Statistical Analysis Plan for the effect of the intervention (early maternal and child supplementation) on growth and body composition of children at 4-6 years

Background of the Study

This study is a follow up of children from a randomized controlled trial which was conducted in the Eastern Region of Ghana. The main study involved women who were at ≤20 weeks gestation and at least 18 years old. They were randomized to receive one of 3 treatments:

a. Daily iron and folic acid during pregnancy and a calcium tablet (placebo) during the first 6 months postpartum and no infant supplementation
b. Daily multiple micronutrient tablet (1-2 RDA of 18 vitamins and minerals) during pregnancy and first 6 months postpartum and no infant supplementation
c. Daily 20 g Lipid-based Nutrient Supplement (LNS) during pregnancy and first 6 months postpartum followed by infant LNS supplementation from 6-18 months postpartum. The maternal LNS had similar micronutrient content as the multiple micronutrient supplement plus calcium, magnesium, phosphorus, potassium and essential fatty acids, while the infant LNS has 22 micronutrients based on infant RNIs.

All arms of the study were followed until infants were 18 months.

In this study, children from the main trial were enrolled when they were between 4 and 6 years. Data collection involved the use of the Deuterium Dilution method for Total Body Water (TBW) determination. Three saliva samples were collected during data collection: baseline and 2 post dose samples (2.5 h and 3h after administering the deuterium dose). Samples were analyzed using the Fourier Transform Infrared Spectrometer to determine peak deuterium enrichment which was then used to calculate TBW from which fat mass and fat free mass were determined. Anthropometric measures (weight, height, triceps skinfold, and circumferences – mid upper arm, waist and hip) were also taken during data collection.

Study Objective

The primary aim of this analysis is to determine the effect of earlier maternal (pregnancy and 6 months postpartum) and infant (6-18 months postpartum) LNS supplementation compared with two maternal nutritional supplements (iron and folic acid and a multiple micronutrient tablet) on height and body composition (fat mass and fat free mass) of their children at 4-6 years. We will also look at the effect of supplementation on other anthropometric indicators and which background characteristics may modify any intervention effects observed.

Hypotheses

a. Children in the LNS group will differ in height and HAZ compared to children in the combined iron and folic acid and multiple micronutrient tablet group at 4-6 years

b. Children in the LNS group will not have significantly higher percent body fat compared to the children in the combined iron and folic acid and multiple micronutrient tablet group at 4-6 years (non-inferiority analysis)

Outcomes and Definitions

The primary outcomes of this study are:

a. Height and height-for-age z-score measured at 4-6 years

b. Percent body fat assessed at 4-6 years

Secondary outcomes assessed at 4-6 years include:

- Stunting (HAZ < -2SD)
- Percent body fat (≥ 20% body fat)
- Weight, weight-for-age z-score, underweight (WAZ < -2 SD)
- BMI-for-age score, overweight (BAZ > 1 SD), obese (BAZ > 2 SD)
- Mid upper arm circumference and MUAC z-score
- Triceps skinfold and triceps z-score, TSFZ > 2 SD
- Percent lean body mass
- Age and sex specific percent body fat

Analysis Principles

Analysis will be based on the intention-to-treat principle. All tests except the non-inferiority analysis will be two-sided, at a 5% level of significance. The non-inferiority hypothesis will be a one-sided analysis.

Sample Size

The sample size was calculated based on detecting an effect size of 0.25 in mean height, resulting in a minimum sample size of 198 per group to detect the difference with 80% power. Based on the number of children who had anthropometric measurements at 18 months in the main trial, 1185, we would be able to detect this difference with 96% power.

For the body composition analysis, sample size was calculated based on an assumed standard deviation of 2.6% in body fat (Prins *et al.*, 2008) and a predetermined non-inferiority margin of 1% in body fat (effect size = 0.4). The estimated sample size was 78 children per group, providing the trial with 80% power and 90% confidence to discard the one-sided inferiority null hypothesis.

Data Cleaning

Data were collected by electronic data collection. Forms were developed using the Open Data Kit (ODK) software and data collected using tablets. Limits for plausible values were built into the forms to prevent the anthropometrists from entering implausible values. The anthropometrists were also provided a diary in which they entered the data for participants whose values were outside the limits. At the end of each week, the data manager ran data cleaning codes written by the statistician and any queries were addressed by the supervisor. The supervisor also manually checked each form at the end of the week and any issues that came up and unanswered queries resulted in individuals being contacted for all measurements to be redone. This was to ensure that all measurements on a particular individual would all be on the same day.

For the enrichment data obtained from measuring deuterium from the saliva samples, the %CV was calculated using average measurements from the 2 post dose time points. If the %CV was greater than 5%, samples were rerun and if samples were not enough, only enrichment at 3 hours was used.

Blinding
The analyst for these analyses will remain blinded to group assignments until all decisions regarding outliers have been made and the initial run comparing treatment groups has been completed.

