iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Impact of long term exposure to lipid-based nutrient supplements on sweet taste preference of Ghanaian children aged 4-6 years.

Statistical Analysis Plan

Prepared by: Harriet Okronipa

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Table of Contents

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

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 number	Version date	Prepared by	Details of significant changes
1.	March 16, 2017	Harriet Okronipa	Original document added on March 12, 2017

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Introduction

Small-quantity lipid-based nutrient supplements (SQ-LNS) belong to a family of products that deliver vitamins and minerals, essential fatty acids, protein and energy in a lipid matrix. They are made from vegetable oil, milk powder, peanut paste, sugar and multiple micronutrients and have been shown to have the potential to improve child growth and development. Between 2009 and 2014, the International Lipid-based Nutrient Supplements study (iLiNS-DYAD trial) was conducted in Ghana to examine the efficacy of SQ-LNS for pregnant and lactating women. 1320 pregnant women ≤20 weeks gestation were enrolled and individually randomized to receive daily, either (a) 60 mg iron plus 400 µg folic acid during pregnancy, and a calcium placebo during the first 6 months postpartum, with no supplementation for offspring during infancy (IFA group) or (b) 1-2 RDA of 18 micronutrients during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy (MMN group) or (c) small quantity lipid-based nutrient supplements (20 g) which contain 22 micronutrients plus some macronutrients, during pregnancy and the first 6 months postpartum, with SQ-LNS for offspring from 6 to 18 months (LNS group).

The SQ-LNS have a moderately sweet taste due to the sugar and milk they contain, and concern has been raised regarding their potential to impact sweet taste preference in children later on in life, given how long the children were exposed to the supplement during the study. Given that children are born with an innate preference for sweet foods, and also that the 20g SQ-LNS contains very little added sugar and also forms only a small percentage of the sweet food intake of this population, we do not think SQ-LNS will influence sweet taste preference. However, given that SQ-LNS is a novel product, it is important that we rule out any negative consequences.

From January to December 2016, we conducted a follow up study of the children (now aged 4-6 years) who participated in the main iLiNS trial with the overall aim of examining the long term impact of the provision of SQ-LNS antenatally and during lactation and infancy on maternal and child health and nutrition outcomes. This SAP describes the non-inferiority analysis to examine the impact of SQ-LNS on child sweet taste preference. We hypothesize that the mean preferred percentage sucrose score of children who were exposed to SQ-LNS in utero, via breastmilk, and from 6 to 18 months of age will not be more than 1.64 % w/v sucrose units higher than that of the non-LNS group.

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Specific objective

1. To examine the impact of early exposure to a small-quantity lipid-based nutrient supplement (SQ-LNS) in utero, via breastmilk, and from 6 to 18 months of age on the sweet taste preference of Ghanaian children aged 4-6 years.

Hypothesis:

1. The mean preferred percentage sucrose score of children who were exposed to SQ-LNS in utero, via breastmilk, and from 6 to 18 months of age will not be more than 1.64 % w/v sucrose units higher than that of the non-LNS group.

Study population, design inclusion and exclusion criteria

This study is a follow-up to the main iLiNS-DYAD trial. All women and children who participated in the main trial (except those who reported miscarriages) were eligible to be included in the follow-up study, irrespective of whether or not they remained in the main trial at endline (women, 6 mo postpartum; children, 18 mo of age). Based on our study objectives, and because we were only interested in the impact of SQ-LNS and not in comparing IFA and MMN groups, we will combine the IFA and MMN groups and compare:

1. Children who were exposed to SQ-LNS in utero, through breastmilk (up to 6 mo postpartum), and from age 6 to 18 months (LNS group).

2. Children who were never exposed to SQ-LNS: their mothers received IFA/MMN (non-LNS group).

For this present analysis, a subsample of mother-child dyads were randomly selected from among the total sample that were eligible for the follow-up study. The selection was done such that in the non-LNS group, we had equal numbers of children from the IFA and MMN groups.

