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# Lipid-based nutrient supplements increase energy and macronutrient intakes from complementary food among Malawian infants[1]

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**List of abbreviations:**

| | |
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| CFs | complementary foods |
| CI | confidence interval |
| DSM | dry skimmed-milk powder |
| i-24-HR | interactive 24-hour dietary recall |
| LAZ | length-for-age z-score |
| LNS | lipid-based nutrient supplements |
| USDA | United States Department of Agriculture |
| WAZ | weight-for-age z-score |
| WLZ | weight-for-length z-score |

**Abstract**

Background: Low intakes of good-quality complementary foods contribute to undernutrition, and consequently negatively impact health, growth and development. Lipid-based nutrient supplements (LNS) are designed to ensure dietary adequacy in micronutrients and essential fatty acids, and provide some energy and high-quality protein. In populations where acute energy deficiency is rare, the dose-dependent effect of LNS on complementary food intakes is unknown.

Objective: The objective of this study was to evaluate the difference in energy and macronutrient intakes from complementary food between a control (no supplement) group and three dose levels of 10g, 20g or 40g/day of LNS.

Methods: We collected repeated interactive 24-hour dietary recalls from caregivers of rural Malawian 9-10 month old infants (n=748) to estimate dietary intakes (LNS and all non-breast milk foods) of energy and macronutrients and their dietary patterns. All infants were participating in a 12-month randomized controlled trial investigating the efficacy of various doses of LNS for preventing undernutrition.

Results: Dietary energy intakes were significantly higher among infants in the LNS intervention groups than in the control group (396, 406, and 388 kcal/day in 10g, 20g and 40g/d, respectively vs. 345 kcal/day; each pairwise $p<0.05$), but there were no significant differences in energy intakes between groups receiving the different LNS doses (10g vs 20g $p=0.72$, 10g vs 40g $p=0.67$, 20g vs 40g $p=0.94$). Intakes of protein and fat were significantly higher in the LNS intervention groups than the control group. There were no significant inter-group differences in median intakes of energy from non-

LNS complementary foods (energy from complementary foods: 357, 347, and 296 kcal/day in 10g, 20g and 40g/d, respectively vs. control: 345 kcal, p=0.11). Conclusion: LNS in doses of 10-40 g/d increase intakes of energy and macronutrients among 9-10 month old Malawian infants, without displacing locally available complementary foods.

Clinical Trial Registry identifier: NCT00945698

Keywords: undernutrition, infants, dietary assessment, lipid-based nutrient supplement, complementary foods

## Introduction

Undernutrition during childhood has lasting negative functional effects across the life span. It is the underlying cause of more than 3 million deaths per year among children under five years old and it undermines the long-term development capacities of individuals and communities (1).

Poor quality complementary foods contribute to infant undernutrition (2). Among certain populations living in low income countries, complementary foods are often low in diversity (3-6), nutrient density (7, 8) and nutrient-rich animal source foods (9, 10). They often do not meet the high nutrient requirements of rapidly growing infants, especially if they have high levels of anti-nutrients that reduce iron and zinc bioavailability (11-13).

Targeted prevention interventions have been shown to be more cost effective than curative treatment programs for reducing undernutrition (14). Several studies have suggested that lipid-based nutrient supplements (LNS) given during the period of complementary feeding could support healthy growth and development (15) or potentially prevent severe stunting (16). LNS are energy-dense, nutrient-rich pastes that when added to infant porridges or eaten plain in the recommended dose are designed to ensure adequate dietary intakes of micronutrients and essential fatty acids (17). However, evidence on efficacy for the prevention of growth faltering has been mixed (17), and research with a variety of formulations is on-going. A lower dose (<20g/d, ~120kcal) LNS could have advantages in settings where infant energy intake is sufficient or near sufficient, as a lower dose supplement (if efficacious) could reduce cost and minimize risk of displacement of breast milk or diverse local foods (17). Few studies have examined the impact of LNS on complementary food intake (15, 18) and no studies, to date, have compared the impact of different doses.

The current study was a sub-study of the iLiNS-DOSE trial in Malawi, which was designed to assess the impact of three different dose levels of LNS, 10g, 20g and 40g/d, on linear growth among infants. It was undertaken in a region (Mangochi) with a low prevalence of wasting (weight-for-length < -2 z-score; 5.9%) and high prevalence of stunting (length-for-age < -2 z-score; 48.3%) (19). We hypothesized that if LNS were consumed as recommended, it would not displace energy contributed by traditional complementary foods.

The sub-study objectives were to: 1) assess whether intake of LNS at 10g, 20g or 40g/d has an impact on the dietary intakes of energy and macronutrients from all non-breast milk complementary foods compared to the control group; 2) examine whether LNS at these different dose levels results in differences in energy intakes from all non-LNS complementary foods (i.e. whether LNS displaced traditional complementary foods); and 3) assess whether there was a difference in energy intakes from specific non-LNS complementary food groups between the intervention groups and the control group.

## Methods

*Study design and participants*

This study was a dietary assessment sub-study nested in the iLiNS DOSE trial. The iLiNS DOSE trial was a 52-week randomized single-blinded efficacy trial conducted with staggered enrolment and follow-up between November 2009 and May 2012. The study included a mixture of semi-rural and rural communities, and spanned the catchment areas of the Mangochi District Hospital and the Namwera Health Centre, Malawi.

