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Effect of supplementation with a lipid-based nutrient supplement on the micronutrient status of children aged 6 to 18 months living in the rural region of Intibucá, Honduras

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Abstract

Background: Lipid-based nutrient supplements (LNS) have been effective in the treatment of acute malnutrition among children. We evaluated the use of LNS supplementation for improving the micronutrient status of young children.

Methods: A 12-month randomized controlled trial was conducted among children aged 6–18 month living in Intibucá, Honduras. Communities (n=18) were randomized into clusters matched by poverty indicators (9 intervention, n=160 and 9 controls, n=140). Intervention participants received LNS. All children received food vouchers and nutrition education. Primary outcomes included measures of micronutrient status: at baseline, 6 and 12 month blood was collected for assessment of folate, iron, zinc, riboflavin and vitamin B_{12} status; hemoglobin was measured every 3 months; and dietary and anthropometry collected monthly. Longitudinal analyses were based on intent-to treat and LNS adherence. Generalized estimating equations were used in the estimation of generalized linear regression models specified for the data.

Results: At 6 months follow-up, children in the intervention group had a lower proportion classified as deficient for B_{12} (43.6%) compared to the control (67.7%; P=0.03). The intervention group had a higher mean concentration for folate at 6 months (P=0.06), and improvements

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Conflict of Interest

The authors have no conflicts of interest to declare.

continued through 12 months for folate (P=0.002) and vitamin A deficiency (P=0.03). This pattern of results, with improved significance, remained in sub-analysis based on LNS adherence.

Conclusion: These data demonstrate that LNS improved select micronutrient status in young non-malnourished Honduran children.

Keywords

lipid-based nutrient supplements; child undernutrition; prevention; micronutrients; Honduras; randomized-controlled trial

About 171 million children aged 0–5 years suffer from chronic undernutrition, mainly in developing countries undergoing economic transitions.[1] In Latin America this represents a total of about 7 million children, where stunting is now much more common than underweight and wasting. [1] Large disparities in economic growth in Latin America have led to higher levels of undernourishment, food insecurity and poor access to quality foods for those living in the rural areas. [2] A diet low in animal-source foods, fruits and vegetables, and high in phytates (beans, maize) may result in micronutrient deficiencies that lead to anemia, poor growth, developmental delays and increased morbidity.[3,4] Efforts to improve micronutrient intake should target children during the first two years of life when the irreversible outcomes of malnutrition may be prevented.[1,3]

Food-based interventions targeting children under 2 have shown some improvements in growth outcomes and micronutrient status.[3] A review of these strategies indicated that interventions pairing supplementation of energy dense products with education have better results compared to educational interventions or food fortification strategies alone.[3]

Lipid-based nutrient supplements (LNS) are energy dense products that provide essential fatty acids and micronutrients. LNS may be advantageous over other types of food-based efforts because they allow more nutrients to be consumed in a serving, do not require major diet behavior changes and include lipids that improve the absorption of fat-soluble vitamins; [5] evidence suggests that LNS products are effective in treating severe child malnutrition,[6–9] and are tolerated and consumed by infants.[5,10,11]

LNS studies in Sub-Saharan Africa, targeting prevention of chronic malnutrition, have reported improvements in weight gain and a reduced incidence of wasting and stunting. [12–15] These ranged from 3–12 month-long-interventions for children 6 to 60 months old using varying doses of LNS. Only one study has examined micronutrients (i.e. zinc and selenium) but no improvements were reported.[12] Given the lack of evidence on the effect of LNS on micronutrient status of children, this study used a cluster-randomized trial to test if LNS supplementation can improve the micronutrient status of children 6–18 months old living in a rural poor region of Central America.

Methods

Setting

The study was conducted in three municipalities (Santa Lucia, Magdalena and San Antonio) in the southwestern part of Intibucá, Honduras, bordering El Salvador. Recruitment for the 12-month intervention was conducted during the dry season (March-April 2009) with follow-up conducted until April 2010. All study participants experienced 6 months each of the dry and rainy season.

