Assessing developmental outcomes of preschool children following three micronutrient supplementation strategies for pregnant and lactating women and their infants in Ghana

Statistical Analysis Plan

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1.0 Background of parent study

The International Lipid-based Nutrient Supplement (iLiNS) DYAD-Ghana study was a randomized, partially double blind, controlled trial conducted from 2009-2014 in the Yilo and Manya Krobo districts of Eastern Ghana, to test the efficacy of three types of micronutrient supplements for preventing malnutrition in pregnant and lactating women and their infants. The three supplementation strategies were (1) lipid-based nutrient supplements (LNS) provided to women during pregnancy and for 6 months postpartum, and to their infants from 6 to 18 mo of age, (2) maternal multiple micronutrient (MMN) supplements during pregnancy and 6 months postpartum, and (3) maternal iron and folic acid (IFA) during pregnancy and calcium placebo tablet during 6 months of lactation. Children in the latter two groups received no supplement during infancy.

1.1 Study site and Participants

The study was conducted in the Yilo and Manya Krobo districts of Eastern Ghana, where the iLiNS DYAD-G1 study took place from 2009-2014. All children within the ages of 4-6 years who participated in the iLiNS randomized controlled trial, and their mothers, were invited to participate in this DYAD-G2 study. The study was conducted when the children were 4-6yrs old.

1.2 Inclusion criteria

To be eligible for follow up, the following criteria for children were used:

a) If they were delivered from the pregnancy for which their mothers were enrolled into the iLiNS DYAD-G1 study

b) Mother or caregiver provided informed consent to participate

1.3 Exclusion criteria

We excluded children from the study if one or more of the following criteria were present:

a) Not residing within the study site (Yilo and Manya Krobo districts) or surrounding towns at a travel distance costing no more than GHc 60 ($ 15) to the study site round trip

b) Mother or caregiver unwillingness to consent to participation

c) If the child's mother had multiple births (more than one child from the pregnancy for which she was enrolled into DYAD-G1) and the child was not randomly selected for inclusion into the study

2.0 Study objective

For the analyses described here, the aim is to:

Investigate the long term effects of the three micronutrient supplementation strategies (LNS, MMN, IFA) on neurocognitive, motor and socio-emotional outcomes at preschool age.

Specific aims

The specific aims of these analyses are to compare infants in three different intervention groups on the following outcomes:

1. **Neuro-developmental outcomes**:
    1. Fine motor
    2. Language (General verbal ability/ IQ)
    3. Non-verbal ability (Visuospatial)
    4. Executive function
    5. Declarative memory/ long term memory
    6. Procedural memory/ implicit learning
    7. Pre-academic skills/early learning
    8. Socio-emotional development

2. **Prevalence of severe and moderate to severe delays** in cognitive, motor and socio-emotional development at preschool age

3. **Physical activity** patterns (sedentary, moderate, and vigorous activity over a seven-day period) at preschool age

3.0 Hypotheses

We hypothesize that:

a) Children in the LNS group will have higher scores on motor, cognitive and socio-emotional function tests at preschool age compared to children in the MMN or IFA groups.

b) The percentage of children with severe and moderate to severe delays in motor, cognitive and socio-emotional development will be lower in the LNS group at preschool age compared to the MMN and IFA groups.

4.0 Power calculations

Aims 1 and 2: Neurodevelopmental outcomes

Allowing for about 20% attrition, we estimated about 950 participants out of the 1,185 mother-child pairs who completed the initial study to be available for follow-up at 4-6 years. There will be about 316 participants for each group, assuming 3 intervention groups (LNS, MMN, IFA). We expect to be able to detect a difference of ≥ 0.25 SD for each of the outcomes (continuous scores) with a power of 80% at a 0.05 level of significance.

Aim 3: Physical activity patterns

For physical activity, to detect an effect size of 0.3 (difference between groups, divided by the pooled SD), assuming 2 groups (LNS vs non LNS (MMN+IFA)), a power of 80% and alpha of 0.05 requires 176 per group, summing up to 352 children. Allowing for up to 10% attrition, approximately 390 participants are needed for this sub study. This calculation is based on the hypothesis that the children in the LNS group who were supplemented for a period of 1 year during infancy would have greater minutes per day spent in moderate-to-vigorous physical activity than those in the non LNS group who were not supplemented during infancy. LNS

contains a complete supply of specific micronutrients and essential fatty acids critical for brain development which differentiates it from the non-LNS supplements given prenatally. Children in the LNS group may be better nourished compared to the non-LNS group. Undernourished children have been shown to be less physically active than their well-nourished counterparts, increasing in activity levels with improvements in nutritional status (Sally Grantham-McGregor & Baker-Henningham, 2005).

