutrient requirements were taken from those summarized in Tables 6 & 7 in[4], except as otherwise indicated

[4] Breast milk energy and nutrient content taken from Table 8 in[5], and values not in that table were taken from [6]

[5] Protein needs taken from Table A3.2a in[4], averaged for boys and girls

[6] Calcium needs- [7]

[7] The bioavailability of iron in complementary foods was assumed to be 10%- [8]

[8] Zinc needs (3 mg/d)- [8]

5.0 Analysis principles

- The primary analysis will be by intention-to-treat. That is, results for all women enrolled (including data on their infants) will be analyzed according to the group to which they were assigned regardless of any protocol violations. A sensitivity analysis will also be carried out to compare children based on the supplement their mothers received during pregnancy.
- Data on subjects, 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.
- All tests will be two-sided, at 5% level of significance.
- Where more than 10% of observations are missing for a dependent variable, we will report the number of observations used in the analysis.

6.0 Dietary data collection procedures

Data on the dietary intakes of infants were collected at 9 months and 15 months. At each of those months, an interactive 24 hour recall [9] was completed on a randomly selected day, and was repeated on the same day in the following week before the child turned 10 or 16 months. Whenever a woman was not available for a recall on the scheduled day, field workers visited the home once every week, to try to complete the recall before the child turned 10 or 16 months, otherwise, the recall was considered missed. No recall was done at more than 9 or 15 completed months.

6.1Handling dietary intake data collected at two recall times.

For energy and nutrient intakes, the data collected for each child, during each of the two recall times (Recall Day 1 and Recall Day 2) at 9 and 15 months will be averaged to determine the single average value for each time point. Where data on only one recall day is available, the energy and nutrient intakes will be based on that one recall only.

6.2 Estimating energy and nutrient intakes

First, a food composition table (FCT), herein referred to as the iLiNS FCT, will be compiled for the iLiNS Dyad-G Project using suitable food composition data sources including the FAO West Africa FCT [10], the USDA FCT [11], and a local FCT (herein referred to as the RIING FCT, because it was compiled for a previous RIING Project at the University of Ghana). The RIING database will be particularly helpful because it was compiled with food intake data and recipes from the same study area. Next, the iLiNS FCT will then be imported into SAS version 9.3 and merged with the dietary intakes data by the unique food codes, to determine energy and nutrient intakes for each participant. Subsequently, mean energy and nutrient densities will be calculated from the two recalls days, or one recall day if the second recall was missed. In cases where the mother reported no intake of complementary foods, it will still be recorded as a valid recall with zero values for energy and nutrients. Energy and nutrient intakes will be reported as mean and standard deviation or median and 1^{st} and 3^{rd} quartiles as appropriate.

7.0 Outliers

- We will check outliers by visually inspecting 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. The decisions made will be documented on a case-by-case basis for future reference.
- Outliers which are plausible or possible will be kept. Variables with outliers will be transformed, and in an extreme situation, a sensitivity analysis will be done to determine if such outliers have undue influence on the results.
- Distribution of continuous outcome 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.

8.0 Software

All analyses will be done using SAS version 9.3 (SAS Inst. Cary, NC, USA)

9.0 Background characteristics

Background characteristics of children will be presented as shown in **Table 1**. Continuous variables will be summarized as mean and SD and binary variables as number of participants (%).

10.0 Comparison of primary and secondary outcomes at 9 and 15 months of age

The effect of intervention on primary and secondary outcomes at 9 and 15 months will be tested using general linear model (SAS PROC GLM) or logistic regression (PROC LOGISTIC), with Tukey adjustment for multiple comparisons. Pairwise differences in means (continuous variable) and relative risks (binary variables) with their 95% CI and p-values will also be calculated. Pairwise relative risks will be calculated using Poisson regression [12]. All analyses will be performed twice, first without controlling for any covariates, and second controlling for pre-specified covariates (see below).Only covariates significantly associated with an outcome at 10% level of significance in a correlation analysis will be included in the final adjusted analysis. This means we may have different sets of covariates for each outcome. By-group analyses for continuous variables will be presented as mean ± SD for unadjusted values, and mean ± SE for adjusted values **(Tables 2 - 3**). Those for binary variables **(Table 4-6)** will be presented as number of participants (%).

10.1 Selection of covariates and effect modifiers in the analysis of primary outcomes

Below we list the primary outcomes to be analyzed (at 9 and 15 months of age), and indicate the covariates and effect modifiers that will be used for each analysis. Effect modifiers will be considered separately in the final regression model to avoid collinearity.

Outcome Variable	Analysis	Covariates	Effect modifiers
Mean energy intakes at 9/15 months	ANCOVA (SAS Proc GLM)	Baseline HH small livestock ownership index Number of under-fives in the HH at baseline Baseline household food insecurity Baseline household socio-economic status Primiparity Maternal height Maternal BMI Maternal age Maternal education Sex of child Season of recall (Nov-Apr) Day food eaten (weekday/weekend)	Baseline HH small livestock ownership index Number of under-fives in the HH at baseline Baseline household food insecurity Baseline household socio-economic status Primiparity Maternal height Maternal BMI Maternal age Maternal education Sex of child Season of recall (Nov-Apr) Day food eaten (weekday/weekend)

10.2 Selection of covariates and effect modifiers in the analysis of secondary outcomes

The table below shows the secondary outcomes to be analyzed (9 and 15 months of age), and the covariates and effect modifiers that will be used. As with the primary outcomes, each of the effect modifiers will be considered separately in the regression model to avoid collinearity.

