

**Effect of three micronutrient supplement strategies for pregnant and lactating women and their infants on developmental milestone acquisition and motor, language, socio-emotional, and executive function scores at age 18 months**

Statistical Analysis Plan Addendum 2 to iLiNS-DYAD-G Main SAP

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Micronutrient supplementation during pregnancy and infancy and developmental milestone acquisition and 18-month development - v2

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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
2	Jan 14, 2015	<p>Added the following outcomes:                      Timing of milestone acquisition (Form C4a)                      Milestones at 6, 12, and 18 months (Form C4b)</p> <p>Changed the per-protocol analysis from:                      “We will perform a per-protocol analysis by examining the interaction between treatment group and adherence to supplement consumption. If the interaction term is significant at <math>p &lt; 0.1</math>, we will further explore the nature of the interaction by examining the effect of treatment group at the 10th, 50th, and 90th percentile of adherence.”                      To:                      “We will perform a per-protocol analysis by examining the effect of the intervention in participants with self-reported high adherence. The cut-off to define high adherence will be determined to be consistent with the analyses on birth outcomes and growth.”</p>

### **1.0 Study Objectives and Hypotheses**

The primary aim of the study was to compare the effects of three types of micronutrient supplements among Ghanaian pregnant and lactating women and to assess the effect of LNS-P&L given to pregnant and lactating women and LNS-20gM provided to children from 6 to 18 months of age on child growth and micronutrient status. A secondary aim was to similarly study the impact of LNS on various other (secondary) outcomes in the same target group.

The aim of the secondary analyses described in this addendum is to compare infants in 3 different intervention groups:

- a) Daily iron and folic acid during pregnancy, and calcium (Ca) only (akin to a placebo) during the first 6 months postpartum, with no supplementation for offspring during infancy
- b) Daily multiple micronutrients (1-2 RDA of 18 vitamins and minerals) during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy
- c) Daily LNS during pregnancy and the first 6 months postpartum (LNS-P&L with similar vitamin and mineral content as the daily multiple micronutrients, plus Ca, P, K, Mg and essential fatty acids), with LNS for offspring (LNS-20gM with 22 vitamins and minerals with concentrations based on RNIs for infants) during infancy

on the following outcomes:

1. 18-month motor development, language development, socio-emotional development, executive function, and interaction with caregivers
2. Prevalence of severe and moderate to severe delay in motor development, language development, socio-emotional development, and executive function at age 18 months
3. The timing of the acquisition of certain developmental milestones, monitored monthly from birth through 18 months of age
4. The proportion of children who had achieved certain motor milestones by 6, 12, and 18 months of age.

The null hypotheses were that:

1. The three groups of infants whose mothers received the three micronutrient treatments will not differ in 18-month scores in gross and fine motor development, language development, socio-emotional development, executive function, and interaction with caregivers, and
2. Infants whose mothers received LNS during pregnancy and lactation, and who received LNS from 6 to 18 months of age will not differ in 18-month scores in gross and fine motor development, language development, socio-emotional development, executive function, and interaction with caregivers from infants in the other two groups.
3. Hypotheses 1-2 will also be examined with regard to the prevalence of severe and moderate to severe delay in motor development, language development, socio-emotional development, and executive function.

4. The timing of developmental milestone acquisition of infants whose mothers were provided with LNS during pregnancy and who were provided with LNS from 6 to 18 months of age will not be different from that of infants of mothers who received either iron-folate or multiple micronutrient supplementation.
5. The proportion of children who had achieved motor milestones at 6, 12, and 18 months of age will not be different in infants provided with LNS during pregnancy and from 6 to 18 months of age as compared to infants of mothers who received either iron-folate or multiple micronutrient supplementation.

## **2.0 Definition of the developmental outcomes**

### 2.1 18-month developmental outcomes

The gross motor score is calculated as the sum of 35 Kilifi Developmental Inventory (KDI) gross motor items, each scored 0 or 1 (sum of *Form C7a Q 5.1-5.2* and *5.6-7.12*). Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.

The fine motor score is calculated as the sum of 34 KDI fine motor items, each scored 0 or 1 following Abubakar et al. (2008). Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.

The psychomotor score is calculated as the sum of 69 KDI fine and gross motor items, each scored 0 or 1. Severe delay is defined in two ways: (1) the bottom 10% of our sample and (2)  $<-3$  SD below the mean according to published norms from Kenya (Abubakar et al. 2008). Moderate to severe delay is defined in two ways: (1) the bottom 25% of our sample and (2)  $<-2$  SD below the mean according to published norms from Kenya (Abubakar et al. 2008).

