Food and drink preferences and consumption of Ghanaian preschool children: Long-term impact of exposure to a slightly sweet lipid-based nutrient supplement early in life.

Statistical Analysis Plan

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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 number	Version date	Prepared by	Details of significant changes
1.	Aug-21-2017	Harriet Okronipa	Original document

iLiNS DYAD-G2 Preschool Follow-up Study: Long-term impact of early exposure to LNS on food and drink preference and consumption of Ghanaian children aged 4-6 years.

Background

Small-quantity lipid-based nutrient supplements (LNS) are slightly sweet nutrient supplements made from vegetable oil, milk powder, peanut paste, sugar and multiple micronutrients and designed to prevent malnutrition during the critical first 1000 days. Between 2009 and 2014, the International Lipid-based Nutrient Supplements study (iLiNS DYAD-G trial) was conducted in Ghana to examine the efficacy of LNS for preventing malnutrition in pregnant and lactating women and their children. 1320 pregnant women ≤20 weeks gestation were individually randomized to receive daily, one of three treatments: (a) 60 mg iron plus 400 μg folic acid during pregnancy, and a low dose 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 contained 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). Women were followed until 6 mo postpartum, and their infants until 18 mo of age.

We conducted a follow-up study of the iLiNS DYAD-G cohort when the children reached age 4–6 y and one of our aims was to examine the long-term impact of exposure to SQ-LNS on the sweet taste and food and drink preferences of children at age 4-6 y. For the analysis described in this document, we aim to examine the impact of long term exposure to SQ-LNS on the food and drink preferences and consumption of Ghanaian children aged 4-6 y.

Main 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 food and drink preferences and consumption of Ghanaian children at age 4-6 years.

Specific objectives

1. To compare the preference for sweet foods/drinks (as reported by child) among LNS exposed and non-exposed groups.
2. To compare consumption of sweet foods/drinks (as reported by caregiver) among LNS exposed and non-exposed groups.
3. To compare consumption of sugar-sweetened drinks (as reported by caregiver) among LNS exposed and non-exposed groups.

Hypotheses to be tested:

1. The mean number of sweet foods/drinks chosen out of a total of 30 food/drink items by children who were exposed to LNS will not be significantly more (more than +0.66 items higher) than the number chosen by children who were never exposed to LNS.
2. The consumption of sweet foods and drinks in the past 7 days preceding the interview (as reported by caregiver) by children in the LNS-exposed group will not be significantly higher (more than 1.96 items higher) than that of children in the non-LNS group.
3. The consumption of sugar-sweetened drinks in the past 7 days preceding the interview by children in the LNS-exposed group will not be significantly higher (more than 1.08 items higher) than that of children in the non-LNS group.

Secondary objectives

1. To compare the mean preference score for sweet foods and drinks (as reported by caregiver) between LNS-exposed and non-exposed children.
2. To compare the consumption of peanut-containing foods between-LNS-exposed and non-exposed groups.

Exploratory analysis

3. To explore the consumption of other foods by children in the LNS exposed and non-exposed groups.

Study population and design

This study is a follow-up to the main iLiNS DYAD-G trial. All women and children who participated in the main trial and are alive (except those who reported miscarriages, misdiagnosed pregnancies and stillbirths) 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). Since women and children in the MMN and IFA groups did not directly receive supplementation and we did not aim to compare the MMN and IFA groups, we combined the two groups into a non-LNS control group.

Data were collected on the full sample of participants for some outcomes and on a subsample for other outcomes. For the latter, we randomly selected a sub-sample of mother-child dyads ensuring that the numbers of children in the IFA and MMN groups were similar within the combined (non-LNS) group.

Outcomes

1. **Child's preference for sweet foods and drinks**
   a. Mean number of photos of sweet food/ drink items chosen by child in two choice situations:
      i. Unlimited choices from among 30 items
      ii. Five favorite items from among those chosen in "i" above
2. **Caregiver report of child's consumption of sweet foods and drinks**
   a. Mean number of times per week child consumed sweet foods and drinks
   b. Mean number of times per week child consumed sugar-sweetened beverages
3. **Caregiver report of child preference for sweet foods and drinks**

   a. Mean preference score for sweet food/drink items. The preference scores will be calculated as follows:

      1. Individual preference scores will be assigned to caregiver responses to the question about "how much child likes specific food/drink items" – (likes very much, 2; likes a little, 1; dislikes, -1; never offered/tried, 0; don't know, 0).

2. The individual preference scores will then be summed up to obtain a total preference score for a particular food group category.