Study Design

This was a cluster randomized controlled trial. 18 communities were paired by region and matched on several poverty indicators; percent of houses with dirt floors, no toilet, 4 or more people per room, and total poverty score (the sum of all the indicators). Clusters were geographically separated to avoid potential cross contamination. One cluster within each pair was then randomized to intervention (n=9) or control group (n=9). Thereafter the mother-infant pairs were enrolled in the study groups according to their cluster randomization.

Our research staff made initial contact with mothers living in the selected communities at community centers, health centers and schools. Recruitment was conducted at each site over a one-month period with one recruitment day per village. Eligible caregiver-child pairs were enrolled from their site of recruitment or from the health clinic on their date of recruitment, making this a convenience sample.

The primary outcomes include micronutrient biomarkers (folate, iron, zinc, vitamin B_{12}, vitamin A, and riboflavin). Secondary outcomes include growth, dietary intake and food insecurity. The study was blinded to study group allocation at the data entry level and at the biomarker analysis level. Given the difficulties of working in this rural setting, delivery of the intervention was not blinded for project staff conducting the assessments.

The Institutional Review Board of the University of North Carolina at Chapel Hill and the Honduran IRB committee approved the study protocol. The Intibucá Ministry of Health endorsed the study's objectives and collaborated in its implementation. An advisory committee monitored the incidence of any adverse effects. Mothers/caretakers gave informed consent for the participation of their child. Participants were referred to a health clinic for iron supplementation if hemoglobin values were <100 g/L.

Study Population

Infants and caretakers (mothers/caretakers >16 years of age) were eligible for the study if the infants were 5–18 months of age at time of recruitment, not participating in a child health brigade that provided vitamin A supplementation, residing within the three study municipalities, had no plans to move outside of the study region in the next 2 months, and had no medical conditions. Infants with congenital anomalies, mental retardation, severe physical handicap, under-nutrition caused by medical conditions, and allergy to peanuts (determined with an allergy reaction test using 5–15 grams of LNS) were ineligible. Infant

with a weight for height z-score ≤ −2 standard deviations below the norm were not eligible and were referred to a qualified health care provider.

Sample Size

We attempted to recruit 150 participants per study group, based on funding as well as on a cluster randomization power analysis (Software Power and Sample Size (PASS), NCSS LLC; http://www.ncss.com. See online Supplementary Material for details.

Intervention

Participants in the intervention group received a monthly supply of a LNS product (*Plumpy'doz* by Nutriset, Malaunay, France) throughout the 12-month study period. *Plumpy'doz* provides 247 kcal, 5.9 g protein, 16.0 g fat, 400 μg vitamin A, 0.9 μg vitamin B_{12}, 9 mg iron and 9 mg zinc in a 46.3 g dose. Caretakers in the intervention group were counseled to feed 3 tsp, 3 times per day (a total of 46.3 g) of LNS to infants 6–12 months and 4.5 tsp. 3 times per day (a total of 70 g) to children 13–30 months. These doses were informed by consultation with experts, recognizing that the product had never been used for this purpose and in this setting. Infants enrolled at 5 months were gradually transitioned to the 46.3 gm dose when solid foods were introduced. Caregivers were advised on correct spoon size and were allowed to mix the product with other foods. The Project Staff counseled mothers to continue breast feeding as normal and not to force feed LNS to the children.

Given the high levels of food insecurity in this region, our study provided food vouchers to all participants, as well as monthly nutritional education sessions. The food vouchers were also intended to offset sharing of the LNS product with other children in the intervention families. Food vouchers were granted based on household size: <4 = L 200 per month, 5–8 = L 300 per month, >=8 = L 400 per month (L= Lempiras; 18L = US $1 during the study period). These were redeemable at local stores for rice, beans, corn, vegetables and fruits which provided only a minimal percentage of the household's food supplies. Use of the food vouchers by study participants and store owners was monitored. Ten culturally- tailored, age-appropriate educational sessions were delivered on nutritional and health topics.