Statistical Analysis
Data will be analyzed using the SAS software version 9.4

Age of the child for analysis will be calculated based on the child's date of birth and date of data collection. Age of the child will be controlled for in all models.

Body composition will be calculated using age and sex specific hydration factors (Appendix 1).

All data obtained will be examined using univariate analysis (graphical plotting) to look at outliers. We will check outliers by visually inspecting Box 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. In analysis of secondary outcomes, 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.

Based on previous studies, we expect the primary outcome variables (mean height – crude height in cm and height-for-age z-score) to be normally distributed. Continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately. If no suitable transformation can be found to optimize normality and homogeneity of variances, analysis will be done on ranked data.

The analysis of the effect of the intervention will begin with testing the null hypothesis of no difference between the two groups using ANCOVA or logistic regression, and controlling for pre-specified covariates including baseline values. Only covariates significantly associated with an outcome at 10% level of significance in a bivariate analysis will be include in the final adjusted analysis. This means we may have different sets of covariates for each outcome.

The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Significant interactions ($p < 0.05$) 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.

Hypothesis A: ANCOVA will be used to analyze the difference in mean height and HAZ between the groups (presented in Table 2). Adjusted analysis will then be performed controlling for potential covariates. A sensitivity analysis will be performed comparing the 3 groups to rule out differences between the IFA and MMN groups.

Hypothesis B: The difference in mean percent body fat between the two groups will be tested by ANCOVA first in unadjusted analysis and then in adjusted analysis controlling for the potential covariates below (Table 3).
For the non-inferiority analysis, the difference between the groups will be determined using GEE linear regression analysis and one-sided 95% confidence intervals. Non-inferiority will be established if the upper bound on the 2-sided 95% CI of the LNS group is below the set margin.

For the secondary outcomes, continuous outcomes will be assessed using ANCOVA and categorical variables by logistic regression controlling for the pre-specified covariates.

Table a: Definition of categorical outcomes

Variable	**Cutoff**	**Reference**
Stunting	HAZ <-2 SD	WHO 2007
Underweight	WAZ <-2 SD	WHO 2007
Overweight	BAZ >1 SD	WHO 2007
Obese	BAZ >2 SD	WHO 2007
Triceps skinfold	TSF >2SD	
Percent body fat	%BF ≥ 20%	

Including paternal height as a covariate will be done in a sub-analysis because the sample size for paternal height is limited.

Covariates: Sex of child
Age of child at follow up
Gestational age at enrollment
Primiparity
Maternal education at enrollment
Maternal BMI at enrollment
Maternal height
Asset score at enrollment
Paternal height

Effect modifiers: Sex of child
Gestational age at enrollment
Primiparity
Maternal education at enrollment
Maternal BMI at enrollment
Maternal height
Asset score at enrollment
Paternal height

Presentation of Results

Table 1: Background characteristics of women and children from the main trial

Variable	IFA+MMN $\bar{x} \pm SD$ [n]	LNS $\bar{x} \pm SD$ [n]	p-value
Maternal Characteristics			
Number of participants			
Age (y)			
Years of formal education			
Married or Cohabiting (% [n])			
Asset score			
Primiparous women (% [n])			
Weight (kg)			
Height (cm)			
BMI (kg/m^2)			
Underweight (BMI <18.5) (% [n])			
Mid upper arm circumference (cm)			
Triceps skinfold (mm)			
Gestational age at enrolment (wk)			
Haemoglobin (g/L)			
Anaemia (% [n])			
Iron deficiency anaemia in pregnancy (% [n])			
Systolic blood pressure (mmHg)			
Diastolic blood pressure (mmHg)			
High Systolic/High Diastolic (>140 syst, >90 diast) (% [n])			
Cholesterol (mmol/L)			
HDL (mmol/L)			
Triglycerides (mmol/L)			
LDL (mmol/L)			
VLDL (µmol/L)			
Coronary risk (ratio)			
Child Characteristics			
Current age of child (y)			
Sex of child (%)			

Table 2: Comparison of continuous anthropometric and body composition measurements

	IFA+MMN $\bar{x} \pm SD$ [n]	LNS $\bar{x} \pm SD$ [n]	p-value	Difference in mean (95% CI)
Weight (kg)				
Weight-for-age z-score				
Height (cm)				
Height-for-age z-score				
BMI-for-age z-score				
MUAC (cm)				
MUAC z-score				
Triceps skinfold (mm)				
Triceps z-score				
Fat mass (kg)				
Lean mass (kg)				
	Mean (95% CI)	**Mean (95% CI)**		
% Fat mass				
% Lean mass				

Table 3: Comparison of categorical outcomes

	IFA+MMN [n]	LNS [n]	p-value
Underweight (WAZ <-2SD)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Stunting (HAZ <-2 SD)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Overweight (BAZ >1 SD)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Obese (BAZ >2 SD)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Body fat (≥ 20%)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted

Appendix 1: Hydration of Fat Free Mass in children (3-6 years)

Age (years)	Boys	Girls
3-4	77.8	78.3
5-6	77.0	78.0