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

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1.0 Introduction

Childhood undernutrition is associated with reduced physical activity, which may lead 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 (Yaméogo et al., 2017; Grantham-McGregor & Baker-Henningham, 2005), and increases in activity levels have been shown with improvements in nutritional status during childhood (Faurholt-Jepsen et al., 2014). When energy intakes are reduced, physical activity may in turn be decreased to preserve energy (Waterlow et al., 1990; Shetty et al., 1999) for other metabolic processes to take place. Additionally, micronutrient deficiencies, particularly iron deficiency have been associated with reduced physical activity in early childhood (Olney et al., 2007; Lozoff et al., 1998).

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 declines with age (Meeks-Gardner at al. 1995; Jahari et al., 2000), and 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 influence of nutrition and health status on physical activity, evidence for the effects of micronutrient supplementation prenatally and during early childhood on behavioral physical activity has 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 nutrient 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 postpartum. 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-DYAD trial and were by then 4-6 years 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 effect of LNS on the physical activity (PA) and sedentary (SED) behavior of the children at preschool age.

The LNS provided to mothers during pregnancy and while breastfeeding, and directly to children from age 6 to 18 months, contained 22 micronutrients, essential fatty acids, proteins and energy, which are critical for growth and brain development. Since children in the MMN and IFA groups did not directly receive supplementation, our main objective was not to compare the MMN and IFA groups, so we combined the two groups into a non-LNS control group.

We hypothesize that the children in the intervention group would have greater physical activity and lower sedentary time than children in the control group.

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 trial 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 was 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 ensuring that the number of children in the IFA and MMN groups were similar within the combined (non-LNS) group. To be eligible for this sub-study, we considered only live births from the main trial who completed the study at age 18 mo. Children who were no longer residing in the study area at the time of follow-up were excluded.

1.2 Measurement and definition of outcomes

All participants were outfitted with a single Actigraph GT3X accelerometer to monitor and measure physical activity over a 1 week period day and night. This was done by trained data collectors blinded to group assignment of the children. Devices were fitted to an elastic belt and fastened to children's right hip during home visits and caregivers where instructed to allow children to wear them continuously day and night unless they experienced discomfort. Activity counts were stored in 60-s intervals or epochs.

Intensity-specific physical activity and sedentary behavior will be reported as percentage values of valid waking wear time.

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 y).

2.1. Specific aims

The specific aims of these analyses are to compare children in the LNS versus control groups on the following outcomes:

Primary outcome:

1. *Mean vector magnitude counts per minute (VM counts/60 s) during waking hours*

   The mean vector magnitude (VM) counts/60 s per participant 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 average mean counts/60 s of each day over all valid days for each child. The difference (95% CI) in mean accelerometer counts/60 s between the intervention group and the control group will then be estimated.

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 y) of vertical axis activity counts of ≥ 2,0296 counts/60 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*

   We will define SED time using validated cutoff points (for children 5-8 y) as vertical axis activity counts ≤ 100 counts/60 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 (or median) time in MVPA over all valid days is ≥60 min/d 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).

2.2 Hypotheses

We hypothesize that:

a). Children in the LNS group would have higher mean VM counts/60 s averaged across all valid days compared to children in the control group.

b). Children in the LNS group would have greater average percent of time spent in moderate-to-vigorous physical activity across all valid days and lower average percent of time spent in SED behavior across all valid days compared to children in the control group.

c). A greater proportion of children in the LNS group would reach an average of 60 min of MVPA/day across all valid days compared to children in the control group.

3.0 Power calculations

To detect an effect size of 0.3 (difference between groups, divided by the pooled SD), assuming 2 groups (LNS vs non-LNS (MMN+IFA)), a power of 80% and alpha of 0.05 requires 176 per group, summing up to 352 children. Allowing for up to 10% attrition, approximately 390 participants were needed for this physical activity sub study. This calculation is based on the hypothesis that the children in the LNS group who were supplemented for a period of 1 year during infancy would have greater physical activity than those in the non-LNS group who were not supplemented during infancy.

4.0 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.

5.0 Statistical analysis

5.1. 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).