Healthy infants under the age of 6 months were identified through community census, and all those who met the inclusion/exclusion criteria were invited to participate in the trial. Inclusion and exclusion criteria were published by Maleta, et.al (20).

Ethical approval was granted from the College of Medicine Research Ethics Committee, Blantyre Malawi and the Pirkanmaa District Hospital Research Ethics Board, Tampere, Finland. The trial was registered with the National Institutes of Health Clinical Trial Registry under the identifier NCT00945698. Ethical permission was also given by the London School of Hygiene and Tropical Medicine for this dietary assessment sub-study.

*Interventions*

Participants were randomized to one of six intervention groups: control, LNS 10g with milk powder, LNS 20g with milk powder, LNS 20g without milk powder, LNS 40g with milk powder and LNS 40g without milk powder. LNS ingredients included soybean oil, dried skim milk (DSM) in the milk-containing LNS, peanut, sugar, and a vitamin/mineral premix. Milk-containing LNS contained 6-12 g DSM, depending on dose. Sugar content was 0.2g, 1.6g and 3.2g in the 10g, 20g, and 40g doses, respectively. All participants had the same length of follow-up (12 months).

For the intervention, caregivers in the LNS groups were advised to feed their children the daily ration of LNS mixed in porridge, over two eating occasions. The message of providing LNS to the infant was reinforced at clinic visits when the infants were 12 and 18 months. At enrollment and at clinic visits when the infant was 12 months and 18 months of age (i.e. every 6 months) caregivers were also given brief messages advising them to breastfeed just as before receiving the supplement, and to feed the child a diverse diet, with the latter message reinforced by use of the Ministry of Health visual representation of a diverse diet. For the purposes of the dietary assessment sub-study, only four intervention groups were considered. Specifically, the milk and non-milk groups were collapsed into one group within each of the 20g and 40g dose levels, and the average energy and nutrient composition per dose was calculated to represent the energy and nutrient content of that dose level. The milk and non-milk LNS groups were pooled within each dose category because we did not expect that the composition itself would affect energy and nutrient intakes from non-LNS complementary foods.

*Sampling*

A sample size of 172 in each group (control, 10g, 20g and 40g/d LNS) was calculated to detect a ≥20% increase in energy intakes from non-breast milk sources of energy (LNS + complementary foods) and a ≥25% displacement (75-81 kcal) in non-LNS complementary food intakes in the 20g and 40g LNS groups compared to the control group, assuming a standard deviation of 131 kcal (21) with 80% power, 95% confidence and an estimated attrition rate of 15%. Initially, infants were randomly selected from each intervention arm to participate in the sub-study. However, because of the high loss-to-follow-up between enrollment at ~6 months and this sub-study at ~9.5 months, additional infants (n=97) were selected from the "basic sub-study group" (i.e. not randomized to any additional sub-study within the main trial). As a result, there was an imbalance in the number of additional infants selected into the LNS 20g and 40g groups compared with the control and LNS 10g groups because the former two groups each included infants from two main study arms (i.e., milk and non-milk LNS groups); whereas, the latter two groups each represented one study arm.

*Dietary Assessment*

Dietary intakes were assessed between April 2010 and October 2011 using a repeat 4-pass interactive 24-hour recall (i24-HR (22)) at 9-10 months of age (week 16 or 17 after enrolment). The first i24-HR was randomly assigned to occur between 0 and 13 days after a planned LNS delivery, and the second i24-HR was done exactly 7 days later in order to capture dietary intakes during each of the two 7-day periods within the fortnightly LNS delivery schedule. i-24-HRs were collected on all days of the week in approximately equal numbers per day in order to avoid a day-of-the week effect on inter-group comparisons.

The i-24HR was designed to assess all non-breast milk foods and beverages consumed by the infant on the previous day. The information was collected from the main-caregiver (in most cases the mother), and portion sizes were asked from the person who fed or observed the infant consuming the recalled food or drink. Two days before the interview day, standardized plastic cups and bowls were dropped off at the household to help with portion size visualization, which was done because of the traditional practice of shared dishes. Data collectors also dropped off a pictorial chart, which contained pictures of various food groups, and the caregivers were asked to prospectively mark on the chart all foods and drinks consumed by the infant within the appropriate food group. The pictorial chart was used to minimize recall errors by asking caregivers to simply check a box next to pictures of the grouped food items. The data collector did a short training session with the caregiver about the correct use of both of these tools.

The i-24HR consisted of four passes. In the first pass, the caregiver was asked to freely list everything the infant consumed during the past full day, including any night feeds other than breast milk. In the second pass, more details about foods/beverages on the initial list were collected, including the time and place of consumption, the person who fed the infant, ingredients that were added to the food or beverage (e.g. milk powder in porridge or sugar in tea) and detailed descriptions of the type of food or drink consumed including brand names, ingredients and preparation methods (e.g. boiled, raw). In the third pass, the data collector asked the caregiver to estimate the portion size served and the amount left-over, using a variety of tools including: salted food models, real foods (e.g. bananas, milk powder, LNS), water (for liquids), unit measures (e.g.number of biscuits). For the main staple food consumed by these infants (i.e., a grain-based porridge), caregivers were asked to select the appropriate consistency from three models of porridge, and this was used to estimate the amount of dry flour consumed. At the end of the third pass, the data collector compared the pictorial chart with the i-24-HR to assess their agreement with one another. Any omissions or intrusions of foods/beverages were discussed and resolved. In the final pass, the data collector summarized the food and beverages recorded and asked the respondent whether it was an accurate representation of what the infant had consumed; this provided the caregiver a final opportunity to correct any misreported or forgotten details of the infant's food and drink intake. At the end of the i-24-HR, caregivers were asked whether the infant was ill on the day of intake, whether the intake was usual, increased or decreased compared to usual, and whether the infant was breastfed on the day of recorded dietary intake.