Blinding

All data collectors and study investigators were blinded to the assigned treatment group. This statistical analysis plan was written before the start of any analysis. All investigators who will be running any analysis will remain blinded until initial analysis of primary outcomes is complete.

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Outcome

Sweet taste preference

Our main outcome is child sweet taste preference, defined as the mean percentage sucrose score on the Monell Forced Choice taste test (1).

Statistical analysis

Analysis principles

1. Analysis will be by intention-to-treat. Results will be analyzed according to the group to which participants were assigned regardless of whether they got the treatment or if they followed protocol.
2. Data for all participants who were lost to follow-up because of death, refusal to continue with the study, or travel from study site will be included in the analysis of household and maternal baseline characteristics, if available.
3. Outcomes will be compared between groups using non-inferiority hypothesis testing.
4. Sweet taste preference scores will be compared between study groups using a non-inferiority margin of 1.64 w/v, which corresponds to an effect size of 0.2.
5. Analysis of treatment effect will be done using negative binomial models. The negative binomial modelling technique outputs differences between groups as ratios or percentage difference instead of mean difference. To make comparisons more efficient and easy to understand, we will convert the non-inferiority margin from the absolute value of 1.64 to a ratio (or percentage difference) by dividing 1.64 by the mean preference score of our sample. Comparisons between groups will be made using this ratio as our non-inferiority margin.
6. Tests will either be one-sided (main outcome comparisons) or two-sided (baseline comparisons) and at 5% level of significance, except as otherwise stated.
7. In reporting our results, we will report the number of observations used in the analysis wherever there are more than 10% of observations missing for a dependent or outcome variable.
8. In addition to the intention-to-treat analysis, we will conduct other analysis by re-running the final models in pre-specified sub-samples described below:
    i. We will re-run the analysis excluding those children whose preferred sucrose solutions for series 1 and series 2 were > 2 concentrations apart.

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Sample size

The subsample size was calculated based on the primary objective: to test the hypothesis of non-inferiority of SQ-LNS regarding long term sweet taste preference as compared to no LNS. The null hypothesis is that the difference in preference score between the LNS and non-LNS group will be 1.64 % w/v or more (non-inferiority margin).

A pilot study in the study area showed the preferred percentage sucrose solution score to be 15.7 ± 8.2 %. Assuming a standard deviation of 8.2 percent sucrose and a pre-determined non-inferiority margin of 1.64 percent sucrose w/v (an effect size of 0.2), a sample size of 620 was estimated to provide the trial with 80% power and 95% confidence to discard the inferiority null hypothesis. Adjusting for 15% attrition rate (including child deaths, loss to follow-up, refusals) brings the total sample size to 713 (356 participants per group).

Study flowchart

A participant flow diagram will be prepared in accordance with the CONSORT 2010 guidelines. The figure (Figure 1 below) will include the numbers and reasons for permanent loss to follow up between the start of the main trial and end of the follow-up study.

Data cleaning

Data cleaning for this follow-up study was done alongside data collection. Range checks were built into direct data entry by enumerators. SAS syntax were written and run weekly to generate a list of queries on plausible but extreme values or inconsistent responses, which were then examined and resolved with the help of the field worker. The corrections/ changes were then recorded in a "data cleaning excel sheet" which was used to generate additional syntax and a corrected data set.

Outliers

Data will be visually examined by means of histograms (for individual continuous variables) and scatter plots (of related variables) to check for outliers after which they will be investigated. Outliers which are clearly implausible or impossible will be corrected if possible or recoded to missing where correction is not possible. Those that are plausible or possible will be kept in the dataset. During analysis, we will transform variables with outliers, and if needed, we will conduct sensitivity analysis by running the models with and without these outliers to examine if these outliers have undue influence on the results.

Software for analysis

All analyses will be done using SAS version 9.4 (SAS Inst. Cary, NC, USA).