5.2 *Data processing*

Data reduction

1. Processing raw data into counts per minute

   The raw accelerometer data will be uploaded to the Actilife software program and converted into vertical axis counts/60 s and vector magnitude counts/60 s.

2. Defining waking time and (excluding sleep time)

   First we will exclude sleep time defined by using the sleep prediction equation developed by Sadeh et al., (1994) and sleep period detection option created by Tudor-Locke et al., (2013).

3. *Excluding non-wear time*

   We will exclude non-wear time defined as strings (consecutive periods) of ⩾20 min of zero counts.

4. Excluding non-valid days

   We will consider a day valid when there is a minimum of ten (10) hours of accelerometer data/day. Only children with ⩾ 3 valid days (minimum of 2 weekdays and 1 weekend day) of data will be included in the analyses.

5. Calculating outcomes

   The reduced data will be used to determine the outcomes.

6.0 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 treatment group. 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 in the subsample with available data with the full sample originally enrolled into the iLiNS Dyad study for similarities on selected sociodemographic characteristics.

7.0 Outliers

For univariate analysis, the distribution of scores will be examined to identify potential outliers. We will visually inspect outliers by creating box and whisker plots or histograms of individual continuous variables, and scatter-plots of related variables. Clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. We will maintain plausible or possible outliers in the data set, and these will be truncated at the 1st and 99th percentile for analysis.

8.0 Data transformation

We will inspect the distribution of outcome variables for normality and transform as necessary. If no suitable transformation is found, nonparametric tests will be employed to analyze data.

9.0 Main *effect of intervention*

For continuous outcomes, an ANCOVA model, following intention-to-treat principles will be used to examine the effects of the LNS intervention on mean VM counts/60 s, and mean percentage of time spent in MVPA and SED behavior. For binary outcomes (percentage of active children in the sample), we will use a logistic regression model. We will adjust for potential covariates listed below. We will compare groups with two models: one model unadjusted for covariates and the second model will be adjusted for any baseline and follow-up listed below in section 11 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.

10.0 Exploratory analysis

Besides the overall averages in all outcomes we will also assess the differences in VM counts/ 60 s, MVPA and SED time between groups separately on:

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

11.0 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

Factors collected at follow-up:

1. Child age

2. Child sex

3. Preschool quality/experience

4. Season of activity measurement

12.0 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

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.