Language development is quantified as

- a. Vocabulary score, calculated as the sum of *Form C7c LANGVOCAB1* through *LANGVOCAB100*. Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.
- b. Expressive vocabulary  $> 10$  words vs.  $\leq 10$  words, derived from the vocabulary score
- c. Word combining (Has the child started combining words into sentences? 0 = not yet, 1 = sometimes, 2 = often) *Form C7c Q 14.1*

Socio-emotional development is calculated as the sum of *Form C7b PSED1* through *PSED19*. Severe delay is defined as the top 10% of our sample (a lower score indicates more advanced socio-emotional development). Moderate to severe delay is defined as the top 25% of our sample.

Executive function is calculated as

- a. A not B task total number correct, *Form C7a Q16.12*. Severe delay is defined as the bottom 10% of our sample. Moderate to severe delay is defined as the bottom 25% of our sample.
- b. A not B task total errors after set 1, *Form C7a Q16.13*
- c. A not B task total trials completed, *Form C7a Q 16.11*. If this variable is not normally distributed, another statistical approach will be used, such as creating a dichotomous variable

Interaction with caregivers is calculated as the sum of the activities with adults in the past three days (*Form C7d Q 6.1.1 through Q 6.6.3*).

## 2.2 Timing of milestone acquisition

The following milestones were monitored monthly by interview with a caregiver:

Pronouncing single words  
Waving goodbye  
Drinking from a cup  
Eating by self  
Walking alone  
Standing alone  
Walking with assistance  
Hands and knees crawling  
Other type of crawling  
Standing with assistance  
Sitting without support

A milestone is considered to be achieved when the child was recorded to have achieved the skill on two consecutive visits. We assume that the child acquired the skill before the first of these two visits, so the age of acquisition is the mean age between the first of the two consecutive visits and the previous visit on which the child had not yet achieved the skill.

We will use right censoring if the child was not recorded to have achieved the milestone by the last visit during the intervention period.

## 2.3 Proportion of children who had achieved milestones at 6, 12, and 18 months of age

The following milestones were assessed at 6, 12, and 18 months of age.

Walking alone  
Standing alone  
Walking with assistance

Hands and knees crawling  
Other type of crawling  
Standing with assistance  
Sitting without support

We will include all children for which these milestones were assessed at the target age plus or minus one month. Thus for milestones assessed at 6 months of age, we will include children assessed at age 5.0 to 7.0 months, for milestones assessed at 12 months of age, we will include children assessed at age 11.0 to 13.0 months, for milestones assessed at 18 months of age, we will include children assessed at age 17.0 to 19.0 months. The outcome is a binary variable indicating whether or not the child had achieved the milestone at that age.

### **3.0 Analysis Principles and Outliers**

The analysis principles and treatment of outliers will be same as that for the primary outcomes. In addition to the intention to treat analysis, we will also perform a per protocol analysis by examining the effect of the intervention in participants with self-reported high adherence. The cut-off to define high adherence will be determined to be consistent with the analyses on birth outcomes and growth.

### **4.0 Hypothesis testing**

The analysis will begin with testing the null hypothesis of no difference between the three treatment groups using ANCOVA for continuous outcomes or logistic regression for binary outcomes, and controlling for pre-specified covariates (see below). For all analyses, if the global null hypothesis is rejected at 0.05 level, then we will perform post-hoc pairwise comparisons of all three groups using Tukey-Kramer adjustment. We will also use Scheffe's test to assess whether the LNS group differs from the non-LNS groups.

For each hypothesis, except the timing of milestone acquisition, three models will be estimated:

1. No covariate adjustment
2. Adjustment for child age at developmental assessment
3. Adjustment for child age at developmental assessment and for any of the variables presented in section 6 showing statistically significant association (at  $p < 0.1$  level) with the developmental score.

For the timing of milestone acquisition, we will estimate models (1) and (3) only. We will not adjust for child age because the outcome is age of acquisition. We will use the survival analysis procedure in SAS (PROC LIFEREG) to estimate the means for normally distributed variables and geometric means for skewed variables. This method takes censoring into account in the calculation of the means and geometric means. The analysis will begin with testing the null hypothesis of no difference between the three treatment groups using survival analysis by SAS

PROC LIFEREG and controlling for pre-specified covariates (see below). If the global null hypothesis is rejected at  $p=0.05$  level, then we will perform pairwise comparisons of all three groups using Tukey-Kramer adjustment. We will also use Scheffe’s test to assess whether the LNS group differs from the non-LNS groups.

