Efficacy of lipid-based nutrient supplements (LNS) for pregnant and lactating women and their infants (iLiNS DYAD-Ghana)

Statistical Analysis Plan

Effect on infant and young child feeding practices at 18 months (added on 06 May 2014, revised cross-site version 2.0 added December 20, 2014)

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1. Version history

Version number	Version date	Prepared by	Description of the completed editions
01.0	06.05.2014	Arimond, Peerson, Okronipa	Original document added 06 May 2014
02.0	06.12.2014	Arimond, Dewey, Peerson, Abbeddou, Okronipa, Kumwenda	This cross-site analysis plan replaces earlier single site plans for iLiNS-DOSE and iLiNS-DYAD-Ghana. Relative to the earlier plans, the analytic approach is the same, but changes are: Analysis is restricted to endline and per protocol analysis is dropped; Details of variable construction are dropped and are captured elsewhere in codebooks; Covariate selection is harmonized with Burkina site (addition of several candidate co-variates); Presentation of results is harmonized across sites.

2. Overview and study objectives

The analysis presented here is nested within a pre-existing iLiNS analysis plans for primary and other secondary outcomes. Refer to the four main analysis plans (iLiNS-DOSE (Malawi), iLiNS-ZINC (Burkina Faso), iLiNS-DYAD-Ghana, iLiNS-DYAD-Malawi) for: inclusion and exclusion criteria for the trial; data cleaning protocols; procedures for breaking code; and procedures for modifying this protocol.

The main objective of data collection related to infant and young child feeding (IYCF) practices at 18 months is to compare a range of practices across intervention groups. Analysis will be within (not across) sites but a cross-site manuscript will be prepared.

This analysis is motivated by concerns that energy-dense LNS may displace breastfeeding and/or nutrient-dense local foods and/or impede dietary diversification with local foods, thus negatively impacting infant feeding practices and development of infant dietary preferences and habits. Effects on IYCF practices could be mediated either by maternal perceptions of different needs

for breast milk or local foods for infants receiving supplements and/or by a change in appetite, demand for breastfeeding, or preference for local foods among infants who consume the supplement.

IYCF practices to be compared across groups include: continued breastfeeding, frequency of breastfeeding, frequency of feeding solid/semi-solid foods[1], consumption of nutrient-dense food groups yesterday and last week, and food group diversity yesterday. We also assessed consumption of other fortified products (other than the project LNS) but preliminary analysis showed that consumption of such products was very rare in two of three sites, so this is not included as an outcome in this analysis.

Specific objectives of analysis

1.1 Primary objective

To compare infant and young child feeding practices and summary diet quality variables across intervention groups.

1.2 Secondary objectives

To provide descriptive data on IYCF practices to contextualize results of the trials, and to aid readers in comparing to IYCF in other settings

3. Hypotheses to be tested

Stated qualitatively: Provision of LNS would not impact infant and young child feeding practices. More specifically, provision of LNS would not cause a change in:

- Breastfeeding (prevalence of any breastfeeding, reported frequency of breastfeeding the previous day)
- Frequency of feeding other solids foods (meals and snacks, or feeding episodes);
- Dietary diversity yesterday measured as food group diversity at or above the WHO cut-off[2];
- Number of nutrient-dense food groups (animal-source foods, fruits and vegetables) consumed yesterday or last week

[1]Frequency of feeding data are available for iLiNS-ZINC (Burkina Faso) and iLiNS-DYAD-Malawi only.

[2] WHO (2008) Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA.

4. Timing and description of IYCF outcome variables at 18 months (endline)

All outcomes are based on caregiver recall of practices in response to structured survey questions. Dietary diversity and consumption of nutrient-dense food groups were assessed through a guided free recall of foods consumed by the child yesterday, and a list-based recall of the number of days food groups were consumed in the 7 days preceding the interview.

This analysis is of endline data in all study sites, with a common target age of 18 months.