*Anthropometric and Socio-demographic Data*

Socio-demographic data (interviewer administered questionnaire) and anthropometric (weight and recumbent length) data were collected at baseline, when the infants were approximately 6 months of age. Weight was measured to the nearest 0.01 kg using an electronic scale (SECA 735; Chasmors Ltd, London England). Recumbent length was measured to the nearest 0.10 cm using rigid recumbent length boards (Harpenden Infantometer, 233 Holtain Limited, Crosswell, Crymych, UK). All anthropometric measurements were made in triplicate and the mean value of the first two measurements was used unless a pre-specified difference was exceeded, in which case the mean of the two closest values was used.

*Data Preparation*

Each i-24HR was entered by trained research personnel, and double checked against the raw data by JH. The grams of food and drinks consumed were estimated from food model weights using conversion factors constructed for this study based on density or per unit measures. Mixed dishes were disaggregated into their raw ingredient gram weights using average recipes calculated from weighed food record recipe data collected from the target population during a validation study of the i-24-HR (n=170 households, *unpublished PhD thesis, J.Hemsworth, 2014, London School of Hygiene and Tropical Medicine*). Energy and nutrient intakes were estimated using food composition data derived from a combination of sources, including the USDA Nutrient Database for Standard Reference, release 24 (23), the West African Food Composition Table (24), Mozambique Food Composition Table (25), manufacturer's websites, and the Tanzanian Food Composition Table (26). Where appropriate, adjustments for nutrient losses from cooking were made using the USDA nutrient retention factors (27). The dose-specific energy and nutrient content of LNS is outlined in Supplemental **Table 1**.

Z-scores for weight-for-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) were calculated from the anthropometric data using the WHO 2006 growth reference standards (28) and the STATA 12 zscore06 function (29). Infants were classified as stunted or wasted if their LAZ or WLZ was <-2SD, respectively.

*Statistical Analysis*

All analyses were performed in STATA-12 (StataCorp, 2011, College Station, Texas, USA). The distributions of dietary variables were first visually examined for normality; and non-normal variables were log transformed or presented as medians ($25^{th}$ and $75^{th}$ percentiles) where appropriate.

Household socioeconomic status (SES) was generated combining the variables maternal occupation, household crowding, housing material, roof material, sanitation facilities, and cooking fuel in a principal component analysis. Quintiles of the first principal component were used to categorize households into 1 of 5 SES levels. The primary outcome was the difference in estimated energy intake from all non-breast milk complementary foods between the control group and each intervention group and between intervention groups. All analyses were done according to intention to treat. A complete case analysis excluding participants with any (1-2) missing portion sizes (n=5) on one or more recalls was performed with no difference in results from the intent to treat analysis. Missing data due to loss to follow-up was considered missing at random (30). Background characteristics (maternal education, age, and occupation; number of household members and number of other under-fives; gender of household head; and SES) were compared between those lost-to-follow-up and those in the analysis sample. Those lost to follow-up (n=179) were lost between enrolment in the main study at 6-months and when we went to perform the dietary assessment visit at 9 months. The reasons for attrition are listed in Figure 1. Background characteristics were also presented across intervention groups, for descriptive purposes.

Analyses of square-root transformed continuous variables (energy and macronutrient intakes) were first completed using an unadjusted analysis of variance(ANOVA). Overall significance between group means was determined by the F statistic. When significant at the 5% level, further pair-wise significance testing determined whether LNS dose level groups differed from control and from each other. No adjustments were made for multiple comparisons. Confounding variables were examined using a univariate regression model with unadjusted total energy (kcal/day) as the outcome and the individual variables as the exposure. Robust confidence estimates were used in place of the square root transformed energy variable to provide a more meaningful beta coefficient and 95% CI. Variables with $p<0.2$ in the univariate model were then used with LNS dose level (control as reference group) in the multivariate analysis. The final model displays the effect of dose, adjusted for other characteristics associated with energy intake. P-values of $<0.05$ were considered significant, for all analyses.

## Results

In total, 1039 recalls were collected from caregivers of 569 infants. One recall was omitted from the analysis since all portion sizes were missing. Mothers of infants whose data were completely missing (n=179) (i.e. visit not completed) had a mean 5 years of education versus 4 years of education in the group from whom the data were analysed (p=0.019), and there were no other significant differences in background characteristics. Among those included in the analyses, participant background characteristics as well as anthropometric characteristics at baseline showed no meaningful differences across groups (**Table 1**).

There was a significant difference in energy intakes from complementary foods (including LNS) comparing each LNS intervention group to the control group, but there were no significant energy intake differences observed between the LNS intervention groups with pair-wise comparisons (**Table 2**). Different trends were seen with the macronutrients. The median protein intakes of the 10g and 20g LNS intervention groups were significantly higher than those of the control group, whereas there was no significant difference in median protein intakes between the 40g LNS group and the control (**Table 2**). When protein was expressed as percent of energy, there was a significant difference comparing both the control and 10g LNS group to the 20g and 40g LNS groups, respectively; it decreased as the dose level increased (**Table 2**). Median fat intake and percentage of energy from fat were significantly higher in all intervention groups than in the control group and were highest in the 40g LNS group (**Table 2**).

