The effects of supplementing maternal and infant diets with micronutrient fortified lipid-based nutrient supplements on physical activity and sedentary behavior at preschool age in Ghana

Statistical Analysis Plan

DECEMBER 15, 2017

UC DAVIS

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	July-25-17	Maku Ocansey	Original document
2	Dec-15-2017	Maku Ocansey	Modified hypothesis, power calculation and statistical analysis to include three intervention group comparisons

1.0 Introduction

Childhood undernutrition is associated with reduced physical activity, which leads to an increased risk of obesity in childhood and adulthood (Guthold et al., 2010). Undernourished children have been shown to be less physically active than their well-nourished counterparts (Grantham-McGregor & Baker-Henningham, 2005), however increases in activity levels have been shown with improvements in nutritional status during childhood (Faurholt-Jepsen et al., 2014). In addition to the physical health benefits, physical activity has also been strongly associated with motor development in undernourished children in the first year of life even though the strength of the association is seen to reduce with age (Meeks-Gardner at al. 1995; Jahari et al., 2000). Physical activity may be associated with improvements in the emotional, social, and cognitive well-being of young children, as it involves gross motor play, the use of imagination and problem solving skills and fosters social interaction with the environment (Burdette & Whitaker 2005).
Although there is some evidence on the positive associations of nutrition or health outcomes and physical activity, evidence for the effects of micronutrient supplementation prenatally and during early childhood on behavioral physical activity have been mixed (Tofail et al., 2008; Aburto et al., 2010; Sazawal et al., 1996; Bentley et al., 1997; Jahari et al., 2000; Meeks-Gardner at al. 1995; Pulakka et al., 2014; Pulakka et al., 2017).

The International Lipid-based Nutrient Supplement (iLiNS) DYAD-Ghana study was a randomized, partially double blind, controlled trial conducted from 2009-2014 in the Yilo and Manya Krobo districts of Eastern Ghana, to test the efficacy of three types of micronutrient supplements for preventing malnutrition in pregnant and lactating women and their infants. The three supplementation strategies were (1) lipid-based nutrient supplements (LNS) provided to women during pregnancy and for 6 months postpartum, and to their infants from 6 to 18 mo of age, (2) maternal multiple micronutrient (MMN) supplements during pregnancy and 6 months postpartum, and (3) maternal iron and folic acid (IFA) during pregnancy and calcium placebo tablet during 6 months of lactation. Children in the latter two groups received no supplement during infancy.

From January to December 2016, we conducted a follow-up study of children who participated in the main iLiNS trial and were by then 4-6years old, to investigate the long-term effects of the

intervention on health and neurodevelopmental outcomes. The aim of this SAP is to describe the analysis to investigate the long-term effects of maternal lipid-based nutrient supplementation (LNS) during pregnancy and 6 months postpartum and infant supplementation from 6 to 18 months of age on the physical activity (PA) and sedentary behavior (SB) at age 4-6 y measured using accelerometers. We hypothesized that LNS supplementation during pregnancy, postpartum and infancy would increase PA and lower SB at 4-6 y compared to maternal iron and folic acid supplementation during pregnancy only, or multiple micronutrient supplementation during pregnancy and through 6 months postpartum.

1.1 Study site, participants, inclusion and exclusion criteria

This follow-up study was conducted in the Yilo and Manya Krobo districts of Eastern Ghana, where the iLiNS DYAD-G1 study took place from 2009-2014. To be eligible for follow-up, all mother-child dyads who took part in the main trial and were willing to participate were included. Mother-child dyads were excluded if mother or caregiver was unwilling to consent to participation or were not residing within the study site (Yilo and Manya Krobo districts) or surrounding towns at a travel distance costing no more than GHc 60 ($ 15) to the study site round trip.

For the present analysis on physical activity, we randomly selected a sub-sample of mother-child dyads eligible for the follow-up study, ensuring that the number of children in the IFA and MMN groups were balanced within the combined (non-LNS) group.

2.0 Study objective

For the analyses described here, the aim is to investigate the long-term effect of LNS on physical activity and sedentary behavior outcomes at preschool age (4-6 yr).

2.1. Specific aims

The specific aims of these analyses are to compare children in the three intervention groups on the following outcomes:

Primary outcome

1. *Daily mean vector magnitude counts per minute (VMCPM)*

The mean vector magnitude (VM) counts per minute in the total sample will be calculated using the Actilife data analysis software v6.13.1, which calculates the mean VM as the square root of the sum of squared activity counts of three axes. We will estimate the difference (95% CI) in mean accelerometer counts/min between the intervention group and the control group.