Data and Blood Sample Collection

Non-fasting blood samples were collected at baseline, 6 months and at the end of the 12 month study period. Venous blood samples were drawn by a nurse using trace element free BD Vacutainer tubes (1.5 mL), 2 hour post-prandial (See online Supplementary Material for details). Hemoglobin was measured at baseline and every three months thereafter from a finger prick using a StatSite-MHgb (Stanbio Laboratory, Boerne, Texas) and recorded to the nearest g/L. The StatSite-MHgb was calibrated three times during the study.

Age-specific cut points for deficiency or suboptimal states were used for hemoglobin[16] and B_{12}.[17] For biomarkers without age-specific cut-points, generally-accepted cut-points were used: <3 ng/ml for folate[18], >8.3 ug/ml for soluble transferrin[19], <65 ug/dl for zinc[20], <0.70 μmol/L of retinol for Vitamin A deficiency[21,22] and <170 nmol/L for riboflavin.[23] For CRP, >3 mg/L indicated acute inflammation. [24]

Sociodemographic, Anthropometric, Diet and Food Security Measures

Baseline demographic information was collected using culturally-appropriate questionnaires. Infant weight and length were measured monthly, without clothing or wet diapers, using an electronic weighing scale to the nearest 10 g for weight, and with an infantometer to the nearest 0.10 cm for length. Growth parameters were assessed using WHO 2006 Child Growth Standards.[25] Monthly 24-hour recalls were conducted by two trained local staff using the multiple-pass approach with the aid of measurement models (i.e. cups, plates) and the Minnesota Nutrition Data System for Research food guide.[26] Additionally, a validated food security questionnaire was implemented at baseline, 6 months and end of study[27]. The results for these secondary outcomes are not included in this analysis.

Statistical Methods

A concern about the potential effect of LNS on breastfeeding led us to determine the proportion of women who self-reported any breast feeding (yes/no) from the 24-hour recall at baseline, 3, 6, 9, and 12 months. Based on the intent-to-treat assumption and LNS adherence, generalized linear regression models were estimated using generalized estimation equations (GEE) to compare treatment and control groups with randomization at the village (group) level. The analyses were performed using SAS procedure GENMOD.

Retinol analyses included CRP levels as a covariate. Micronutrient biomarkers in their continuous form were log-transformed and complete case analysis was used for each outcome variable. Binary outcome variables were created for biomarker analyses based on deficiency cut points described above. Hemoglobin values were adjusted for altitude using the correction by Dirren et al.[28]

Sub-analyses were done with regard to adherence to the LNS consumption protocol assessed from mother's report of child's dietary intake collected by 24 hour recalls at 3, 6, 9 and 12 months. Two variables described adherence, one based on the study's protocol called "protocol" adherence (at least 46 g/d for children <12 months and at least 70 g/d for those ≥12 months), and the other based on "any consumption" of the product. These variables were coded dichotomously, with adherence coded as 1. We computed the average amount of LNS consumed only among those children coded as adherent.

Missing data occurred primarily due to participants' missed visits (see figure 1). The amount of missing data was similar between treatment groups at each time point though missed visits were more frequent toward the end of the study. Multiple imputation was used to examine the influence of missing data using the MI procedure in SAS with no difference in results between the imputed and intent-to-treat analyses (See online Supplementary Materials for details).[29–33] Therefore, only the intent-to-treat and subanalyses results are presented in this paper. SAS (version, 9.3; SAS Institute, Cary, NC) was used for all data analyses.