The significant predictors of energy intake from complementary foods (+LNS), in both the univariate and multivariate linear regression analyses were: LNS intake, breastfeeding status, maternal education, agricultural season (rainy season) and reported lower than usual food intake on the day of the i-24-HR. Breastfeeding and reported lower intakes were negatively associated with total energy intake from complementary foods (+LNS), whereas other variables were positively associated with it (**Table 3**). The multivariate model showed that participation in an LNS intervention group was associated with a 51 to 55 kcal/d increase in non-breast milk energy intakes, which is approximately equivalent to the energy contributed by the 10g LNS dose (55 kcal/day) if fully consumed.

There were no significant differences in overall energy intakes from non-LNS complementary foods, comparing the control group pairwise to any of the LNS groups; however, pairwise comparisons within the LNS groups showed that the 10g LNS group was significantly higher than the LNS 40g group ($p<0.05$) in overall energy intakes from non-LNS complementary foods (**Table 4**). Further, when examined by food group sources of non-LNS complementary foods, the mean energy contributed by legumes, nuts and seeds was significantly lower in the 40g LNS group than in the control group ($p=0.041$). Energy contributed by LNS was significantly different both between the control and each of the LNS intervention groups and significantly different between each of the LNS groups (all contrasts $p<0.01$) (**Figure 2**). Starchy staples contributed between half to two thirds of the energy from complementary foods (+LNS). LNS was the second or third highest source of energy for the LNS groups and the food groups “added sugar” and “sweetened snacks” together were the second or third highest sources of dietary energy for the control and LNS groups; these contributed 33-40 kcal/d of added sugar (~8-10 g/d) and 26-40 kcal from sweetened snacks (~2-3 biscuits/d or 6-10 g/d).

## Discussion

To our knowledge, this is the first and only study to evaluate the impact of different doses of LNS, including small doses, on energy intake from complementary foods among young infants. We found that compared to the control group, infants in the LNS groups had higher energy intakes from non-breast milk foods (complementary foods + LNS); there were, however, no significant between LNS-group differences in energy intakes. Compared to the control group, infants in the LNS groups had similar energy intakes from non-LNS complementary foods, suggesting that there was minimal or no displacement of other complementary foods by LNS. Finally, compared to the control group, infants in the LNS groups had similar energy intakes from individual food groups, except for the legumes food group, which was lower in the 40 g LNS group than the control group.

On average, energy intakes from LNS across intervention groups were 51-55 kcal per day. This is in contrast to the intended range of the additional 55-241 kcal per day that would have been consumed with full adherence across the intervention groups. At this age, approximately half of breastfed infants' energy requirements are contributed by complementary foods (300 kcal/day), assuming "average" breast milk intake (2). The energy intakes we observed in all groups, including the control group, were above this estimated average energy contribution from complementary foods. The dietary protein densities that we observed in all groups were well above the WHO desired level from complementary foods, assuming average breastmilk intakes (2). The control group had a median protein density of 2.4g /100kcal and LNS groups had median protein densities between 2.3 and 2.4g /100kcal compared with the desired level of 1.1 g/100kcal (2). Fat intake increased dose-dependently across the LNS intervention groups. Even though the median percentage of energy from fat from complementary foods in each group was below the acceptable level of ~35% energy from fat for infants 6-24 months of age (31), most of the infants were breastfed so these percentages under-estimate the actual total percent of energy from fat.

Two other smaller trials evaluated energy intakes from complementary foods and LNS; however, in both studies the aim was not to evaluate how various doses impact energy intakes. An intervention in Ghana used 20g (~108 kcal) of LNS per day as one of three dietary interventions (15). Energy intakes from complementary foods at 9 months of age in all three groups were lower than what we observed; ranging from 140 kcal/d to 230 kcal/d in the LNS group. Thakwalakwa and colleagues presented the dietary intakes of 8-18 month old moderately malnourished infants in Malawi given 43g (~220kcal) LNS per day; and showed significantly higher energy intakes in the LNS group versus the control group (18). The magnitude of the difference in energy intakes we observed between the control group and LNS intervention groups, however, was lower than in both of these studies. Lower energy needs among our study infants compared to the other Malawian study where infants were underweight at enrolment (i.e., mean WLZ score of <-1.0), and different supplement delivery schedules (weekly instead of biweekly) and other contextual differences in the Ghana study might have contributed to these inter-study differences. In contrast, the estimated energy intakes from non-LNS complementary foods were similar to those reported for 9-10 month old infants in Zambia (32) (353 [299, 407]) and earlier reports from Malawi (12) (358 [292, 472]), suggesting that energy intakes from non-LNS complementary foods were not over-estimated in our study.

We found no significant difference between the control group and intervention groups in energy intakes from non-LNS foods. This suggests that, in all groups, LNS was consumed in addition to the traditional fare, and is consistent with the earlier results from Ghana (15).