Tolerance for late endline visits varied by site and study, and child age (expressed as a deviation from the site-specific median age for endline) will be included as a potential covariate in all sites.

In Malawi, late endline visits were allowed, to minimize loss to follow-up for primary outcomes. For this analysis, data are excluded if collected more than 28 days early or more than 42 days late. The rationale for allowing a wide tolerance is that feeding practices may change more slowly by 18 months of age compared to earlier time points. Also, in Malawi the endline clinic visit was planned to occur exactly one year after the date of enrollment. However, so long as it occurred within one month of the target date, the child received a 2 week supply of LNS. So, if the endline FFQ occurred within ~6 weeks (42 days) of target, the child should have had LNS in the week prior to the FFQ.

In Ghana, IYCF practices data were usually measured within a week of the infant turning 18 months of age. In instances where the mother/caregiver travelled, field workers were allowed to complete data collection up to one month from the scheduled date. This was rare at endline and there are no exclusions in Ghana.

In Burkina Faso, the target for endline visits was 39 +/- 2 weeks, after enrollment, with enrollment at age ~9 months. In special cases, early endline visits were allowed from 35 weeks onward (e.g. if the family planned to travel) and late visits were allowed up to 43 weeks after enrollment; as in Malawi, in these cases the child continued to receive LNS until the endline visit where IYCF practices were surveyed.

Outcomes:

1. Infant still breastfed at 18 months (%)
2. Infant breastfed 6 or more times yesterday (%)

3. Frequency of feeding adequate (WHO indicator, %)[3]
4. 4 or more food groups yesterday (WHO indicator; %)
5. Mean or median # ASF groups yesterday (of 5)[4]

[3] Burkina and DYAD-Malawi only.

[4] The 5 ASF groups are: 1) organ meats; 2) other meat/poultry; 3) fish; 4) eggs; and 5) dairy.

6. Mean or median # fruit/vegetable groups yesterday (of 5)[5]

7. Mean ASF score, last seven days (of 28)[6]
8. Lowest ASF score tertile (%)
9. Mean fruit/vegetable score, last seven days (of 35)[7]
10. Lowest fruit/vegetable score tertile (%)

5. Approach to analysis and exclusions specific to this analysis

All tests will be two-sided, at 5% level of significance.

If specific outcome variables are missing for more than 10% of infants (with denominator being total records available for the time point) we will report the number of observations used per specific outcome analysis.

Analysis will be by intention-to-treat.

Observations outside the tolerances for visit timing stated above will be excluded from analysis. There will be no other exclusions.

Note: Data available in the DYAD-Ghana trial are divided into three "periods" based on their relationship to an error in allocation of treatments. Women in "period 1" received the same supplement throughout pregnancy, though it was not the intended supplement (reversal of MMN and IFA groups); women in "period 2" received the incorrect supplement at enrollment, but started receiving the intended supplement at some point during the pregnancy; women in "period 3" received the correct supplement throughout pregnancy and lactation. At no point was LNS confused with the two tablets (IFA and MMN).

Note: For iLiNS-DOSE, comparisons will be made across groups receiving different quantities of LNS, but "milk" and "no milk" LNS of the same quantity (20g and 40g) will be grouped together.

[5] The 5 fruit and vegetables groups are: 1) vitamin A-rich orange/yellow vegetables; 2) dark green leafy vegetables; 3) other vegetables; 4) vitamin A-rich fruits; and 5) other fruits.

[6] Score sums four groups over seven days; groups are similar to those for yesterday, but organ meats and other flesh foods are grouped together.

[7] Score sums five groups over seven days; groups are the same as those for yesterday.

6. Statistical methods

5.1 Software

All analyses will be done using SAS version 9.3 (SAS Inst. Cary, NC, USA) or Stata version 10.1 or higher (StataCorp, TX, USA).