4. **Caregiver report of child's consumption of peanut-containing food**
   a. Mean number of times per week child consumed peanut-containing foods.

5. **Caregiver report of child consumption of other foods**
   a. Mean number of times per week child consumed other food groups.

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 (LNS vs. non-LNS) regardless of whether they got the treatment or if they followed protocol.
2. Most outcomes will be compared between groups using non-inferiority hypothesis testing.
3. Given our large sample size we expect that our count data will behave continuously enough to satisfy typical ANCOVA model assumptions. If this is not the case, then count data will be assessed with negative-binomial modeling techniques.
4. Preference for sweet food/drinks will be compared between study groups using a non-inferiority margin of 0.66 for number of sweet items chosen, 1.96 for number of times sweet foods/drinks consumed in the past 7 d and 1.08 for number of times sugar-sweetened drinks consumed in the past 7 d. These non-inferiority margins correspond to an effect size of approximately 0.2.
5. Tests will either be one-sided (non-inferiority tests) or two-sided (baseline, other outcome comparisons) and at 5% level of significance, except otherwise stated.
6. 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.

7. In addition to the intention-to-treat analysis, we will conduct additional sensitivity analysis by re-running the final models in pre-specified sub-samples described below:
    i. For the outcome "Mean number of sweet food/ drink items chosen by child", we will re-run the analysis to include a food item only if the child recognized and 'knew' it (Photo game). "Knew" here will be defined as child 'knowing' a food/ drink item with or without prompting.
8. As part of the iLiNS follow-up studies, we measured child sweet taste preference using a psychophysical tool known as the Monell 2-series, forced-choice paired-comparison test. This tool, as part of its design, provides the means to objectively identify children who give random responses because they do not understand the test. As an additional **exploratory analysis**, for the outcome "mean number of sweet food/ drink items chosen by child", we will re-run the analysis to include only the subset of children who provided reliable responses during the sweet taste test. We think that these children may be more mature and cognitively more capable of understanding both the photo game and the sweet taste tests.

Sample size/ power calculations

1. *Specific objective 1: To compare the preference for sweet foods/drinks (as reported by child) among LNS exposed and non-exposed groups.*

This outcome was measured in a subsample. We aimed to detect an effect size of 0.2 (1), which is equivalent to a non-inferiority margin of 0.66. This yielded a sample size of 620 assuming a standard deviation of 3.3 items, an 80% power and an alpha of 0.05. Accounting for 25% attrition brought the total sample size to 775 (388 per group).

Data were successfully obtained from 624 children, which gave us 80% power to detect a mean difference of 0.658 between groups in the preference for sweet foods and drinks (as reported by child) assuming a standard deviation of 3.3 items (obtained from early preliminary data). This mean difference is close to our desired non-inferiority margin of 0.66.

Specific objectives 2: to compare the consumption of sweet foods/drinks (as reported by caregiver) among LNS exposed and non-exposed groups).

We aimed to detect an effect size of 0.2, which is equivalent to a non-inferiority margin of 1.96.

Data were successfully obtained from 985 children out of the 1222 children eligible for follow-up, which gave us a 90% power to detect a mean difference of 1.83 between groups in the preference for sweet foods/drinks (as reported by caregiver) assuming a standard deviation of 9.8 items (obtained from early preliminary data) and an alpha of 5%.

Specific objectives 3: to compare the consumption of sugar-sweetened drinks (as reported by caregiver) among LNS exposed and non-exposed groups

We aimed to detect an effect size of 0.2, which is equivalent to a non-inferiority margin of 1.08

Data were successfully obtained from 985 children out of the 1222 children eligible for follow-up, which gave us a 90% power to detect a non-inferiority margin of 1.01 between groups in the preference for sugar-sweetened drinks (as reported by caregiver) assuming a standard deviation of 5.4 items (obtained from early preliminary data) and an alpha of 5%.

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.

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.

Data cleaning

Data were collected using electronic data collection devices (tablets). Questionnaires were developed using the Open Data Kit (ODK) software. To the extent possible, 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 (and/or study respondent). The corrections or 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).