While few studies have examined displacement of complementary food by supplements, several have focused on potential breast milk displacement. Diets that have a higher energy density can increase energy intake from complementary foods and this can be inversely related to breast milk intake (33). However, in a sub-set of this sample of infants (n=400), there was no evidence of displacement of breast milk by LNS at any dose, when assessed using a stable isotope mother to infant dose technique (34). This result is consistent with three other studies from Zambia (32), the Democratic Republic of Congo (35), and a smaller trial Malawi (36).

The absolute energy intake per food group did not differ between the control group and any of the intervention groups, except for a significant difference in the intake of groundnuts and other legumes in the pairwise comparison between the LNS 40g group and control group (p=0.030). These results suggest that small doses of LNS do not displace energy from non-breastmilk food groups; whereas a small displacement may occur with the largest dose of LNS. A displacement of legumes, however, does not result in an important change in traditional dietary patterns since it was replaced by LNS which is also legume-based. A displacement in legumes (especially groundnuts) was also noted with the intervention porridge in Zambia (32).

**Study limitations and strengths**

Potential measurement error can and does arise from many different sources in the 24-hour recall. However, the purpose of the study was to compare dietary intake differences across groups, which means similar measurement error will exist among the intervention and control groups, except possibly for the estimation of LNS intakes. A systematic error in LNS intake estimations would not affect the estimates of non-LNS complementary foods, and thus would not alter our conclusion that LNS did not displace other complementary foods, at least in the 10g and 20g groups. However, if LNS intake was overestimated, this would have inflated the estimates of total energy intake in the LNS groups but not in the control group. We thus acknowledge that there is some uncertainty regarding the extent to which LNS increased total energy intake from complementary foods in the intervention groups.

In the 40g LNS group there was an apparent 49 kcal displacement of energy from complementary foods, which our study was not powered to detect as significant. However, because our analyses suggest LNS may have displaced legumes in the 40g LNS dose group, these results may be less of a concern since the LNS is also legume-based. Finally, we also cannot rule out the possibility that some of our significant findings, such as the displacement of energy from legumes in the 40g LNS group, could have been due to chance, given the number of statistical tests performed (37).

This study also had several strengths. We assessed the dietary impact of three different dose levels of LNS, which addresses important concerns that LNS may displace local foods or negatively impact diversification of infant diets (38). In dietary assessment, where measurement error is inevitable, we paid close attention to minimizing it, including the use of a pictorial chart to reduce recall error (22), weekly feedback sessions with the data collectors to discuss challenges in portion-size estimations, “quizzes” for the data collectors to ensure that their approach for measuring portion sizes was consistent, and a rigorous method for measuring key food portion sizes, such as LNS. Specifically, the use of food models improves portion size estimations by allowing caregivers to visually and manually estimate portion sizes. Special care was also taken to estimate the consistency of the porridge consumed, which would influence dietary energy intake estimates.

## Conclusions

Energy intakes from all non-breast milk complementary foods were significantly lower in the control group compared with the three LNS intervention groups, but there were no significant differences between LNS dose groups. Secondly, there were no significant differences in energy intakes from non-LNS foods between the control and the LNS groups.These results suggest that LNS, especially in small dose quantities (10g and 20g) does not alter traditional dietary patterns among rural Malawian children. These results are part of a much larger and dynamic picture of growth, illness, and infant development are but one piece of a larger effort to assess the potential for use of LNS in the prevention of undernutrition among infants.

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## Author Contributions:

J.H, C.K., M.A., K.M., J.P., S.A.V., U.A., K.G.D., P.A. & E.L.F designed the research and significantly contributed to the aim and structure of manuscript; J.H. & C.K. conducted the research; A.M.R. & S.F. provided statistical guidance and assistance with methods; J.H & E.L.F analyzed data or performed statistical analysis; J.H drafted the paper with inputs from M.A. & E.L.F; J.H. & E.L.F had primary responsibility for the final content. All authors have read and approved the final manuscript.

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**Figure Legend:**

Figure 1: Flow Diagram of participant recruitment, enrollment and completion of the dietary assessment sub-study

Figure 2: Total absolute mean energy (kcal) contributed by complementary food groups as a proportion of all non-breast milk intakes (CFs) per LNS dose intervention group

[a] significantly different between LNS 40g and control at p=0.030
[b] significantly different between control and intervention groups and between intervention groups at p<0.001

Table 1: Anthropometric and Background Characteristics of Participants at Baseline (6 months)

