Study design

This study forms part of a larger community-based randomized controlled trial in Ghana, the International Lipid-Based Nutrient Supplements-DYAD (iLiNS-DYAD) Study, which aims to evaluate the effects of a lipid-based nutrient supplement (LNS) on the nutritional status of Ghanaian pregnant and lactating women, and the effects of LNS on the growth and nutritional status of their children.

Study participants are pregnant women attending antenatal clinics in select hospitals/clinics in the Yilo Krobo and Manya Krobo Districts of the Eastern Region of Ghana. Women who meet an established set of inclusion criteria are recruited and randomly assigned to one of three intervention groups, as shown in Table 1 below.

Table 1. Intervention groups in the iLiNS-DYAD Ghana Study

Group	Pregnant women	Lactating women
1	Daily iron (60mg) and folic acid (400 µg) tablet during pregnancy (IFA)	Daily calcium tablet (placebo) during first 6 months of lactation
2	Daily multiple micronutrient tablet during pregnancy (MMN)	Daily MMN tablet during first 6 months of lactation
3	Daily lipid-based nutrient supplement during pregnancy (LNS)	Daily LNS during first 6 months of lactation

For the present study, there will be regular follow-up of the women from enrolment (≤ 20 weeks of gestation) through delivery and the first 6 months of lactation.

Data collection

Background demographic information is collected from the women at enrolment using a semi-structured questionnaire. Information on maternal intake of vitamin A-rich foods and oils fortified with vitamin A is collected at enrolment, at 36 weeks gestation and 6 months postpartum, using food frequency questionnaires. Information on maternal intake of nutrient supplements containing vitamin A is collected biweekly including baseline, 36 weeks gestation and 6 months postpartum. Data on intake of high dose postpartum vitamin A supplements are collected at delivery and at 8-10 weeks after birth. Data on adherence are recorded biweekly by interview of participants and verified by collection and count of remaining intervention supplements.

Maternal blood samples are collected at baseline, 36 weeks of gestation and 6 months postpartum for the analysis of the acute phase proteins C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP). Breast milk samples are collected from the women at 6 months postpartum for the analysis of breast milk retinol and fat.

All samples are collected using standard procedures. Breast milk retinol will be analyzed using reverse-phase high performance liquid chromatography (HPLC); breast milk fat will be analyzed using the creamatocrit method; and serum CRP and AGP will be analyzed using an auto-analyzer (based on a turbidimetric assay).

General statistical procedures

Data cleaning

The initial rounds of data cleaning are being performed at the Ghana field site. Additional data cleaning will be performed before statistical analysis by generating graphical plots of distribution of variables (histograms, stem and leaf plots, scatterplots) to look for the normal distribution of variables and to determine if there are outliers. Queries will be communicated to the Ghana field site for clarification and correction.

Modifying analysis plan

When new hypotheses arise out of new data that have been collected or added, addendums will be made to the analysis plan, with clear documentation of their rationale.

Analysis principles

Analysis will be by intention-to-treat. Results for all women enrolled will be analyzed according to the group to which they were assigned regardless of any protocol violations. Data on subjects who were lost to follow-up or refusal to continue with the study will be included in the analysis if available.

In addition, a per protocol analysis will be performed including subjects meeting minimum criteria for adherence to study protocol. Good adherence will be defined as consumption on $\geq$ 70% of supplement days and minimum adherence will be defined as consumption on > 50% of supplement days.

Blinding

All initial statistical analyses will be performed with blinding to the group assignments; dummy codes will be assigned to the different intervention groups. Blinding will be maintained until the final results are released by the study statistician.

Software

All statistical analyses will be done using SAS, version 9.3 (SAS Institute, Cary, NC).

Paper 1: Effect of lipid-based nutrient supplements (LNS) providing daily low doses of vitamin A during pregnancy and lactation on maternal breast milk retinol concentration at 6 months postpartum, and interactions with high dose postpartum maternal vitamin A supplementation

Objectives

Examine the effect of LNS containing daily low doses of vitamin A given to Ghanaian pregnant and breastfeeding women on breast milk retinol levels at 6 months postpartum; and the interaction of treatment group with high dose postpartum maternal vitamin A supplementation on maternal breast milk retinol levels at 6 months postpartum.