iLiNS-DYAD G2 Preschool Follow-up Study

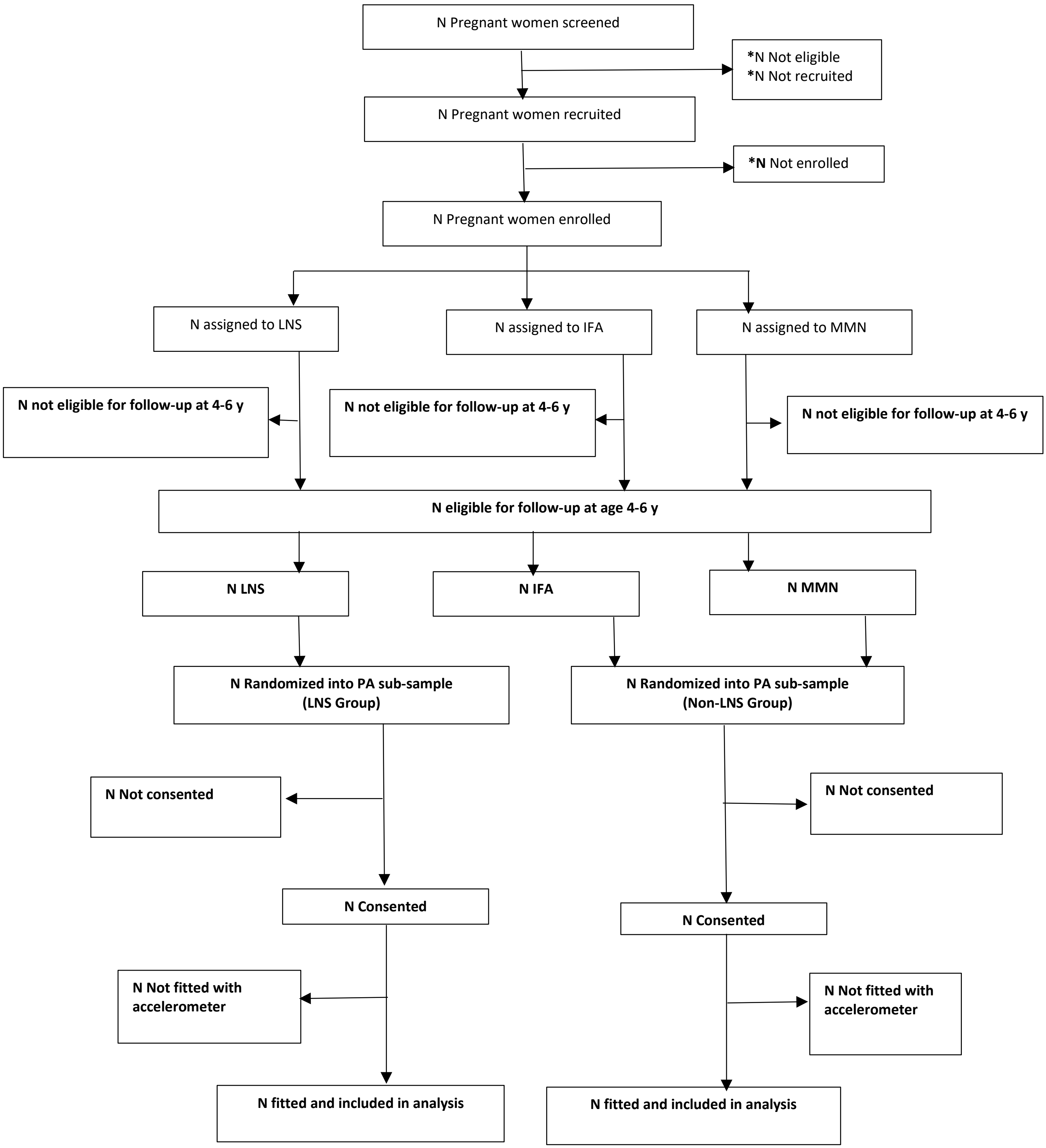

FIGURE 1 Study profile.
LNS, lipid-based nutrient supplement; IFA, iron and folic acid; MMN, multiple micronutrients.
LNS group, women received 20 g LNS daily during pregnancy and 6 mo lactation. Infants received 20 g LNS daily from 6-18 mo of age; Non-LNS group, women received either IFA during pregnancy 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** n Mean ± SD [n] or % [n/total]	**Non-LNS** n 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
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

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

	Treatment group		Comparison between groups		
	LNS	Non-LNS			
	n=	n=			
Variables	Mean (SD)	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 behavior (%)	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

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.

Yameogo, C. W. Cichon, B. Fabiansen, C. Iuel-Brockdorf, A. S. Shepherd, S. et al. (2017). Correlates of Physical Activity among Young Children with Moderate Acute Malnutrition. J Pediatr. 181 235-241

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.

Herman KM, Craig CL, Gauvin L, Katzmarzyk PT. Tracking of obesity and physical activity from childhood to adulthood: the Physical Activity Longitudinal Study. International Journal of Pediatric Obesity 2009;4(4):281-8.
Shetty PS. Adaptation to low energy intakes: the responses and limits to low intakes in infants, children and adults. Eur J Clin Nutr. 1999; 53, 14–33.

Olney DK, Pollitt E, Kariger PK, et al. Young Zanzibari children with iron deficiency, iron deficiency anemia, stunting, or malaria have lower motor activity scores and spend less time in locomotion. J Nutr . 2007; 137, 2756–2762.

Waterlow JC (1990) Energy-sparing mechanisms: reductions in body mass, BMR and activity: their relative importance and priority in undernourished infants and children. In: Activity, Energy Expenditure and Energy. Requirements of Infants and Children, pp. 239–251 [B Schürch and NS Scrimshaw, editors]. Lausanne: International Dietary Energy Consultancy Group.

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.

Tudor-Locke C, Barreira TV, Schuna JM, Mire EF, Katzmarzyk PT. Fully automated waist-worn accelerometer algorithm for detecting children’s sleep-period time separate from 24-h physical activity or sedentary behaviors. Appl Physiol Nutr Metab 2014;39(1):53-57