Secondary outcomes

1. *Percentage of time spent in moderate-to-vigorous physical activity (MVPA)*
   This will be defined using validated cutoff points (for children 5-8 yrs) of vertical axis activity counts of ≥ 574 counts/15 s (Evenson et al., 2008). This will be averaged over all valid days within a 7-day period and the averaged value (per participant) will be used in the analysis.
2. *Percentage of time spent being sedentary (SED)*
   We will define sedentary time using validated cutoff points (for children 5-8 yrs) as vertical axis activity counts ≤ 25 counts/15 s (Evenson et al., 2008).This will be averaged over all valid days and the average value (per participant) will be used in the analysis.
3. *Percentage of active children in the sample*
   The proportion of children whose mean time in MVPA over all valid days is ≥60 minutes will be considered active, based on the guidelines of the U.S. National Association for Sports and Physical Education (NASPE, 2009) and the WHO global recommendations for physical activity and health (WHO, 2010).

3.0 Definition of outcomes

For both primary and secondary outcomes:

Intensity-specific PA and SED behavior will be reported as min/day and as percentage values of valid wear time.

4.0 Hypotheses

We hypothesize that:

a). Children in the LNS group would have higher mean daily VMCPM compared to children in the MMN or IFA groups.
b). Children in the LNS group would have greater minutes per day spent in moderate-to-vigorous physical activity and lower minutes per day spent in sedentary behavior compared to children in the MMN or IFA groups.
c). A greater proportion of children in the LNS group would reach 60 min of MVPA/day compared to children in the MMN or IFA groups.

5.0 Blinding

All participants were outfitted with a single Actigraph accelerometer to monitor and measure all outcomes. This was done by trained data collectors blinded to group assignment of the children.

6.0 Power calculations

To detect an effect size of 0.33 SD or greater in mean vector magnitude (VM) counts/min, which was the primary outcome of this sub-study, assuming 3 intervention groups, a power of 80% and alpha of 0.05 requires 132 per group, summing up to 396 children needed for this physical activity sub study.

7.0 Analysis principles

Analysis will be performed by intention-to-treat and results will be analyzed according to the group to which participants were originally assigned regardless of whether they got the treatment or if they followed protocol. We will include data on participants lost to follow-up or who refused to continue the study if available.

8.0 Statistical analysis

8.1. Software

Data will be processed using the Actilife wear-time validation, scoring and sleep detection tools in the Actilife v6.13.3 software. All statistical analyses will be done using SAS version 9.4 (SAS Inst. Cary, NC, USA).

8.2 *Data processing*

Data reduction

Activity counts for each 1-min interval will be uploaded to the Actilife software program to determine total counts per hour of monitoring and the number of intervals of sedentary, light, moderate-to-vigorous and vigorous physical activity per hour. We will consider a day valid when there is a minimum of ten (10) hours of accelerometer data/day.

Sleep/ non-wear time

We will define non-wear time as strings (consecutive periods) of ⩾20 min of zero counts. Only children with ⩾ 3 valid days (minimum of 2 weekdays and 1 weekend day) of data will be included in the analyses.

For sleep analysis, age-specific count cutoffs corresponding to sleep period will be derived from the algorithm sleep prediction equation developed by Sadeh et al., 1994 and sleep period detection option created by Tudor-Locke et al, 2013 to calculate each epoch as asleep or awake.

9.0 Background characteristics by intervention group

For some maternal and child variables measured, the available values at the time of screening, recruitment or enrolment in the initial study (e.g. baseline maternal BMI, education), will be considered as background characteristics. For other variables, the available values at time of enrollment into the current follow-up study (child age, child height etc.) will be considered as background characteristics and will be presented in a table, by the three treatment groups. Group characteristic comparisons will be described based on several socio-demographic variables, using

frequencies and percentages to summarize categorical variables and mean and standard deviations for continuous variables. We will compare children fitted with accelerometers during follow-up with those unavailable for accelerometer fitting even though they were randomly selected for this sub-study, on sociodemographic characteristics.