| | | Control | 10g LNS | 20g LNS | 40g LNS |
|---|---|---|---|---|---|
| | n | 170 | 170 | 200 | 208 |
| Age (months) | mean ± SD | 9.8 ± 0.4 | 9.8 ± 0.4 | 9.7 ± 0.5 | 9.7 ± 0.5 |
| Female | n (%) | 85 ± 50 | 86 ± 50.9 | 97 ± 48.5 | 106 ± 51.0 |
| Socio-demographic Background Characteristics | n | 159 | 159 | 188 | 192 |
| Maternal age (years) | mean ± SD | 25.6 ± 6.1 | 26.0 ± 6.4 | 26.5 ± 6.1 | 26.7 ± 6.3 |
| Maternal education (years) | mean ± SD | 4.7 ± 3.3 | 4.6 ± 3.7 | 4.4 ± 3.6 | 4.9 ± 3.8 |
| Maternal height (cm) | mean ± SD | 155 ± 5 | 156 ± 6 | 156 ± 6 | 156 ± 6 |
| Female-headed household | n (%) | 14 (8.9) | 18 (11.3) | 28 (14.9) | 19 (10.0) |
| Two or more children under 5 years old in household | n (%) | 89 (58.2) | 85 (54.5) | 90 (48.7) | 96 (51.9) |
| Maternal occupation | n (%) | | | | |
| Farming/Fishing | | 92 (57.9) | 90 (56.6) | 97 (51.6) | 109 (56.8) |
| House wife | | 53 (33.3) | 57 (35.8) | 70 (37.2) | 70 (36.5) |
| Indoor / office work | | 5 (3.1) | 3 (1.9) | 12 (6.4) | 3 (1.6) |
| Other | | 5 (3.1) | 4 (2.5) | 4 (2.1) | 5 (2.6) |
| Unknown | | 4 (2.5) | 5 (3.1) | 5 (2.7) | 5 (2.6) |
| Anthropometry at Baseline | n | 170 | 170 | 200 | 208 |
| 6 month LAZ | mean ± SD | -1.37 ±1.02 | -1.41 ±1.11 | -1.41 ±1.01 | -1.46 ±1.20 |
| *Percent Stunted (<-2 LAZ)* | | *30.8* | *29.2* | *31.2* | *30.6* |
| 6 month WAZ | mean ±SD | -0.70 ±1.11 | -0.78 ±1.12 | -0.64 ±1.13 | -0.83 ±1.20 |
| *Percent Underweight (<-2* | | *9.9* | *14.6* | *11.7* | *13.9* |

| | | | | | |
|---|---|---|---|---|---|
| *WAZ)* | | | | | |
| 6 month WLZ | mean ±SD | 0.32 ±1.08 | 0.27 ±1.08 | 0.42 ±1.10 | 0.25 ±1.04 |
| *Percent Wasted (<-2 WLZ)* | | *1.2* | *1.2* | *0.5* | *2.4* |

Table 2: Energy and Macronutrient Intake from all Complementary Foods by LNS group [1, 2,3]

| | Control | 10g LNS | 20g LNS | 40g LNS | P-value[1,2] |
|---|---|---|---|---|---|
| Energy (kcal/day) | 345 (247, 463)[a] | 396 (309, 532)[b] | 406 (300, 535)[b] | 388 (304, 548)[b] | <0.001 |
| Protein (g/d) | 8.2 (5.7, 11.4)[a] | 9.3 (7.4, 12.0)[b] | 9.4 (6.5, 12.3)[b] | 9.0 (6.2, 11.7)[ab] | 0.040 |
| % energy from protein | 9.6 (8.4, 10.7) [a] | 9.8 (8.7, 10.7)[a] | 8.9 (8.0, 10.0)[b] | 8.6 (7.4, 9.8)[b] | <0.001 |
| Fat (g/d) | 7.0 (3.9, 10.8)[a] | 10.1 (6.9, 15.4)[b] | 11.9 (7.6, 17.2)[b] | 13.0 (8.7, 19.1)[c] | <0.001 |
| % energy from fat | 18.0 (13.6, 23.6)[a] | 22.6 (18.6, 27.3)[b] | 26.8 (19.7, 32.4)[c] | 29.7 (24.1, 35.3)[d] | <0.001 |

[1] Data are presented as median ($25^{th}$, $75^{th}$ percentile), control n=123, 10g/d LNS n=130, 20g/d LNS n=158, 40g/d LNS n=157

[2]Labeled medians without a common letter differ, P<0.05

[3]The p-value is the overall p-value of effect of dose as exposure on energy and nutrient intakes.

LNS: Lipid-based nutrient supplement

Table 3: Factors associated with energy Intake of Complementary Foods including various doses of LNS versus control (kcal/day)

| | Univariate | | | Multivariate[1] | | |
|---|---|---|---|---|---|---|
| | β coefficient | 95% CI | p-value | β coefficient | 95% CI | p-value |
| LNS 10g | 63 | 20, 107 | | 51 | 6, 97 | 0.044 |
| LNS 20g | 71 | 28, 115 | 0.002 | 55 | 10,100 | |
| LNS 40g | 73 | 30, 116 | | 54 | 9, 100 | |
| Infant breast fed on day of recall | -340 | -470, -200 | <0.001[3] | -340 | -500, -190 | <0.001 |
| PCA – SES [2] 2nd | 57 | 5, 110 | | 37 | -12, 86 | 0.457 |
| 3rd | 47 | -4, 98 | 0.183[3] | 28 | -19, 75 | |
| 4th | 28 | -19, 74 | | 2 | -44, 48 | |
| Highest quintile | 36 | -15, 86 | | -1 | -52, 49 | |
| Maternal Education, (years) | 11 | 6, 16 | <0.001[3] | 10 | 5, 16 | <0.001 |
| Reported decreased appetite | -78 | -120, -36 | <0.001[3] | -81 | -123, 38 | <0.001 |
| Season, (Rainy Oct-April) | 35 | 2, 67 | 0.04[3] | 36 | -12, 69 | 0.030 |
| WAZ at 6 months | 12 | -3, 28 | 0.121[3] | 15 | -1, 30 | 0.06 |

[1] Dose as the exposure, including decreased appetite, season, breastfeeding status, socio-economic status (PCA), maternal education, WAZ at 6 months, and maternal height as covariates.

Data are untransformed for regression analysis, but robust confidence estimates were used to control for non-normality of data. The R-squared in the multivariate model was 0.16.