5.0 Definition of outcomes

Table 1.0: List of Primary and Secondary developmental outcomes

Test score	Primary outcomes	Secondary outcomes
Body part naming	Cognitive factor z-score	Language mean z-score
Body part identification		
Comprehension of instructions		
Block design		Visuospatial/attention mean z-score
Visual search chicken time per correct target		
Visual search kitten time per correct target		
Paired associate learning		Declarative memory mean z-score
Paired associate recall		
Head-toe Inhibition		Head-toe Inhibition z-score
PEDS pre-academic skills		Pre-academic z-score
Delay gratification		Delay gratification
RACER Inhibition		RACER Inhibition
RACER Declarative memory		RACER Declarative memory
RACER Procedural memory		RACER Procedural memory
Pegboard dominant hand	Motor average z-score	N/A
Pegboard non-dominant hand		
SDQ total difficulties	SDQ total difficulties z-score	
SDQ prosocial		SDQ prosocial z-score
Behavior Rating Scale		Behavior rating scale z-score

5.1 Primary outcomes

The three main outcomes are Motor average z-score, Cognitive factor z-score and SDQ total difficulties z-score. Z-scores will be calculated in 3-month age bands, with a mean of 0 and a standard deviation of 1 in each age band. We will create composite scores for motor and socioemotional domains by calculating the average of each child's test z-scores from the various subtests under that domain (Tables 1 & 3). For the cognitive domain, we will use factor analysis

to create a composite factor score, if all scores load on one latent factor. In the case of having several factors, we will perform Principal Component Analysis (PCA) to be able to explain as much of the total variance in the variables as possible.

5.2. Secondary outcomes

- Developmental outcomes

  For the secondary developmental outcomes, we will average the z-scores for each of the sub-domains as secondary outcomes, as shown in Tables 1 and 4.

  The percentage of children with severe and moderate to severe delay in our sample will be defined as the bottom 10% (lowest decile) and 25% (lowest quartile) of our sample respectively, of scores in each of the domains.

- Physical activity outcomes

  We will measure three indicators of physical activity as secondary outcomes:

  i. Mean vector magnitude accelerometer counts/15s

  The mean Vector Magnitude (VM) accelerometer counts/15s in the total sample will be calculated using the ACTi life data analysis software V. 6.13.1, which calculates the mean VM as the square root of the sum of squared activity counts of three axes. We will estimate the difference (95% CI) in mean accelerometer counts/15s between the intervention group (LNS) and the control group (IFA+MMN).

  ii. Percent time spent in moderate-to-vigorous physical activity (MVPA). This will be calculated using vertical axis cut points of ≥ 419 counts/15s (Trost et al. 2011). This will be averaged over all valid days within a 7-day period and the averaged value (per participant) will be used in the analysis. We will consider a day valid when there is a minimum of ten hours of accelerometer data between 6:00 am and 8:00 pm, after excluding strings of ⩾20 min of zeroes. Only children with ⩾4 valid days (Minimum of 3 weekdays and 1 weekend day) of data will be included in the analyses.

  iii. Percent time spent being sedentary

  This will be averaged over all valid days and the average value (per participant) will be used in the analysis. We will define sedentary time as vertical axis activity counts ≤48 counts/15 s (Trost et al. 2011).The children whose mean time in MVPA over all valid days is ≥90 minutes will be considered active, based on the guidelines of the U.S. National Association for Sports and Physical Education (NASPE 2009).

6.0 Analysis principles

Analysis will be performed by intention-to-treat. We will include data on participants lost to follow-up or who refused to continue the study if available.

We will also perform two per protocol analyses based on self-reported high adherence to supplementation during the main trial as follows:

We will first include children of mothers who self-reported greater than or equal to 80% adherence (based on previous main trial analyses) to supplement consumption during pregnancy. Secondly we will include children of mothers who self-reported greater than or equal to 80% adherence during the entire period of pregnancy up to 6 months postpartum.