Hypothesis

1. Ghanaian women receiving lipid-based nutrient supplements (LNS) or multiple micronutrient supplements (MMN) providing a daily low dose of vitamin A (800 µg RE) during pregnancy and the first 6 months of lactation will have significantly higher mean breast milk retinol levels at 6 months postpartum, compared to women who do not receive daily low dose vitamin A during pregnancy and the first 6 months of lactation.

2. Mean breast milk retinol levels at 6 months postpartum among Ghanaian women in each of the 3 intervention groups will not be affected by intake of high dose postpartum vitamin A supplements.

Primary outcome

Maternal breast milk retinol concentration (µmol/g fat) at 6 months postpartum.

Data analyses

Background and demographic information will be presented by treatment group. A list of the variables of interest is presented in table 2. Means and standard deviations, or medians and ranges, will be presented for continuous variables; frequencies and percentages will be calculated for categorical variables, as outlined in table 3.

Plots will be made of the distribution of variables using histograms, stem and leaf plots, and scatterplots to look for outliers and normal distribution of variables. Shapiro-Wilk test will be used to assess normality. Skewed data will be transformed to normalize distributions prior to statistical testing.

ANOVA or ANCOVA will be used to compare breast milk retinol levels at 6 months postpartum among the three intervention groups; means and 95 % confidence intervals (CI) will be reported, as shown in table 4, for both unadjusted and adjusted data.

Interactions between daily low dose and high dose postpartum vitamin A on breast milk retinol will be assessed using ANOVA or ANCOVA, with treatment group and intake of high dose postpartum vitamin A supplements as main effects, and an interaction term included in the model. If the interaction term is significant (at 10% level of significance), the direction of the relationship between breast milk retinol and

intervention group will be assessed separately among women receiving high dose postpartum vitamin A and those who do not.

Table 2. List of variables, potential covariates and effect modifiers

Dependent / outcome variable	**Covariates**	**Effect modifiers**
Breast milk retinol concentration at 6 months postpartum	High dose vitamin A supplementation postpartum	High dose vitamin A supplementation postpartum
	Household food insecurity	Household food insecurity
	Household socio-economic status	Household socio-economic status
	Primiparity	Primiparity
	Maternal BMI at enrolment (adjusted for gestational age)	Maternal BMI at enrolment (adjusted for gestational age)
	Hemoglobin concentration at baseline	Hemoglobin concentration at baseline
	Gestational age at enrolment	Gestational age at enrolment
	Maternal age	Maternal age
	Maternal education	Maternal education
	Season at enrolment being dry season (Nov-Apr)	Season at enrolment being dry season (Nov-Apr)

Table 3. Baseline characteristics of study participants by intervention group

Variable	**IFA* n=**	**MMN* n=**	**LNS* n=**	**p-value**
Age	Mean (±SD)	Mean (±SD)	Mean (±SD)	
Socioeconomic status	Mean (±SD)	Mean (±SD)	Mean (±SD)	
Years of formal education	Mean (±SD)	Mean (±SD)	Mean (±SD)	
Initial body mass index (BMI)	Mean (±SD)	Mean (±SD)	Mean (±SD)	
Primiparity	%	%	%	
Season at enrolment being dry season	%	%	%	

*Intervention groups: IFA=Iron and Folic Acid; MMN=Multiple Micronutrient Supplement; LNS=Lipid-based Nutrient Supplement

Table 4. Comparison of breast milk retinol levels at 6 months postpartum among intervention groups

	IFA n=	**MMN n=**	**LNS n=**	**p-value**	**Comparison of IFA and MMN**		**Comparison of IFA and LNS**		**Comparison of MMN and LNS**	
					Difference in means (95% CI)	p-value	Difference in means (95% CI)	p-value	Difference in means (95% CI)	p-value
Breast milk retinol (µmol/L)	Mean (±SD)	Mean (±SD)	Mean (±SD)							
Breast milk retinol (µg/g fat)	Mean (±SD)	Mean (±SD)	Mean (±SD)							