Comparison of maternal, household and child characteristics of participants

Background characteristics will be compared between groups using ANOVA for continuous variables or a chi-square test for categorical variables.-We will compare characteristics between participants who had outcome data and those who did not have outcome data because they were loss to follow-up (or were not tested). For some maternal and household variables, available values at the time of maternal enrolment into the main study, prior to first intake of study supplement (e.g maternal age, education, primiparity) will be considered as background characteristics. Other factors measured at time of enrolment into follow up (e.g child age, sex) will also be considered as background characteristics and presented in a table. In analyzing these characteristics:

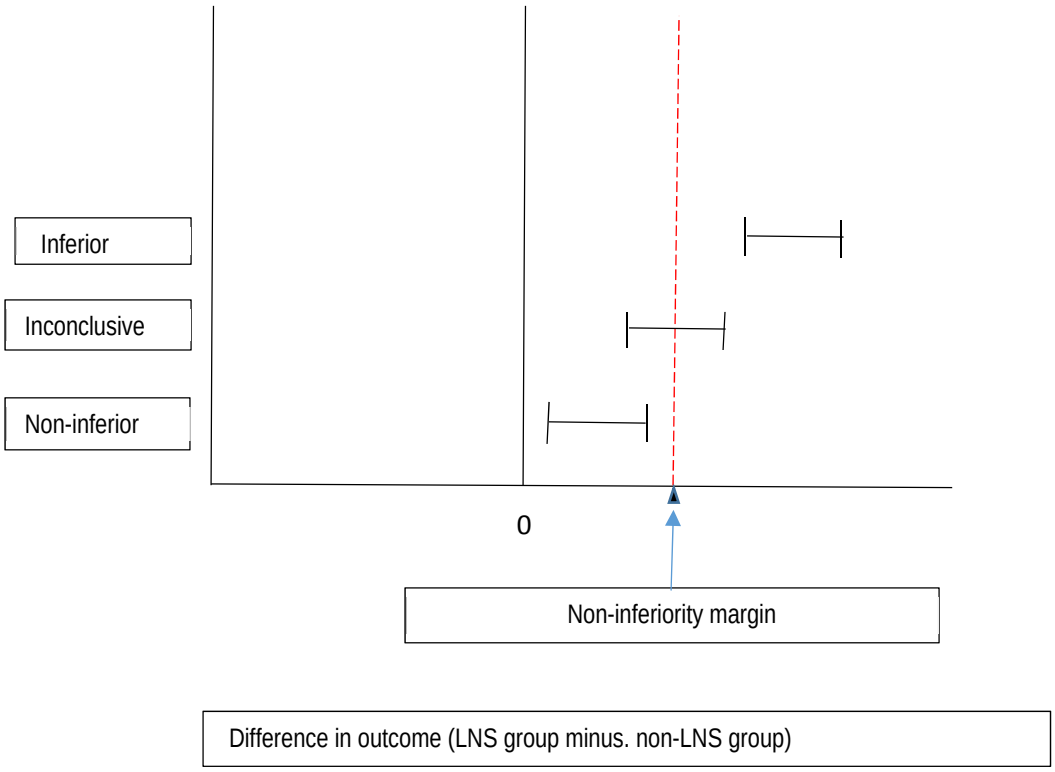
1. Means and SE (or SD) or medians and 25th and 75th percentiles will be used to describe continuous data.
2. Frequencies and percentages will be used to summarize categorical data. We will calculate percentages based on the number of participants for whom data are available.
3. Where data for certain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

Analysis of the impact of the intervention

Linear regression, ANCOVA or negative binomial modelling techniques will be used to test the null hypothesis of inferiority between the two groups and to examine differences in other outcomes between 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 (or mean ratio) between the two groups. The 95% confidence interval will be reported. Results (from non-inferiority tests) 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** 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**.


Inferior
Inconclusive
Non-inferior
0
Non-inferiority margin
Difference in outcome (LNS group minus. non-LNS group)

Potential covariates

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. Covariates will be included if they show significant association with the outcome at P<0.1. Potential covariates that will be examined include:

1. Maternal and household factors collected at baseline
    - Maternal years of formal education at enrolment
    - Maternal estimated pre-pregnancy BMI at enrolment
    - Maternal age at enrolment
    - Primiparity
    - Household Assets Index at enrolment
    - Distance to market

2. Factors collected at follow-up
    - Child sex
    - Child age

Potential effect modifiers

The following will be examined as potential effect modifiers with an interaction term included in the models. When an interaction is found to be significant ($p < 0.1$), it 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. If effect modification is present, results will be reported using suitable graphs (e.g. bar graph for a categorical effect modifier or line graph for a continuous effect modifier).

1. Maternal and household factors collected at baseline
    - Maternal years of formal education at enrolment
    - Maternal estimated pre-pregnancy BMI at enrolment
    - Maternal age at enrolment
    - Primiparity

- Household Assets Index at enrolment
- Distance to market

2. Child factors collected at follow-up
   - Child sex
   - Child age

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. Non-parametric tests will be employed if no suitable transformations are found.