5.2 Background characteristics and loss to follow-up

Selected background characteristics will be examined for the analysis sample compared to those lost to follow-up. Differential attrition will be assessed with chi-square tests. The same background characteristics will be examined by group for analysis samples.

5.3 Analysis of the effect of the intervention

General comments:

a. The hypothesis stated in section 3 is a non-equivalence hypothesis. However, the study was not powered for IYCF practices outcomes and we are severely underpowered for equivalence analyses, particularly for dichotomous outcomes which comprise the majority of outcomes in this analysis. Therefore the more traditional approach in the nutrition literature of analyzing for significant differences will be followed in the first instance. This limitation will be clearly explained in the discussion section of any publication.

b. For quasi-continuous variables, we will supplement this with an equivalence approach to hypothesis testing, to help inform conclusions from this analysis (see below).

Analysis of the effect of the intervention will follow these steps:

a. In Ghana only, we will test group-by-period interactions for each outcome. In the absence of group-by-period interactions, observations from participants in all periods will be included in the analysis, and analysis will be performed both for groups as allocated (reflecting the supplement received during early lactation up to six months post-partum) and for groups based on first supplement received. If there are significant group-by-period interactions for a specified outcome, period 3 data only will be used for that outcome.

b. We will check for collinearity by running models with all covariates (see below) and examining variance inflation factors (VIF). VIF above 10 are problematic and one or more covariates will be removed after discussion of which to drop; this decision can be made considering subject matter and/or data constraints (e.g. number of missing values per covariate).

c. In each site, we will assess pre-specified covariates (see below) for relationship to each outcome. Covariates significantly associated with an outcome (criterion: $p <$

0.10) will be included in models for that outcome; final covariates can vary by outcome.

d. We will test the null hypothesis of no difference among the three treatment groups using ANCOVA or logistic regression, with and without controlling for significant covariates.

e. If the global null hypothesis is rejected at 0.05 level for any outcome, then we will perform post-hoc pairwise comparisons of all three groups (Ghana) or four groups (Malawi DOSE) using appropriate adjustments for multiple comparisons to examine contrasts of interest.

f. In the DYAD trials, if the IFA and MMN groups are not different for a specific outcome, we will test differences between LNS and non-LNS groups (IFA and MMN, grouped together) (using all data from periods 1-3 in Ghana). In iLiNS-DOSE, if the global null hypothesis and pairwise comparisons by dose are not significant, we will test differences between all LNS groups (together) and non-LNS group.

g. The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Each interaction will be assessed separately, in models including all significant covariates. In DYAD and DOSE trials, if two-group comparisons are tested under f., effect modifiers will be tested within two-group models only.

h. 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.

i. Confidence intervals will be adjusted for multiple comparisons.

j. Equivalence analysis: For quasi-continuous outcomes (number of time points with exclusive or predominant breastfeeding; number of fruit/vegetable groups consumed yesterday; number of animal-source food groups consumed yesterday, and fruit/vegetable and animal-source food scores for last week) equivalence will be assessed based on defined margins. Margins for food groups yesterday will be ±1.0 (one more or one fewer fruit/vegetable group yesterday; one more or one fewer animal-source food group yesterday). For scores for last week, the margin will be ±4 points (~= a difference of one fruit/vegetable or animal-source food group on four or more of the last seven days). We will assess equivalence in the context of ANCOVA models, controlling for the same pre-specified covariates as noted above. Equivalence will be determined to exist if the 90% confidence

interval for the difference between the means is entirely contained within the negative and the positive values of the equivalence margin. In DYAD and DOSE trials, if two-group comparisons are tested under f., equivalence testing will be between two groups.

5.5 Covariates in main effects models

In theory, a variety of community-, household-, maternal-, and child-level characteristics could affect child feeding practices independently of the intervention. Data are available for the covariates listed below.

All covariates are as measured at baseline, with the exception of season, and child sex (DYAD studies) and age. Season of measurement is included due to potential seasonal effects on access to diverse foods (and through this, on feeding practices).