10.0 Main *effect of intervention*

For all outcomes, an ANCOVA model, following intention-to-treat principles will be used to examine the effects of the LNS intervention on mean *VMCPM*, MVPA and sedentary behavior. We will adjust for potential covariates listed below. We will first test the null hypothesis of no difference between the three intervention groups using ANCOVA for continuous outcomes and logistic regression for binary outcomes. For all analyses, post-hoc pairwise comparisons of the three intervention groups will be performed using Tukey-Kramer adjustment for continuous or binary variables. We will define significant pairwise comparisons as p-value < 0.05. If there are no significant differences between the IFA and MMN groups, we will combine the groups into a single control (non-LNS) group to estimate two-group comparisons (LNS vs control).

We will compare groups with two models: one model unadjusted for covariates and the second model will be minimally adjusted any baseline, follow-up or other factors listed below in section 12 that are statistically significantly associated at the p<0.1 level with each of the outcomes. For any covariates that were collected after baseline, we will first check whether they are different between groups before including in the model since they could be potential mediators. Tests will either be one-sided (main outcome comparisons) or two-sided (baseline comparisons) and at 5% level of significance, except as otherwise stated.

11.0 Exploratory analysis

Besides the overall averages in all outcomes we will also assess the differences in VMCPM, MVPA and SED time between groups separately on:

1. Weekdays (Monday through Friday)
2. Weekends (Saturday and Sunday)

12.0 Potential covariates

The following covariates are to be included in the ANCOVA or logistic regression models when they show a statistically significant association with the outcome (P<0.1):

Factors collected at baseline:

1. Birth order
2. Maternal age
3. Maternal education
4. Household asset index
5. Household food insecurity index

Factors collected at follow-up:

1. Child age
2. Child sex
3. Preschool attendance
4. Season of activity measurement

13.0. Potential effect modifiers

The following variables will be examined as potential effect modifiers with an interaction term in the ANCOVA model:

Factors collected at baseline:

1. Child sex
2. Maternal age
3. Maternal education
4. Primiparity
5. Household asset index

Factors collected at follow-up:

6. Home stimulation composite score
7. Maternal depression
8. Child LAZ
9. Child WLZ

We will test the interaction between the effect modifiers and intervention groups. Significant interactions ($p < 0.1$) 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.

References

Guthold R, Cowan MJ, Autenrieth CS, Kann L, Riley LM. Physical activity and sedentary behavior among schoolchildren: a 34-country comparison. J Pediatr 2010;157(1):43-9 e1. doi: 10.1016/j.jpeds.2010.01.019.

Grantham-McGregor S, Baker-Henningham H. Review of the evidence linking protein and energy to mental development. Public health nutrition 2005;8(7a):1191-201.

Faurholt-Jepsen D, Hansen KB, van Hees VT, Christensen LB, Girma T, Friis H, Brage S. Children treated for severe acute malnutrition experience a rapid increase in physical activity a few days after admission. J Pediatr 2014;164(6):1421-4. doi: 10.1016/j.jpeds.2014.02.014.

Meeks Gardner J, Grantham-McGregor SM, Chang SM, Himes JH, Powell CA. Activity and behavioral development in stunted and nonstunted children and response to nutritional supplementation. Child development 1995;66(6):1785-97.

Jahari A, Saco-Pollitt C, Husaini M, Pollitt E. Effects of an energy and micronutrient supplement on motor development and motor activity in undernourished children in Indonesia. European journal of clinical nutrition 2000;54:S60-8.

Burdette HL, Whitaker RC. Resurrecting free play in young children: looking beyond fitness and fatness to attention, affiliation, and affect. Arch Pediatr Adolesc Med 2005;159(1):46-50.

Tofail F, Persson LA, Arifeen SE, Hamadani JD, Mehrin F, Ridout D, Ekstrom E-C, Huda SN, Grantham-McGregor SM. Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. American Journal of Clinical Nutrition 2008;87:704-11.

Aburto NJ, Ramirez-Zea M, Neufeld LM, Flores-Ayala R. The effect of nutritional supplementation on physical activity and exploratory behavior of Mexican infants aged 8-12 months. European Journal of Clinical Nutrition 2010;64:644-51.

Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. Pediatrics 1996;98(6 Pt 1):1132-7.

Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA, Ruel MT, Brown KH. Zinc supplementation affects the activity patterns of rural Guatemalan infants. Journal of Nutrition 1997;127(7):1333-8.

Jahari AB, Saco-Pollitt C, Husaini MA, Pollitt E. Effects of an energy and micronutrient supplement on motor development and motor activity in undernourished children in Indonesia. Eur J Clin Nutr 2000;54 Suppl 2:S60-8.