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). Non-inferiority graphs for outcomes will also be presented.

iLiNS DYAD-G2 Preschool Follow-up Study: Long-term impact of early exposure to LNS on food and drink preference and consumption of Ghanaian children aged 4-6 years.

Figure 1: Study Flow chart

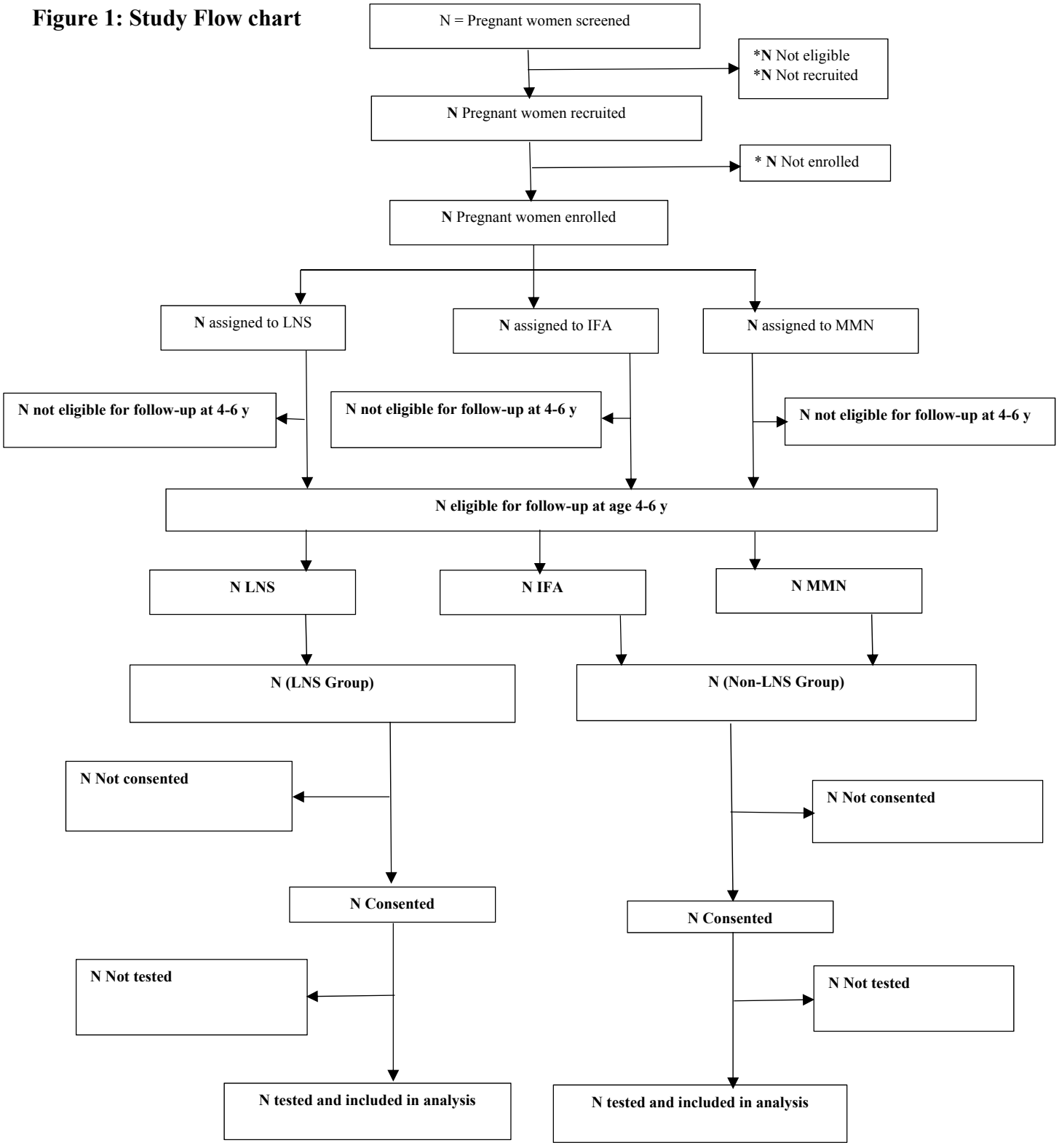


FIGURE 1 Study profile.
LNS, lipid-based nutrient supplement; IFA, iron and folic acid; MMN, multiple micronutrients.
LNS group, women received 20 g LNS daily during pregnancy and 6 mo lactation. Infants received 20 g LNS daily from 6-18 mo of age; Non-LNS group, women received either IFA during pregnancy and placebo for 6 mo postpartum or MMN capsules during pregnancy and 6 mo lactation. Infants did not receive any supplement.
*Details reported in a previously published study {Adu-Afarwuah, 2016; Adu-Afarwuah, 2015}