7.0 Statistical analysis

7.1. Software

All analyses will be done using SAS version 9.4(SAS Inst. Cary, NC, USA) or Stata version 10 (StataCorp, TX, USA).

7.2. **Imputation of developmental scores**

In case of missing item data, the method described in Raghunathan et al. (2001) will be adopted to impute missing item scores based on other items in that same test or subscale. If a large percentage of data is missing for any item, we will exclude that item from the calculation of the total score. In cases where data from an entire test are missing, that child will be excluded and the number of participants included in the analysis will be indicated.

8.0 Outliers

For univariate analysis, the distribution of scores will be examined to identify potential outliers. We will visually inspect outliers by creating box and whisker plots or histograms of individual continuous variables, and scatter-plots of related variables. Clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. We will maintain plausible or possible outliers in the data set, and these will be truncated at the 1st and 99th percentile for analysis.

9.0 Data transformation

We will inspect the distribution of outcome variables for normality and transform as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

10.0 Background characteristics by intervention group

For some maternal and child variables measured, the available values at the time of screening, recruitment or enrolment in the initial study (e.g. baseline maternal height, weight), will be

considered as background characteristics. For other variables, the available values at time of enrollment into the current follow-up study (child age, Hb concentration etc.) will be considered as background characteristics and will be presented in a table, by treatment group. Group characteristic comparisons will be described based on several socio-demographic variables, using frequencies and percentages to summarize categorical variables and mean and standard deviations for continuous variables. We will compare participants tested during follow-up with those unavailable for follow-up or lost to follow-up on sociodemographic characteristics.

11.0 Main *effect of intervention*

For each developmental domain, we will first test the null hypothesis of no difference between the three treatment groups using ANCOVA for continuous variables and logistic regression for categorical (binary) outcomes. For multilevel or non-normally distributed count outcomes, we will use the negative binomial regression or the PROBIT model for analysis. If a variable is non-normal and cannot be transformed to normality, as a last resort non-parametric methods will be employed.

For all analyses, post-hoc pairwise comparisons of all three groups will be performed using Tukey-Kramer adjustment for continuous variables or contrast statements for categorical variables. We will accept significant pairwise comparisons when the p-value is <0.05.

If there are no differences between the IFA and MMN groups we will combine the groups into a non-LNS group to assess whether it differs from the LNS group. Because children born to LNS mothers were the only group also given LNS for a period of 1 year, we will examine differences between them and the rest of the children in this cohort. Due to the same reasons stated in section 4.0 above, we anticipate that the differences between the LNS and non-LNS supplements could lead to differences in development in these children.

We will compare groups with three models: one model will be minimally adjusted for child age as a covariate, the second model will be additionally adjusted for gender, data collector and any baseline factors listed below in section 12 that are statistically significantly associated at the $p<0.1$ level with the developmental score. Thirdly, we will adjust for any factors collected at follow-up listed below that are statistically significantly associated at the $p<0.1$ level with the developmental score. For any covariates that were collected after baseline, we will first check whether they are different between groups before including in the model since they could be potential mediators.

If the variables collected at follow-up are not different between groups, we will run one fully adjusted model.

12.0 Potential covariates

The following covariates are to be included in the ANCOVA or logistic regression models when they show a statistically significant association with the outcome ($P<0.1$):

Factors collected at baseline:

1. Birth order
2. Exposure to multiple languages
3. Maternal age
4. Maternal education
5. Maternal Pre-pregnancy BMI
6. Maternal hemoglobin
7. Household asset index

Factors collected at follow-up:

1. Preschool quality/experience
2. Data collector
3. Home /environmental stimulation
4. Maternal depressive symptoms
5. Maternal agency
6. Maternal cognition

13.0. Potential effect modifiers

The following variables will be examined as potential effect modifiers with an interaction term in the ANCOVA or logistic regression model:

Factors collected at baseline:

1. Child gender
2. Maternal age
3. Maternal education
4. Primiparity
5. Maternal BMI
6. Maternal Hemoglobin
7. Household asset index

Factors collected at follow-up:

1. Home stimulation composite score

We will test the interaction between the effect modifiers and intervention groups. 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.