Meeks Gardner J, Grantham-McGregor SM, Chang SM, Himes JH, Powell CA. Activity and behavioral development in stunted and nonstunted children and response to nutritional supplementation. Child development 1995;66(6):1785-97.

Pulakka A, Ashorn U, Cheung Y, Dewey K, Maleta K, Vosti S, Ashorn P. Effect of 12-month intervention with lipid-based nutrient supplements on physical activity of 18-month-old Malawian children: a randomised, controlled trial. European journal of clinical nutrition 2014.

Pulakka, A, Cheung, YB, Maleta, K, Dewey, KG, Kumwenda, C, Bendabenda, J, Ashorn, U, Ashorn, P. Effect of 12-month intervention with lipid-based nutrient supplement on the physical activity of Malawian toddlers: a randomised, controlled trial. British Journal of Nutrition. 2017.

Lozoff B, Klein NK, Nelson EC, et al. (1998) Behavior of infants with iron-deficiency anemia. Child Dev 69, 24–36.

National Association for Sport and Physical Education (2009) Active Start: A Statement of Physical Activity Guidelines for Children from Birth to Age 5, 2nd ed. Sewickley, PA: American Alliance for Health, Physical Education, Recreation and Dance.

World Health Organization. 2010. Global recommendations on physical activity for health. WHO Press, World Health Organization. Switzerland.

Sadeh, A. Sharkey, K. M. Carskadon, M. A. Activity-based sleep-wake identification: an empirical test of methodological issues. Sleep. 1994.

Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG: Calibration of two objective measures of physical activity for children. J Sports Sci 2008, 26:1557–1565.