Since child age at each visit can vary (see exclusions above), child age at time of measure (deviation from median age at endline) will be included as a potential covariate in models for all outcomes.

Before making final decisions on inclusion of covariates, completeness of data for the covariates will be considered and covariates will be excluded if loss of sample size is judged too large.

- Enrollment site (DYAD-Malawi only)
- Season of measurement (definition of season varies by site)
- Characteristics of households
    - Distance to nearest weekly market, in meters[8]
    - Baseline HH asset score[9]
    - Baseline HH small livestock score[10]
    - Baseline HH food security category from HFIAS[11]

[8] For continuous covariates highly skewed in one or more sites, we will test for trend by grouping the variables in 4 groups, and assigning the group median as the value for a new variable for each case in the group. This new variable will be the covariate. The grouping rule will be quartiles where possible; in cases where there is very heavy lumping on "0", the first group will be all 0's and groups 2-4 will be tertiles among the non-zero values.

[9] As above, for distance to market.

[10] As above.

[11] Categories as in Coates et al., 2007.

  - Number of under-fives in the HH at baseline (categorical variable, differing by site)[12]

- Characteristics of mother
  - BMI[13]
  - Age
  - Education (categorical variable, differing by site)
  - HIV status (DYAD-Malawi only; unknown in DOSE and ZINC; excluded if known to be positive in DYAD-Ghana)
  - Ethnicity or language (categorical variable, differing by site)
  - Marital status (categorical variable, differing by site)
- Child's characteristics
  - Child age (deviation from median age at endline)
  - Child sex
  - HAZ at baseline (DOSE and ZINC only)[14]
  - WHZ at baseline (DOSE and ZINC only)

5.6 List of potential effect modifiers to be examined

Most of the covariates identified will also be evaluated for their potential to interact with intervention group; exceptions are distance to market, maternal BMI, and child age deviation.

[12] A high proportion of data are missing for DYAD-Malawi, and parity at baseline (nulliparous Y/N) will be used instead.

[13] In DYAD studies: will use predicted BMI at 13.7 wk of gestation, for Malawi; BMI at enrollment for Ghana, because baseline BMI was not related to gestational age at enrollment (R-squared = 0.007).

[14] As DYAD interventions began antenatally, there are no baseline values for infant anthropometry.

7. Design of tables and figures

Tables will vary slightly by site reflecting different study designs and covariate categories. The tables listed below will be examined by the manuscript writing group, and final decisions on how to best consolidate results across sites for presentation in a manuscript will follow later.

Table 1. Comparison of analysis sample to those lost to follow-up

Table 2. Comparison of baseline characteristics and two concurrent covariates, by intervention group

Table 3. Associations of covariates to outcomes

Table 4. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values[15]

Figure 1. Participant flow

Additional Tables and Figures as needed to describe or illustrate interactions. For the manuscript, Table 4 may be supplemented by selected descriptive Figures for cross-site comparison of outcomes.

On the following pages, Tables show an example format from the DYAD-Ghana design.

[15] For DYAD-Ghana, this table will be presented for groups as assigned, groups based on first type of supplement received, and potentially also for a two-group comparison (LNS vs. non-LNS). For DYAD-Malawi, this will be presented for three groups and potentially also for a two-group comparison (LNS vs. non-LNS). For ZINC, this table will show a two-group comparison. For DOSE, this table will show a four-group comparison (0, 10, 20, and 40 g LNS groups) and potentially also a two-group comparison (LNS vs. non-LNS).