Outcome Variable	Analysis	Covariates	Effect modifiers
Mean nutrient densities at 9/15 months Protein (g/100kcal) Iron (mg/100kcal) Zinc (mg/100kcal) Calcium (mg/100kcal) Vitamin A (µg/100kcal) Vitamin C (mg/100kcal) Vitamin B6 (mg/100kcal) Vitamin B12(mg/100kcal) Thiamine (mg/100kcal) Niacin (mg/100kcal) Riboflavin (mg/100kcal) Folate(mg/100kcal **MMDA at 9/15 months** **Percentage of children with MMDA < 50% at 9/15mo**. **Percentage of children MMDA≥ 75% at 9/15mo.**	ANCOVA (SAS Proc GLM)	Baseline household food insecurity Baseline household socio-economic status Number of under-fives in the HH at baseline Baseline HH small livestock ownership index Primiparity Maternal height Maternal BMI Maternal age Maternal education Sex of child Season of recall (Nov-Apr) Day food eaten (weekday/weekend)	Baseline household food insecurity Baseline household socio-economic status Number of under-fives in the HH at baseline Baseline HH small livestock ownership index Primiparity Maternal height Maternal BMI Maternal age Maternal education Sex of child Season of recall (Nov-Apr) Day food eaten (weekday/weekend)

Tables

Table 1: Baseline characteristics of study participants by intervention group

Variable	**IFA** ($\bar{x}$ ± SD) [n]	**MMN** ($\bar{x}$ ± SD) [n]	**LNS** ($\bar{x}$ ± SD) [n]%
Maternal age	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]
Socioeconomic status	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]
Years of formal education	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]
Initial body mass index (BMI)	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]
Primiparity	%	%	%
Season at enrolment = dry season	%	%	%
Sex of child = male	%	%	%

Table 2: Comparison of the difference in energy intakes at 9 and 15 months between intervention groups

	IFA ($\bar{x}$ ± SD) [n]	**MMN ($\bar{x}$ ± SD) [n]**	**LNS ($\bar{x}$ ± SD) [n]**	**p-value**	**Comparison of IFA and MMN**		**Comparison of IFA and LNS**		**Comparison of MMN and LNS**	
					Difference in means (95% CI)	p-value	Difference in means (95% CI)	p-value	Difference in means (95% CI)	p-value
Mean energy intake(kcal) at 9 months	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]	x.xxx	x.xx (x.xx - x. xx)	x.xxx	x.xx (x.xx - x. xx)	x.xxx	x.xx (x.xx - x. xx)	x.xxx
Mean energy intake(kcal) at 15 months	xx.x± x.x [xxx]	xx.x± x.x [xxx]	xx.x± x.x [xxx]	x.xxx	x.xx (x.xx - x. xx)	x.xxx	x.xx (x.xx - x. xx)	x.xxx	x.xx (x.xx - x. xx)	x.xxx

Table 3: Comparison of the differences in nutrient densities at 9 and 15 months between intervention groups

		IFA ($\bar{x}$ ± SD) [n]	**MMN ($\bar{x}$ ± SD) [n]**	**LNS ($\bar{x}$ ± SD) [n]**	**P-value**	**Comparison of IFA and MMN**		**Comparison of IFA and LNS**		**Comparison of MMN and LNS**	
						Difference in means (95% CI)	**P-value**	**Difference in means (95% CI)**	**P-value**	**Difference in means (95% CI)**	**P-value**
Protein (g/100kcal)	9 mo.										
	15 mo.										
Iron (mg/100kcal)	9 mo.										
	15 mo.										
Zinc (mg/100kcal)	9 mo.										
	15 mo.										
Calcium (mg/100kcal)	9 mo.										
	15 mo.										
Vitamin A (µg/100kcal)	9 mo.										
	15 mo.										
Vitamin C (mg/100kcal)	9 mo.										
	15 mo.										
Vitamin B6 (mg/100kcal)	9 mo.										
	15 mo.										
Vitamin B12 (mg/100kcal)	9 mo.										
	15 mo.										
Thiamine (mg/100kcal)	9 mo.										
	15 mo.										
Niacin (mg/100kcal)	9 mo.										
	15 mo.										
Riboflavin (mg/100kcal)	9 mo.										
	15 mo.										
Folate (mg/100kcal)	9 mo.										
	15 mo.										

Table 4: Comparison of the mean micronutrient density adequacy (MMDA) of complementary diets across intervention groups at 9 and 15 months

	IFA ($\bar{x}$ ± SD) [n]	MMN ($\bar{x}$ ± SD) [n]	LNS ($\bar{x}$ ± SD) [n]	P-value	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value
9 mo.										
15 mo.										

Table 5: Comparison of the percentage of children with mean micronutrient density adequacy (MMDA) of complementary diets <50% across intervention groups at 9 and 15 months

	IFA n (%)	MMN n (%)	LNS n (%)	P-value	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value
9mo.										
15mo.										

Table 6: Comparison of the percentage of children with mean micronutrient density adequacy (MMDA) of complementary diets ≥75% across intervention groups at 9 and 15 months

	IFA n (%)	MMN n (%)	LNS n (%)	P-value	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value
9mo.										
15mo.										

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