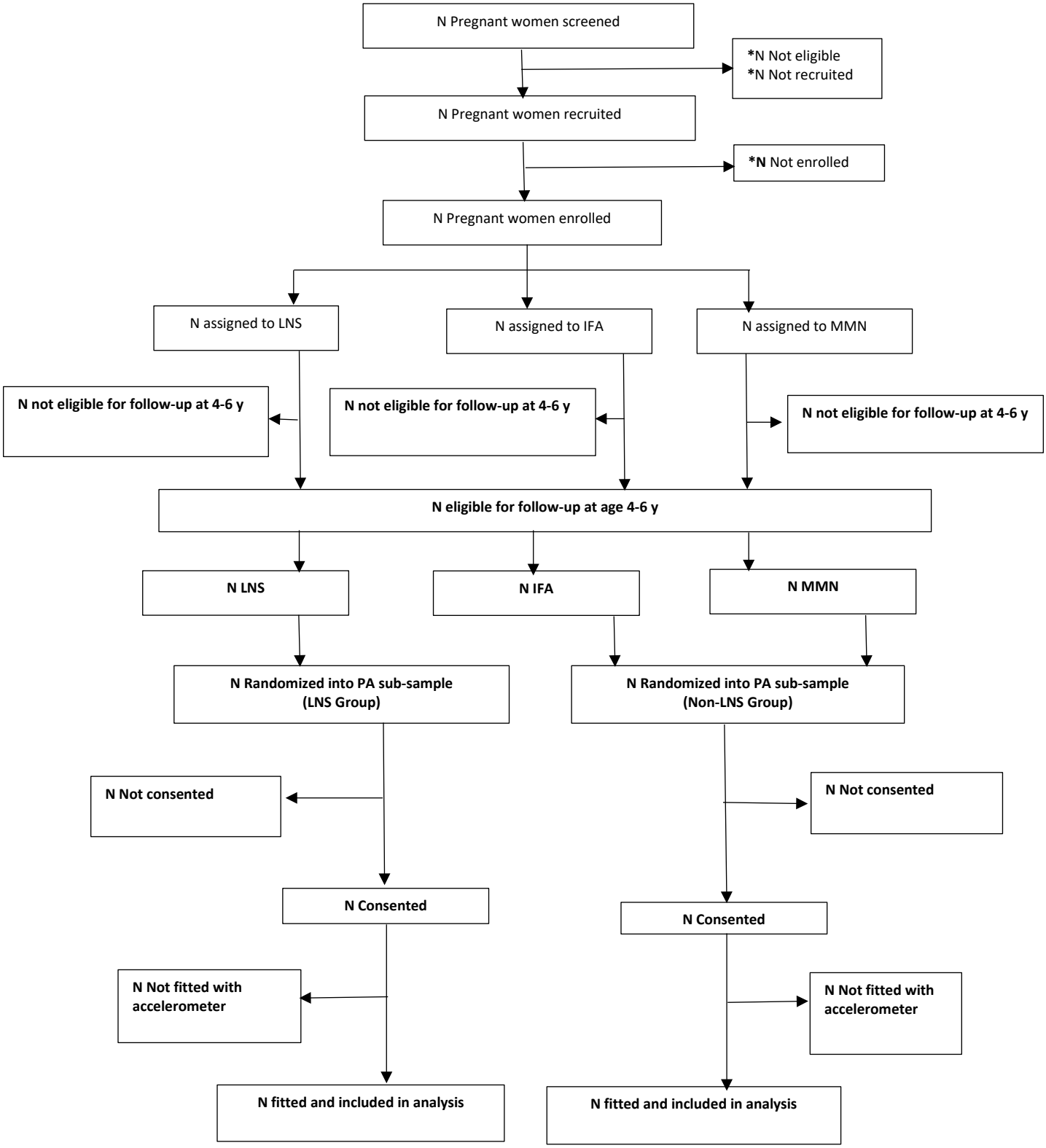

FIGURE 1 Study profile.
LNS, lipid-based nutrient supplement; IFA, iron and folic acid; MMN, multiple micronutrients.
LNS group, women received 20 g LNS daily during pregnancy and 6 mo lactation. Infants received 20 g LNS daily from 6-18 mo of age; Non-LNS group, women received either IFA during pregnancy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

Table 1: Selected characteristics of women and children by intervention group at baseline & follow-up

Variable	LNS	Non-LNS	
	n	n	
	Mean ± SD [n] or % [n/total]	Mean ± SD [n] or % [n/total]	P-value
Baseline maternal age (y)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Baseline maternal education (y)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Baseline household asset score[1]	x.xx ± x.xx [xxx]	x.xx ± x.xx [xxx]	x.xxx
Primiparous (%)	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Child male (%)	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Birth wt (kg)	x.xx ± x.xx [xxx]	x.xx ± x.xx [xxx]	x.xxx
Age at follow-up (y)	x.xx ± x.xx [xxx]	x.xx ± x.xx [xxx]	x.xxx
LAZ @ 6mo	x.xx ± x.x [xxx]	x.xx ± x.x [xxx]	x.xxx
WLZ @ 6mo	x.xx ± x.x [xxx]	x.xx ± x.x [xxx]	x.xxx
Child Hb @ 6mo (g/L)	xxx.x x.x [xxx]	xxx.x x.x [xxx]	x.xxx
LAZ @ 4-6y	x.xx ± x.x [xxx]	x.xx ± x.x [xxx]	x.xxx
WLZ @ 4-6y	x.xx ± x.x [xxx]	x.xx ± x.x [xxx]	x.xxx
Child Hb @ 4-6y	xxx.x x.x [xxx]	xxx.x x.x [xxx]	x.xxx
Season of physical activity measurement (%)			
Season 1	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Season 2	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Season 3	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Season 4	xx.x [xxx/xxx]	xx.x [xxx/xxx]	x.xxx
Home stimulation score at 4-6y	x.xx ± x.x [xxx]	x.xx ± x.x [xxx]	x.xxx

LNS=Lipid-based Nutrient Supplement. Non-LNS= Iron & folic acid +multiple micronutrient capsules (control group).

[1]Proxy indicator for household socioeconomic status ; higher value represents higher socioeconomic status

Hb= Hemoglobin level. LAZ = Length-forage z score. WLZ= weight-for-length z score

Table 2: Physical activity at 4-6 years by intervention group

	Treatment group		Comparison between groups		
Variables	**LNS n= Mean (SD)**	**Non-LNS n= Mean (SD)**	**Mean (95% CI)**	**Unadjusted P-value**	**P-value adjusted for baseline and other covariates**
Vector magnitude (counts/min)	xxx (xx)	xxx (xx)	x(x to x)	x.xxx	x.xxx
Time in MVPA (%)	xxx (xx)	xxx (xx)	x (x to x)	x.xxx	x.xxx
Time in SED (%)	xxx (xx)	xxx (xx)	x (x to x)	x.xxx	x.xxx

LNS=Lipid-based Nutrient Supplement. Non-LNS= Iron & folic acid +multiple micronutrient capsules (control group)

MVPA= moderate-to-vigorous physical activity. SED= Sedentary behavior