[2] The PCA-SES is a composite score of socio economic status based on the following: maternal occupation, housing material, roof material, water source, source of household electricity, type of cooking fuel used, type of sanitary facility, and number of household members per room (crowding index).

[3] These variables were included as possible intervention confounding variables in the multivariate analysis, because their p-values in the univariate analysis were <0.2.

CI: confidence interval, LNS: Lipid-based nutrient supplement, PCA-SES: Principle component analysis, socio economic status, WAZ: weight-for-age z-score

Table 4: Energy and macronutrient intakes from non-LNS complementary foods by LNS group[1,2,3]

| | Control | 10g LNS | 20g LNS | 40g LNS | P-value[1,2] |
|---|---|---|---|---|---|
| Energy (kcal/d) | 345 (247, 463)[ab] | 357 (281, 469)[a] | 347 (238, 474)[ab] | 296 (228, 426)[b] | 0.11 |
| Protein (g/d) | 8.2 (5.7, 11.4)[ab] | 8.5 (6.7, 11.2)[a] | 8.2 (5.7, 11.9)[ab] | 7.5 (5.0, 10.0)[b] | 0.04 |
| Fat (g/d) | 7.0 (3.9, 10.8)[ab] | 6.3 (4.1, 10.6)[ab] | 6.8 (4.1, 11.6)[a] | 5.5 (3.3, 8.6)[b] | 0.20 |

[1] Data are presented as median (25th, 75th percentile), control n=123, 10g/d LNS n=130, 20g/d LNS n=158, 40g/d LNS n=157

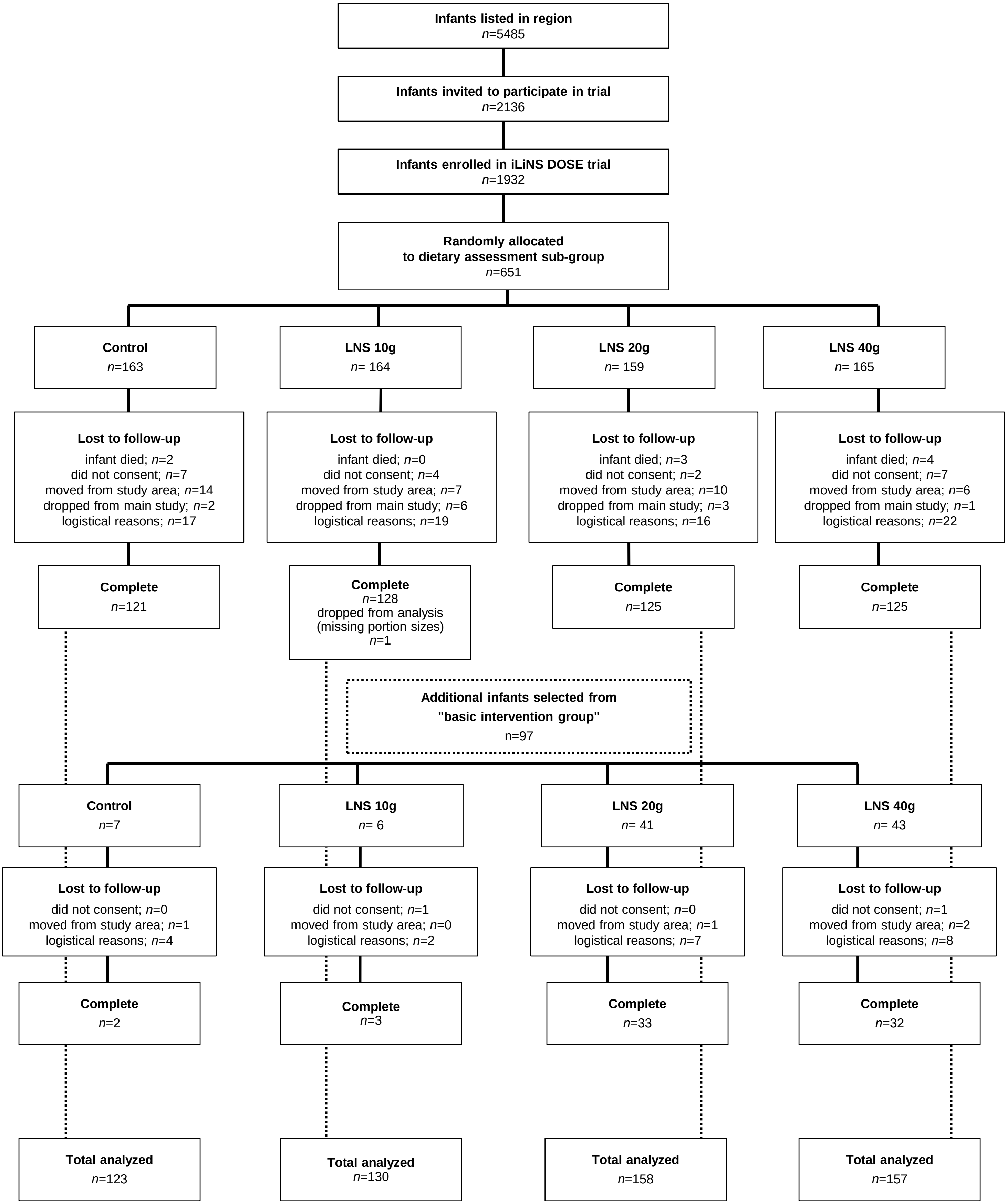
[2] Labeled medians without a common letter differ, P<0.05.