Baseline characteristics of participants and baseline comparisons

Baseline characteristics will be compared between groups using ANOVA for continuous variables or a chi-square test for categorical variables. If there are dropouts (selected for the subsample but who were not tested for various reasons), we will compare baseline characteristics between participants who will be included in the analysis and the dropouts. Baseline characteristics will be selected household and maternal characteristics at baseline, that is, all available values for these selected variables at the time of maternal enrolment into the main study, prior to first intake of study supplement. Child age will however be the age of child at the time of sweet taste preference testing. In analyzing baseline characteristics:

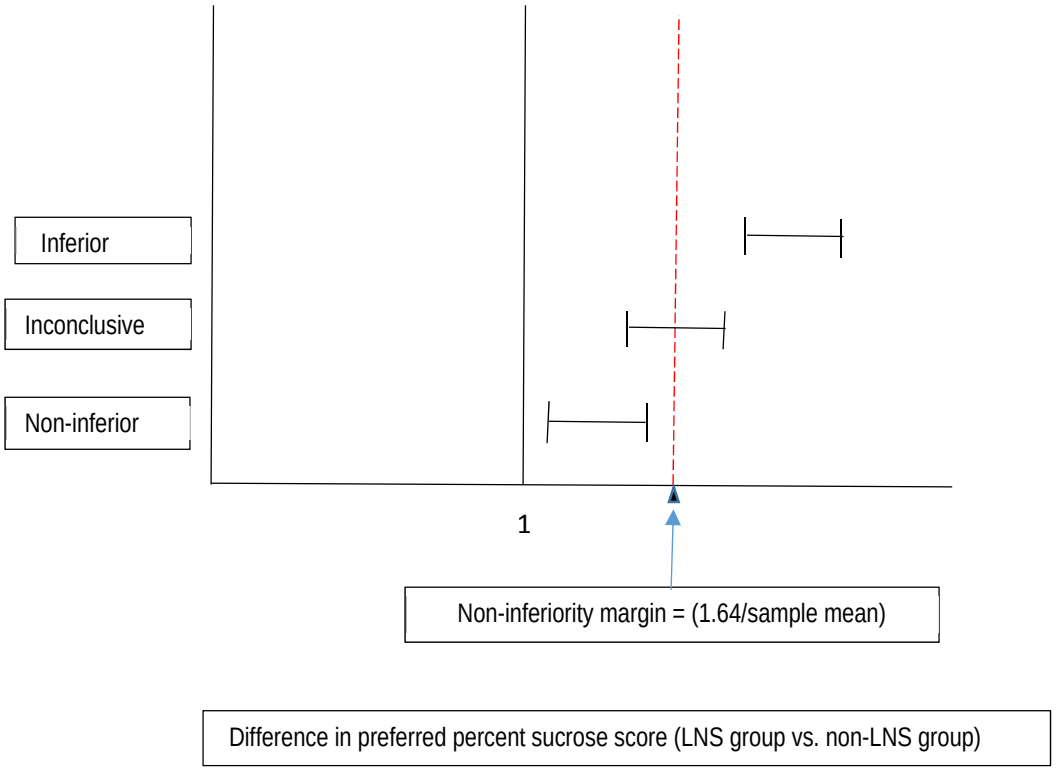
1. Means and SE (or SD) or medians and 25^{th} and 75^{th} percentiles will be used to describe continuous data.
2. Frequencies and percentages will be used to summarize categorical data. We will calculate percentages based on the number of participants for whom data are available.
3. Where data for certain participants are missing (>10 %), the number of participants included in the analysis will be indicated.

Analysis of the impact of the intervention

Negative binomial models will be used to test the null hypothesis of inferiority between the two groups. We will first run the models without covariates, then run them again controlling for pre-specified covariates. Only covariates significantly associated with the outcome at 10% level of significance in bivariate analysis will be included in the final adjusted analysis. Results will be presented as the difference

in means between the two groups. The 95% confidence interval will be reported. Results will be interpreted as follows (and graphed below):

1. If the entire 95% CI is below the non-inferiority margin, we conclude that **non-inferiority** has been established
2. If the lower level of the 95% CI is greater than the non-inferiority margin, we conclude that the treatment group is **inferior** (worse in sweet taste/ have higher preference for sweet taste) to the control group.
3. If the upper level of the 95% CI is greater than the non-inferiority margin and the lower level is less than the non-inferiority margin, we conclude that the result is **inconclusive**.