Table 2: Selected characteristics of women by original intervention group at baseline and follow-up at 4-6yr

	IFA	MMN	LNS	P-value	Test
Variable	Mean ± SD or % [n]	Mean ± SD or % [n]	Mean ± SD or % [n]		
Baseline maternal age (yr)	xx.x ± x.x [x]	xx.x ± x.x [x]	xx.x ± x.x [x]	x.xx	ANOVA
Baseline maternal weight(Kg)	x.x ±x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xx	ANOVA
Baseline maternal BMI (Kgm^{-2})	x.x ±x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xx	ANOVA
Baseline Gestational age (wk)	xx.x±x.x [xxx]	xx.x±x.x [xxx]	xx.x±x.x [xxx]	x.xx	ANOVA
Baseline maternal education (yr)	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xx	ANOVA
Father's education (yr)	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xx	ANOVA
Maternal hemoglobin concentration (g/L) at baseline	x [xxx/xxx]	x [xxx/xxx]	x [xxx/xxx]	x.xx	Chisq
Baseline household asset index	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xx	ANOVA
Mean adherence (% of supplements consumed during pregnancy & lactation)	xx.x [xxx]	xx.x [xxx]	xx.x [xxx]	x.xx	Chisq
Primiparity (% of women during intervention)	xx.x[xxx]	xx.x [xxx]	xx.x[xxx]	x.xx	Chisq
Maternal cognitive z-score at follow-up	x.xx ± x.xx [xxx]	x.xx ± x.xx [xxx]	x.xx ± x.xx [xxx]	x.xx	-
Home stimulation score at follow-up	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xx	ANOVA

Table 3: Primary outcomes: Motor, cognitive and socio-emotional *z* scores at the end of the intervention period

Domain (z-scores)		IFA	MMN	LNS	Minimally-adjusted		Adusted for covariates	
	n	Mean ± SD			Estimate (95% CI)	P	Estimate (95% CI)	P
Cognitive	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Motor	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Socio-emotional	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx

Table 4: Secondary outcomes: Mean motor, cognitive and socio-emotional *z* scores at the end of the intervention period

Domain (z-scores)		IFA	MMN	LNS	Minimally-adjusted		Adusted for covariates	
	n	Mean ± SD			Estimate (95% CI)	P	Estimate (95% CI)	P
Language[1]	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Visuospatial/attention[2]	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Declarative memory[3]	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Pre-academic skills	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
SDQ Prosocial	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Behavior Rating Scale	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
RACER PC-based								
Inhibition	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Procedural memory	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx
Declarative memory	xxx	x.xx ± x.xx	x.xx ± x.xx	x.xx ± x.xx	x.xx (x.xx to x.xx)	x.xx	x.xx (x.xx to x.xx)	x.xx

[1]Body part naming, Body part identification & Comprehension of instructions [2]Block design, Visual search chicken time per correct target & Visual search kitten time per correct target
[3]Paired associate learning & Paired associate recall.

Table 5: Minimally-adjusted and adjusted point estimates and 95% confidence intervals for non-continuous outcomes in children 4-6yrs

	LNS		**MMN**		**IFA**		**Combined**	
# of Treats	Minimally adjusted predicted probabilities (95% CI)	Adjusted predicted probabilities (95% CI)	Minimally adjusted point estimates (95% CI)	Adjusted predicted probabilities (95% CI)	Minimally adjusted predicted probabilitiess (95% CI)	Adjusted predicted probabilities (95% CI)	Minimally predicted probabilities (95% CI)	Adjusted predicted probabilities (95% CI)
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)
3	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)
4	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)	x.xx (x.xx-x.xx)

Table 6: Physical activity by intervention group

Variable	n	LNS	Non-LNS	P (diff btw all groups)	Minimally adjusted	
					Difference (CI)	P
Mean (s.d.) VM counts/15 s	xxx	xxx (xx)	xxx (xx)	x.xx	x(xx - xx)	x.xx
Mean (s.d.) vertical axis counts/15 s	xxx	xxx (xx)	xxx (xx)	x.xx	x(xx - xx)	x.xx
Mean (s.d.) % of time in MVPA, VM	xxx	xxx (xx)	xxx (xx)	x.xx	x(xx - xx)	x.xx
Mean (s.d.) % of time in MVPA, vertical axis	xxx	xxx (xx)	xxx (xx)	x.xx	x(xx - xx)	x.xx