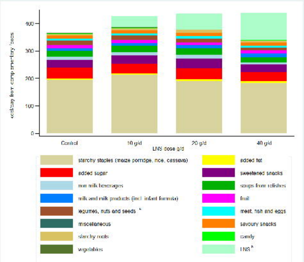
[3] The p-value is the overall p-value of effect of LNS dose as exposure on non-LNS energy and macronutrient intakes.

Supplementary Table 1: Energy and Nutrient Content of LNS by Dose

| | LNS-10g | LNS-20g[1] | LNS-40g[1] |
|---|---|---|---|
| Daily ration (g) | 10 | 20 | 40 |
| Total energy (kcal) | 55 | 117 | 241 |
| Protein (g) | 1.3 | 1.75[1] | 3.5[1] |
| Fat (g) | 4.7 | 9.5[1] | 18.9[1] |
| Linoleic acid (g) | 2.22 | 4.44 | 8.88 |
| α-Linolenic acid (g) | 0.29 | 0.58 | 1.16 |
| Vitamin A (μmol RAE) | 1.40 | 1.40 | 1.40 |
| Vitamin C (mg) | 30 | 30 | 30 |
| Thiamine (mg) | 0.3 | 0.3 | 0.3 |
| Riboflavin (mg) | 0.4 | 0.4 | 0.4 |
| Niacin (mg) | 4 | 4 | 4 |
| Folic acid (μg) | 80 | 80 | 80 |
| Pantothenic acid (mg) | 1.8 | 1.8 | 1.8 |
| Vitamin B6 (mg) | 0.3 | 0.3 | 0.3 |
| Vitamin B12 (μg) | 0.5 | 0.5 | 0.5 |
| Vitamin D (μg) | 5 | 5 | 5 |
| Vitamin E (mg) | 6 | 6 | 6 |
| Vitamin K (μg) | 30 | 30 | 30 |
| Iron (mg) | 6 | 6 | 6 |
| Zinc (mg) | 8 | 8 | 8 |
| Copper (mg) | 0.34 | 0.34 | 0.34 |
| Calcium (mg) | 240 | 240 | 240 |
| Phosphorus (mg) | 208 | 208 | 208 |
| Potassium (mg) | 265 | 265 | 265 |
| Magnesium (mg) | 50 | 50 | 50 |
| Selenium (μg) | 20 | 20 | 20 |
| Iodine (μg) | 90 | 90 | 90 |
| Manganese (mg) | 1.2 | 1.2 | 1.2 |
| Phytate (mg) | 28 | 56 | 112 |

[1] the energy and nutrient content of both LNS products were identical except for protein and fat. For protein and fat, the mean food composition values of milk and non-milk LNS were used
Protein: LNS 20g-milk: 2.5g, LNS 20g-non-milk: 1.0g; LNS 40g-milk LNS 40g-milk: 5.0g, LNS 40g-non-milk: 2.0g;
Fat: LNS 20g-milk: 9.5g, LNS 20g-non-milk: 9.4g; LNS 40g-milk: 19.0g, LNS 40g-non-milk: 18.8g;


Infants listed in region
n=5485
Infants invited to participate in trial
n=2136
Infants enrolled in iLiNS DOSE trial
n=1932
Randomly allocated
to dietary assessment sub-group
n=651
Control
n=163
LNS 10g
n= 164
LNS 20g
n= 159
LNS 40g
n= 165
Lost to follow-up
infant died; n=2
did not consent; n=7
moved from study area; n=14
dropped from main study; n=2
logistical reasons; n=17
Lost to follow-up
infant died; n=0
did not consent; n=4
moved from study area; n=7
dropped from main study; n=6
logistical reasons; n=19
Lost to follow-up
infant died; n=3
did not consent; n=2
moved from study area; n=10
dropped from main study; n=3
logistical reasons; n=16
Lost to follow-up
infant died; n=4
did not consent; n=7
moved from study area; n=6
dropped from main study; n=1
logistical reasons; n=22
Complete
n=121
Complete
n=128
dropped from analysis
(missing portion sizes)
n=1
Complete
n=125
Complete
n=125
Additional infants selected from
"basic intervention group"
n=97
Control
n=7
LNS 10g
n= 6
LNS 20g
n= 41
LNS 40g
n= 43
Lost to follow-up
did not consent; n=0
moved from study area; n=1
logistical reasons; n=4
Lost to follow-up
did not consent; n=1
moved from study area; n=0
logistical reasons; n=2
Lost to follow-up
did not consent; n=0
moved from study area; n=1
logistical reasons; n=7
Lost to follow-up
did not consent; n=1
moved from study area; n=2
logistical reasons; n=8
Complete
n=2
Complete
n=3
Complete
n=33
Complete
n=32
Total analyzed
n=123
Total analyzed
n=130
Total analyzed
n=158
Total analyzed
n=157



400
300
200
100
0
kcal/day from complementary foods
Control
10 g/d
20 g/d
40 g/d
LNS dose g/d
starchy staples (maize porridge, rice, cassava)
added fat
added sugar
sweetened snacks
non milk beverages
soups from relishes
milk and milk products (incl. infant formula)
fruit
legumes, nuts and seeds [a]
meat, fish and eggs
miscellaneous
savoury snacks
starchy roots
candy
vegetables
LNS [b]