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Tables

Table 1: Child, maternal and household characteristics, by intervention group[1] for participants who were included in analysis				
Variable[2]	**All groups combined (n=X)**	**LNS Group (n=X)**	**Non-LNS Group (n=X)**	**P-value[3]**
Child characteristics at birth				
Sex n (% male)				
Length-for-age z-score (LAZ)				
Child characteristics at 18 mo				
Length-for-age z-score (LAZ)				
Child characteristics at age 4-6 y follow-up				
Age at testing (y)				
Height (cm)				
Weight (kg)				
Height-for-age z-score (HAZ)				
BMI-for-age z-score (BMIZ)				
Mothers (baseline characteristics) at time of original trial				
Age (y)				
Education (y)				
Married or cohabiting (%)				
Pre-pregnancy BMI (kg/m^2)				
Primiparity (%)				
Household Assets Score (#)[4]				
Distance to market (m)				
Speaks krobo language at home (%)				
Child characteristics at age 4-6 y follow-up				
Sex (% male)				
Age (y)				
Weight (kg)				
Height (m)				
BMI-for-age z-score (BMIZ)				

[1]LNS = small-quantity lipid-based nutrient supplement (LNS) group; Non-LNS = Non-LNS group, no exposure to LNS

[2]Data will be presented as n (%); mean ± SD or median (Q1, Q3) depending on the distribution.

[3]Group differences were compared using ANOVA for continuous variables and the chi-squared test for categorical variables

[4]Proxy indicator for socio-economic status; a higher value represents a higher socioeconomic status

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Table 2: Sweet food and drink and peanut preference and consumption among 4-6 y old Ghanaian children who participated in the iLiNS DYAD-G2 follow up study, by intervention group[1]					
Variable[2]	**LNS**	**Non-LNS**	**LNS vs. No LNS Mean ratio (95% CI)[3]**	**Percent Difference (of mean ratio) (95% CI)**	**P-value**
Child's preference for sweet food and drink					
Mean number of sweet food/ drink items chosen by child from among 30 items (unlimited choices)					
Mean number of sweet food/ drink items chosen by child (out of top 5 favourite food/drink items)					
Caregiver report of child's consumption of sweet food and drink					
Mean number of times child consumed sweet foods and drinks in the last 7d					
Mean number of times child consumed sugar-sweetened beverages in the last 7d					
Caregiver report of child preference for sweet food and drink					
Preference score for sweet food/drink items					
Caregiver report of child's consumption of peanut-containing food					
Mean number of times child consumed peanut-containing foods in the last 7d					
[1]LNS= small-quantity lipid-based nutrient supplement (LNS) group; Non-LNS = Non-LNS group, no exposure to LNS [2]Data will be presented as mean ± SD or Median (Q1, Q3) depending on the distribution [2]Differences between groups will be examined using negative binomial modelling techniques					

iLiNS DYAD-G2 Preschool Follow-up Study: Long-term impact of early exposure to LNS on food and drink preference and consumption of Ghanaian children aged 4-6 years.

Supplementary table 1: Food and drink consumption[1] (# times consumed per week) among 4-6 y old Ghanaian children who participated in the iLiNS DYAD-G2 follow up study				
Variable[2]	**All groups combined (n=X)**	**LNS Group[3] (n=X)**	**Non-LNS Group[3] (n=X)**	**P-value**
Sweet foods and drinks				
Sugar sweetened drinks and beverages				
Sweet snacks				
Milk				
Fruits				
Vitamin-A rich fruits				
Other fruits				
Vegetables				
Vitamin-A rich				
Other vegetables				
Legumes, nuts and seeds				
Animal-source foods				
Meat				
Fish				
Eggs				

[1]Data presented as number of times a particular food/drink item consumed in the week preceding the interview.
[2]Data are presented as mean ± SD
[3]LNS = small-quantity lipid-based nutrient supplement (LNS) group; Non-LNS = Non-LNS group, no exposure to LNS

References

1. Cohen J. Statistical power analysis in the behavioral sciences. Hillsdale (NJ): Lawrence Erlbaum, 1988.