Potential covariates

Independent of the intervention, child sweet taste preference could be influenced by a variety of household, maternal, and infant factors. Each pre-specified covariate (listed below), will be examined for completeness of data, and a decision will be made for the exclusion of any covariate with a high proportion of missing data. Potential covariates that will be examined include:

- Maternal years of formal education
- Maternal estimated pre-pregnancy BMI
- Maternal marital status
- Maternal age
- Parity
- Child age at testing
- Child sex
- Household Assets Index (baseline)
- Household food insecurity index (baseline)
- Distance to market
- Ethnicity

Data transformations

We will examine the residuals of continuous outcome variables to assess if they conform to the normal distribution using the Shapiro-Wilk test. Shapiro-Wilk statistic values >0.97 will be considered acceptable. Any continuous outcome whose residuals are not normally distributed will be transformed appropriately.

Figures and Tables

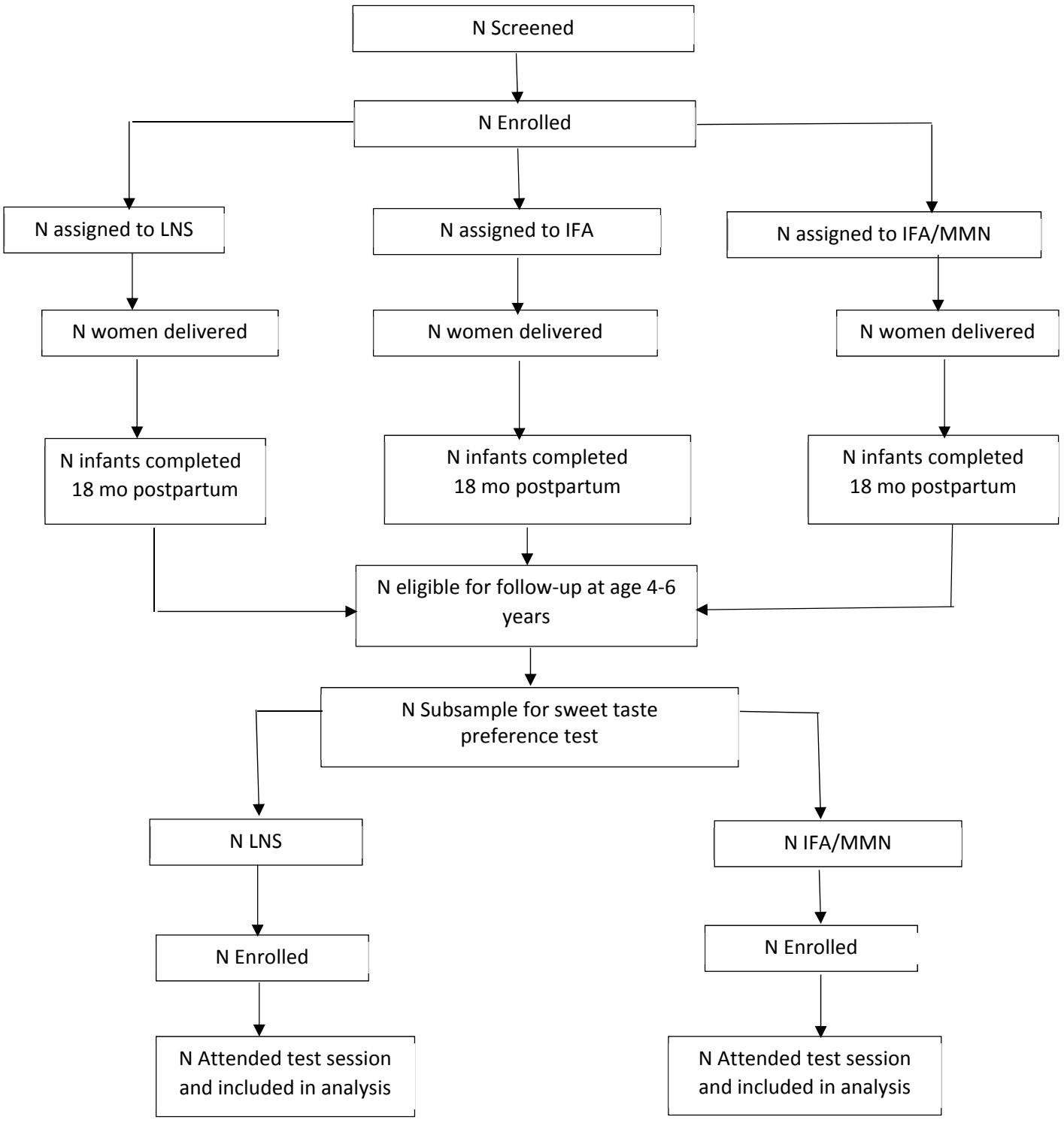
The following figures and tables are only drafts. They will be examined and final decisions on presentation of information will be made by the manuscript writing group later.