Table 1. Comparison of analysis sample to those lost to follow-up

		Lost to follow-up n=		**In analysis sample** n=		**All enrolled** n=			
		Mean/%	SD	Mean/%	SD	Mean/%	SD	P-value	Test
Distance to market	Score[a]								Chi-sq
Asset index	Score[a]								Chi-sq
Small livestock score	Score[b]								Chi-sq
HFIA category (%)									Chi-sq
	Food secure								
	Mildly food insecure								
	Moderately food insecure								
	Severely food insecure								
Other U5 at baseline (%)	Yes								Chi-sq
Maternal education (%)									Chi-sq
	None								
	Some 1° (1-5 y)								
	Completed 1°, some 2° (6-8 y)								
	Upper 2° (9-11 y)								
	Completed 2° or more (12+ y)								
Maternal age (y)									ANOVA
Maternal BMI (kg/m2)									ANOVA
Language spoken in HH (%)									Chi-sq
	Krobo/Ga								
	Ewe								
	Other								
Marital status (%)	Married								Chi-sq
Child sex (%)	Male								Chi-sq

[a] Source variables were highly skewed. Quartiles were created and the median score for the quartile was assigned to all households within each quartile.

[b] The source variable was heavily lumped on "0" and highly skewed. Four groups were created for zero, and tertiles of none-zero values. The median score for the group was assigned to all households in the group (0, or tertile medians).

Table 2. Comparison of baseline characteristics and two concurrent covariates, by intervention group (as assigned)

		IFA n=		**MMN** n=		**LNS** n=		**ALL** n=			
		Mean/ %	SD	Mean/ %	SD	Mean/ %	SD	Mean/ %	SD	P-value	Test
Season of interview (%)	Dry season										Chi-sq
Distance to market	Score[a]										Chi-sq
Asset index	Score[a]										Chi-sq
Small livestock score	Score[b]										Chi-sq
HFIA category (%)											Chi-sq
	Food secure										
	Mildly food insecure										
	Moderately food insecure										
	Severely food insecure										
Other U5 at baseline (%)	Yes										Chi-sq
Maternal education (%)											Chi-sq
	None										
	Some 1° (1-5 y)										
	Completed 1°, some 2° (6-8 y)										
	Upper 2° (9-11 y)										
	Completed 2° or more (12+ y)										
Maternal age (y)											ANOVA
Maternal BMI (kg/m2)											ANOVA
Main language in HH (%)											Chi-sq
	Krobo/Ga										
	Ewe										
	Other										
Marital status (%)	Married										Chi-sq
Child sex (%)	Male										Chi-sq
Age deviation (d)											ANOVA

[a] Source variables were highly skewed. Quartiles were created and the median score for the quartile was assigned to all households within each quartile.

[b] The source variable was heavily lumped on "0" and highly skewed. Four groups were created for zero, and tertiles of none-zero values. The median score for the group was assigned to all households in the group (0, or tertile medians).

Table 3. Associations of covariates to outcomes

		stillbf	bf_6	dd24GE4	asf24sum	asf24sum	frveg24sum	frveg24sum	asf7sum	frveg7sum	i18_asfT1	i18_frvegT1
		logit	logit	logit	ANOVA	OLOGIT	ANOVA	OLOGIT	ANOVA	ANOVA	logit	logit
Season of interview	dryseas											
Distance to market	distance											
Asset index	asset											
Small livestock score	smlivestock											
HFIA category	hfia_cat											
Other U5 at baseline	other_u5yn											
Maternal education	schooling											
Maternal age	moth_age											
Maternal BMI	mombmi											
Main language in HH	HHlanguage											
Marital status	marital											
Child sex	male											
Age deviation	age_dev											

P-values are from simple bivariate ANOVA or logit or ologit models. Covariates included in models testing for effect of group are:

Still breastfed (not reported fully weaned) (for each outcome, list covariates significant at P < 0.10)
Breastfed 6+ times/yesterday
4+ food groups yesterday (WHO indicator)
ASF food groups yesterday, range 0-5
fruit/veg food groups yesterday, range 0-5
ASF score last 7 d, range 0-28
Fruit/vegetable score last 7 d, range 0-35
Lowest tertile for 7 d ASF score
Lowest tertile for 7 d fruit/vegetable score

Table 4a. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values - groups as assigned[a]