Results

Of the 300 children enrolled in the study, 160 were in the intervention group and 140 in the control group. Sample collection rates at baseline, 6 months, and 12 months are shown in Figure 1. After verification of eligibility criteria our final sample size was 298. Table 1 details the socio- demographic and selected maternal characteristics of the participants. Because the randomization occurred at the village, and not at the participant, level, we would not expect the treatment groups to have similar characteristics. Mean (±SD) length-for-age, weight-for-age z, and weight-for-length z scores were −0.56 ± 1.05, −0.35 ± 0.93, and −0.08 ± 0.99 for the control children respectively and −0.72 ± 1.06, −0.53 ± 0.96, and −0.19 ± 0.91for the intervention children; these were not statistically different (data not shown). The mean food insecurity score for the population at baseline was 33 ± 5.4 (range 19 to 42) which indicates moderate food insecurity.[34]

Adherence to the intervention

Overall, there was low adherence to the study protocol of consuming *Plumpy' doz*. At study month 3, protocol adherence was 5% (Table 2). Adherence increased to 9% at study month 6 and then declined. The mean daily intake of LNS by children with protocol adherence ranged from 82 g to 105 g. However, approximately 73% of children reported any LNS consumption from study months 3 to 9 and this decreased to 69% at month 12. The average amount consumed by children in this category ranged from 34 g to 50 g, achieving stability after month 6 (Table 2). Given the low adherence to the suggested LNS dose, the sub-analysis was limited to the main study outcomes of the 'any' dose category.

The pattern of self-reported breastfeeding was different between the study groups (See Supplemental Table #1 for details). At baseline and at three months, there were no differences between study groups in the proportion of caregivers who reported any breastfeeding. However, beginning at month 6, a greater reduction in breastfeeding was observed in the intervention group compared to the controls (64% control versus 56% in the intervention group) and this continued throughout the remaining intervention period.

Micronutrient status

There were no subjects identified as folate deficient in either group at any time during the study. At baseline there were significantly lower concentrations of retinol and thus a higher frequency of vitamin A deficiency in the control group compared to the intervention group (Table 3). Additionally we observed higher levels of CRP in the intervention group compared to the control. No other significant differences between intervention and control groups were noted.

At the 6th month time point, a significant difference between groups was found for the mean concentration of vitamin B_{12} and retinol; the latter was adjusted for baseline level and time-specific CRP levels (Table 3). Additionally, the intervention group had a significantly lower proportion of children classified as deficient for B_{12}. The intervention group had a higher mean concentration for folate compared to controls (P=0.06).

At the 12th month time point, mean folate concentration continued to be higher in the intervention than the control group (Table 3). The trend towards higher levels of B_{12} in the intervention group (285.3±2.04), compared to the control group (227±2.16) continued (P=0.08). Children in the intervention group continued to have lower risk of vitamin B_{12} and A deficiency.

The subanalysis taking into account the adherence reflected those found with the intent-to-treat analysis (See online Supplementary Materials for details).

Adverse effects

Throughout the 12 month study duration, no participants experienced any clinician-documented adverse effect and none experienced a serious adverse effect.

Comment

This trial of an LNS product conducted in Intibucá Honduras for children 6–18 months resulted in improved mean vitamin B12, folate and retinol concentrations after 6 months of the intervention. After one year of the intervention, a significant difference between the intervention and control groups continued for folate concentrations and the intervention group had a significantly lower prevalence of vitamin A deficiency not attributed to baseline values or CRP levels. Furthermore, the trend of improvement for B_{12} continued. Subanalysis based on adherence with the LNS product mirrored the results found in the intent-to-treat analysis, with improved significance.

This study shows that the LNS supplement was able to improve mean folate concentration for the children; mean plasma folate concentration ranged from 16 to19 ng/mL and normal folate concentration for children usually ranges between 2.7 to 17 ng/mL.[35] Folate deficiency was not detected at any time point in either group. We speculate that the control group may have had increased consumption of folic acid fortified wheat (as a result of increases in corn prices) or high intake of folate rich foods such as beans due to the vouchers. In Honduras more than 80% of wheat flour is fortified with iron, folic acid and vitamins B_1, B_2 and niacin as established by a national mandate implemented in 2002.[36]

Our study population had a baseline prevalence of anemia (50.0% control and 39.6% intervention) of severe public health significance as defined by WHO.[37] In contrast to studies conducted in Africa among moderately malnourished children, results from this study did not show improvements in iron status as measured by hemoglobin or transferrin receptors.[5,10] This difference in the study populations, moderately malnourished versus non-malnourished children, may explain our lack of findings for iron status improvement.

