Statistical Analysis Plan for the effect of the intervention (early maternal and child supplementation) on blood pressure 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.

The primary outcome for the follow up study, when children were between 4 and 6 years, was height and height-for-age z-score (HAZ). In this analysis, we look at the effect of the intervention on blood pressure of the children at 4-6 years. We use socioeconomic data, anthropometric data and blood pressure measurements as part of this analysis.

Study Objective

There is considerable evidence for an inverse relationship between birth weight and blood pressure (BP) later in life. Because fetal growth is largely a reflection of nutrient and oxygen supply to the fetus, it is widely assumed that prenatal nutrition is the most important programming stimulus (1).

The objective of these analyses is to determine the effect of maternal (pregnancy and 6 months postpartum) and infant (6-18 months of age) LNS supplementation compared with two maternal nutritional supplements (iron and folic acid and a multiple micronutrient tablet) on systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the children at 4-6 years. We will also examine the effect of supplementation on other blood pressure indicators and which background characteristics may modify any intervention effects observed.

Hypotheses

a. Children in the LNS group will have lower blood pressure compared to children in the combined iron and folic acid and multiple micronutrient tablet group at 4-6 years

Outcomes and Definitions

The primary outcomes of this study are:

a. Systolic blood pressure at 4-6 years
b. Diastolic blood pressure at 4-6 years

Secondary outcomes assessed at 4-6 years include:

- Systolic blood pressure z-score

- Diastolic blood pressure z-score
- High systolic blood pressure (SBP $\geq 90^{th}$ percentile)
- High diastolic blood pressure (DBP $\geq 90^{th}$ percentile)

Analysis Principles
Analysis will be based on the intention-to-treat principle. All tests will be two-sided, at a 5% level of significance.

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 determined we would have at least 80% power to detect a difference of the same effect size in all our other outcomes.

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 data collectors from entering implausible values. 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.

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. Field staff were also blinded to group assignments.

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.

Blood pressure percentiles and z-scores will be calculated using the equations given in the National Heart Lung and Blood Institute fourth report (2) on diagnosis, evaluation and treatment of high blood pressure in children and adolescents.

All data obtained will be examined using univariate analysis (graphical plotting). We will check for 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.

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 on the primary outcomes 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 (see below). 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. For the secondary outcomes, continuous outcomes will be assessed using ANCOVA and categorical variables by logistic regression controlling for the pre-specified covariates. For all outcomes, a sensitivity analysis will be performed comparing the 3 groups to rule out differences between the IFA and MMN groups.

The effects of potential effect modifiers (listed below) 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.

In a per protocol analysis, we will also test the effect of the intervention limiting the analysis to mothers who self-reported ≥ 80% adherence to the supplements during the combined pregnancy and lactation period, using the same methods as described above.

Covariates: Sex of child
Gestational age at enrollment
Primiparity
Maternal BMI at enrollment
Asset score at enrollment

Effect modifiers: Sex
Maternal BMI at enrollment
Maternal systolic blood pressure and diastolic blood pressure at enrollment
Primiparity

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			
Age (y)			
Gestational age at enrolment (wk)			
Years of formal education			
Married or Cohabiting (% [n])			
Asset score			
Primiparous women (% [n])			
Weight (kg)			
Height (cm)			
BMI (kg/m^2)			
Overweight (BMI $\geq$ 25) (% [n])			
Systolic blood pressure (mmHg)			
Diastolic blood pressure (mmHg)			
Child Characteristics			
Current age of child (y)			
Sex of child (%)			
Birth weight (kg)			
Weight at 4-6 years (kg)			
Height at 4-6 years (m)			
HAZ at 4-6 years			
Fat mass (kg)			
Fat free mass (kg)			

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)
Systolic blood pressure (mmHg)				
SBP z-score				
Diastolic blood pressure (mmHg)				
DBP z-score				

Table 3: Comparison of categorical outcomes

	IFA+MMN [n]	LNS [n]	p-value
Hypertensive (SBP $\geq 90^{th}$ percentile)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Hypertensive (DBP $\geq 90^{th}$ percentile)			
Prevalence (%)			
OR (95% CI)			unadjusted
			adjusted
Hypertensive (SBP $\geq 90^{th}$ percentile or DBP $\geq 90^{th}$ percentile)			
Prevalence (%)			unadjusted
OR (95% CI)			adjusted

1. Adair LS, Kuzawa CW, Borja J. Maternal energy stores and diet composition during pregnancy program adolescent blood pressure. Circulation. 2001;104(9):1034-9.
2. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(2 Suppl 4th Report):555-76.