	IFA (n=)		MMN (n=)		LNS (n=)		ALL (n=)			
	Proportion or mean	SD	Proportion or mean	SD	Proportion or mean	SD	Proportion or mean	SD	Unadjusted p-value[b]	Adjusted p-value[c]
Still breastfed										
Breastfed 6+ times/yesterday										
4+ food groups yesterday (WHO)[d]										
# ASF food groups yesterday, range 0-5[e]										
# fruit/veg food groups yesterday, range 0-5[f]										
ASF score last 7 d, range 0-28[g]										
Fruit/veg score last 7 d, range 0-35[h]										
Lowest tertile for 7 d ASF score										
Lowest tertile for 7 d fruit/veg score										

Table 4b. Outcomes at 18 months, unadjusted proportions or means, unadjusted and adjusted P-values - "flipped" groups (as received at enrollment for IFA and MMN)

Still breastfed										
Breastfed 6+ times/yesterday										
4+ food groups yesterday (WHO)[d]										
# ASF food groups yesterday, range 0-5[e]										
# fruit/veg food groups yesterday, range 0-5[f]										
ASF score last 7 d, range 0-28[g]										
Fruit/veg score last 7 d, range 0-35[h]										
Lowest tertile for 7 d ASF score										
Lowest tertile for 7 d fruit/veg score										

Table 4c.Two-group analyses: Outcomes at 18 months, unadjusted proportions and means, unadjusted and adjusted P-values - groups as assigned

	IFA + MMN (n=)		LNS (n=)		ALL (n=)			
	Proportion or mean	SD	Proportion or mean	SD	Proportion or mean	SD	Unadjusted p-value[b]	Adjusted p-value[c]
Still breastfed								
Breastfed 6+ times/yesterday								
4+ food groups yesterday (WHO)[d]								
# ASF food groups yesterday, range 0-5[e]								
# fruit/veg food groups yesterday, range 0-5[f]								
ASF score last 7 d, range 0-28[g]								
Fruit/veg score last 7 d, range 0-35[h]								
Lowest tertile for 7 d ASF score								
Lowest tertile for 7 d fruit/veg score								

[a] IFA=iron folic acid group (standard care); MMN=multiple micronutrient group; LNS=lipid-based nutrient supplement group; ASF=animal-source food.

[b] P-values from ANOVA and LOGIT models for dichotomous. P-values for OLOGIT for food groups yesterday were similar and all NS.

[c] Models adjusted for all significant covariates in bivariate models, see above.

[d] At least 4 out of the following 7 food groups: grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A rich fruit and vegetables; other fruits and vegetables.

[e] The 5 ASF groups are: 1) organ meats; 2) other meat/poultry; 3) fish; 4) eggs; and 5) dairy.

[f] The 5 fruit and vegetables groups are: 1) vitamin A-rich orange/yellow vegetables; 2) dark green leafy vegetables; 3) other vegetables; 4) vitamin A-rich fruits; and 5) other fruits.

[g] Score sums four groups over seven days; groups are similar to those for yesterday, but organ meats and other flesh foods are grouped together.

[h] Score sums five groups over seven days; groups are the same as those for yesterday.

Table 5. Evaluation of effect modification – significance of interaction terms

		stillbf	**bf_6**	**dd24GE4**	**asf24sum**	**asf24sum**	**frveg24sum**	**frveg24sum**	**asf7sum**	**frveg7sum**	**i18_asfT1**	**i18_frvegT1**
		logit	logit	logit	ANOVA	OLOGIT	ANOVA	OLOGIT	ANOVA	ANOVA	logit	logit
Season of interview	dryseas											
Asset index	asset											
Small livestock score	smlivestock											
HFIA category	hfia_cat											
Other U5 at baseline	other_u5yn											
Maternal education	schooling											
Maternal age	moth_age											
Main language in HH	Hhlanguage											
Marital status	marital											
Child sex	male											