Vitamin A (retinol) deficiency also met criteria for severe public health significance[38] and was higher in the control group at baseline (45% control vs. 27% intervention). This intervention reduced vitamin A deficiency to moderate levels (≥10% to <20%) among children in the intervention group, values comparable to those reported in a Honduran national survey in 1996 .[38] Serum retinol concentration was significantly improved in the intervention group at the 6 month time point and to a lesser degree at the 12 months after controlling for baseline values and CRP. The ability to detect larger differences between

groups could have been masked by concomitant vitamin A supplementation by the health clinic prevention programs. Although the study guidelines instructed clinical staff not to provide additional vitamin A supplementation to children participating in the study, it was difficult to monitor adherence to these instructions. To the best of our knowledge, this is the first LNS trial to measure and report significant increases in retinol among intervention participants.

The risk of zinc deficiency in this population is high (37.5–62.3% at baseline).[39] This trial aimed to supplement children with 9 to 14 mg of zinc daily, the upper level of suggested intake.[20,40,41] High consumption of phytates in their dietary staples (i.e. beans, corn), and low adherence to LNS may explain the consistently high prevalence of zinc deficiency throughout the study. Studies using food fortification have also shown no reduction in the prevalence of low serum zinc concentrations[42–44], which may be explained by poor absorption, or interference by the iron fortificant.[45] A lack of effect on plasma zinc levels was also reported in other studies.[10,12]

The lower than expected levels of adherence to the LNS protocol partly may be explained by measurement error or sharing of the product within the household. Adherence was measured using one 24-hour recall collected each month which did capture true consumption over the entire month if mothers ran out of LNS the day before. However, the very low proportion of participants reporting protocol adherence suggests this is not the only explanation. Furthermore, qualitative process evaluations showed that sharing or improper use of the product (i.e. spoon sizes) was present only among a small subgroup of households. Nevertheless, approximately 73% of participants reported consuming an average dose of 50 g/d with positive effects on vitamin B_{12}, folate and retinol. Adherence results indicated that a higher dose of 70 g/d was not achievable by the Honduran children 12–36 months living in a rural area.

This study reported a reduction in breast milk consumption among intervention participants, specifically after study month 6. These findings contrast those reported in Malawi and the Democratic Republic of Congo which reported no displacement of breast milk by complementary foods.[46,47] However, both of these studies used short follow up periods with small sample sizes and comparison groups that provided an equivalent source of energy; our control participants did not receive any other equivalent source of energy dense foods, therefore providing a more realistic scenario for comparison. This reduction of breast milk consumption among intervention participants is expected for children increasing the amount of complementary foods in their diet.[48]

A limitation of the study design was the integration of food vouchers and complementary feeding education to both groups. This most likely contributed to the improvement in food security we saw over time (data not shown), and limited our ability to detect larger changes. A second limitation was the inability to truly match the villages on all socio-economic indicators. The sampling clusters differed in some socio-economic characteristics which may influence adherence to the trial and health outcomes. Finally, although we were unable to mask mothers to the intervention group and they were able to travel from an intervention to control village, data entry and laboratory personnel were masked to group allocation.