The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Significant interactions ( $p < 0.1$ ) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification.

## 5.0 Background Characteristics of Participants and Baseline Comparisons

The following group characteristics will be compared:

Group characteristics	From
Child sex	Covariate/Effect modifier
Mother's Age at enrollment (y)	Main SAP maternal
Mother's education (years of formal education)	Main SAP maternal/child
Mother's height (m)	Main SAP maternal/child
Mother's BMI (kg/m <sup>2</sup> )	Main SAP maternal/child
Women with a low BMI (< 18.5 kg/m <sup>2</sup> )	Main SAP maternal
Mother's mid upper arm circumf. (cm)	Main SAP maternal/child
Mother's Hemoglobin (g/L)	Main SAP maternal
Mother Anemic at enrollment (% [n])	Main SAP maternal
Mother's ZPP (μmol/mol heme)	Main SAP maternal
Elevated ZPP at enrolment (ZPP > 60)	Main SAP maternal
Iron deficiency anemia at enrolment (% [n])	Main SAP maternal
Positive rapid test for malaria (% [n])	Main SAP maternal
Parity	Main SAP child
Primiparous women (% [n])	Main SAP maternal
Gestational age at enrolment (wk)	Main SAP maternal/child
Proxy(s) household socioeconomic status (Assets index / Housing index)	Main SAP maternal/child
Proxy variable for household food insecurity	Covariate/Effect modifier
Season at enrolment being dry season (Nov-Apr)	Covariate/Effect modifier
One or several proxy indicators for diet quality	Covariate/Effect modifier
Mother's marital status married or cohabiting (% [n])	Main SAP maternal/child
Number of persons in the household	Main SAP child

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Children <5 y in the household

Main SAP child

Child age at developmental assessment

Covar Dev Assessment

Analysis of background characteristics will be the same as that for the main outcomes:

- We will use frequencies and percentages to summarize categorical data. Percentages will be calculated based on the number of participants for whom data are available.
- Continuous variables will be summarized using either mean and SD for continuous variables, geometric mean and SD for skewed variables, or median and range.
- Where data for certain participants are missing, the number of participants included in the analysis will be indicated.

### 6.0 Potential Covariates

All variables listed above in section 5.0 will be considered as covariates plus the following, where appropriate:

Family care indicators score, if this is not different between groups

Child's mood during testing, if this is not different between groups

Child's interaction with tester during testing, if this is not different between groups

Child's activity level during testing, if this is not different between groups

Child's primary language

Number of languages to which child has been exposed (1 or more than 1)

The data collector who conducted the developmental assessment or interview

Only covariates significantly associated with an outcome at 10% level of significance in a bivariate analysis will be included in the final adjusted analysis. This means we may have different sets of covariates for each outcome.

### 7.0 Potential effect modifiers

In accordance with the main SAP, the following variables will be considered as effect modifiers:

- a. Composite variable for food security and/or socio-economic status
- b. Primiparity
- c. Maternal height
- d. Maternal BMI at enrollment (adjusted for gestational age)
- e. Maternal anemia at enrollment
- f. Gestational age at enrollment
- g. Season at enrollment
- h. Maternal age
- i. Maternal education
- j. Child sex
- k. One or several proxy indicators for diet quality

In addition, we will consider the following variables as effect modifiers:

- a. Family care indicators z-score

## 8.0 Calculating developmental scores and z-scores

If a large percentage of data is missing for any item, we will exclude that item from the total score. For all other missing item scores, we will impute the scores based on the other items in the same subscale. We will use the imputation method described in Raghunathan et al. (2001).

Z-scores of developmental variables will be calculated based on the distribution of the iLiNS-DYAD-G sample, by standardizing the distribution to a mean of 0 and standard deviation of 1.

### References

- Abubakar, A., Holding, P. A., Van Baar, A., Newton, C. R. J. C., & Van de Vijver, F. J. R. (2008). Monitoring psychomotor development in a resource-limited setting: An evaluation of the Kilifi Developmental Inventory. *Annals of Tropical Pediatrics*, 28, 217-226.
- Raghunathan, T. E., Lepkowski, J. M., Van Hoewyk, J., & Solenberger, P. (2001). A multivariate technique for multiply imputing missing values using a sequence of regression models. *Survey Methodology*, 27(1), 85-95.