Figures:

Figure 1 will be the study flow chart (shown below) and figure 2 will be the non-inferiority graph shown above.

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Figure 1: Study Flow chart



iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Tables

Table 1: Comparison of baseline[1] characteristics and other covariates, by intervention group[2]		
Variable[3]	**LNS**	**No LNS**
Number of participants		
Maternal age (y)		
Maternal education (y)		
Married or cohabiting (%)		
Maternal pre-pregnancy BMI (kg/m^2)		
Parity (#)		
Ethnicity (%)		
Child male sex (%)		
Child age at follow-up (mo)		
Household Assets Score (#)		
Household Food Insecurity Score (#)		
Distance to market (#)		

[1]Baseline characteristics listed (except child age) were obtained prior to maternal enrolment into the main study (prior to first intake of study supplement). Child age is the age of the child at the time of sweet taste preference testing during follow-up

[2]LNS=lipid-based nutrient supplement; Non-LNS = exposure to IFA/MMN but no LNS

[3]Data will be presented as mean ± SD or Median (Q1, Q3) depending on the distribution

Table 2: Task performance, by intervention group[1]			
Variable[2]	**LNS**	**Non-LNS**	**P-Value[3]**
Total time required			
Series 1			
Series 2			
Number of pairs (trials) required to reach criterion			
Series 1			
Series 2			
Both series			
Agreement between series 1 and 2 [n (%)]			
Preferred same solution			
Preferred one concentration higher/lower			
Preferred two concentrations higher/lower			
Preferred more than two concentrations higher/lower			

[1]LNS=lipid-based nutrient supplement; Non-LNS = exposure to IFA/MMN but no LNS

[2]Data will be presented as mean ± SD or Median (Q1, Q3) depending on the distribution

[3]Differneces between groups will be tested using ANOVA or the chi-squared test for continuous and categorical variables, respectively

iLiNS DYAD-G2 Preschool Follow-up Study: Impact of long term exposure to LNS on sweet taste preference of Ghanaian children aged 4-6 years.

Table 3: Preferred sucrose concentration, by intervention group[1]

	LNS	Non-LNS	LNS vs. No LNS Difference in means (95% CI)[2]
Intensity of sucrose most preferred			
Unadjusted			
Adjusted			

[1]LNS=lipid-based nutrient supplement group; Non-LNS = exposure to IFA/MMN but no LNS

[2]Differneces between groups will be tested using the negative binomial regression method

REFERENCE

1. Mennella JA, Lukasewycz LD, Griffith JW, Beauchamp GK. Evaluation of the Monell forced-choice, paired-comparison tracking procedure for determining sweet taste preferences across the lifespan. Chem Senses. 2011 May;36:345-55.