In summary, this study is the first LNS efficacy trial to be reported in Latin America. Caregivers were highly engaged in the process and had low attrition rates throughout the study period. Acceptability of the product was good and overall consumption of LNS by children that averaged close to 50 g per day is reasonable given the uniqueness of the product. Clearly, our expectation of 70 g for older children was too high. These results add to the current literature on the feasibility and efficacy of LNS strategies using integrated approaches to prevent malnutrition. Results from this study could inform future development of effectiveness LNS trials for hard-to-reach rural populations in Latin America.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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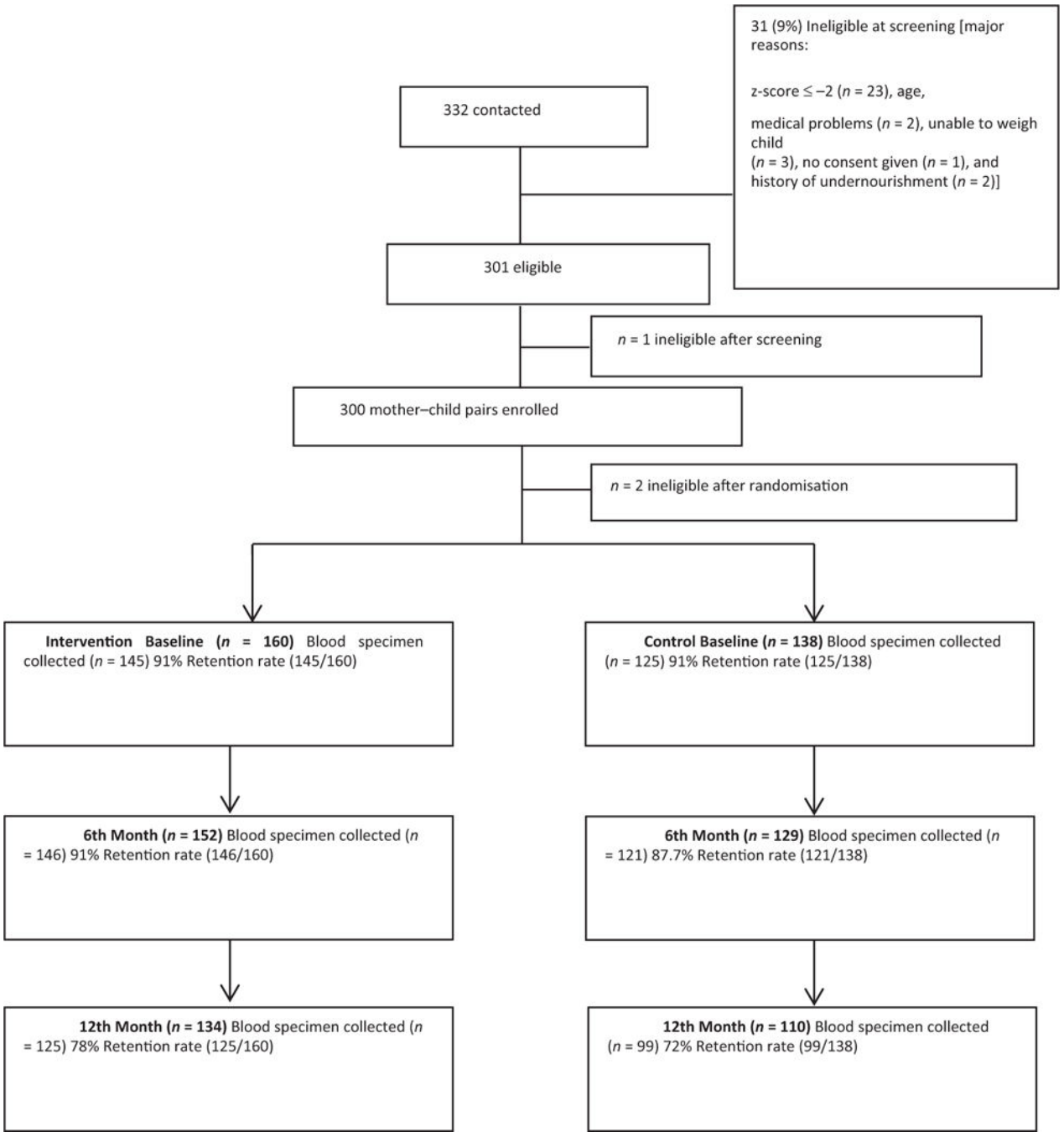

Figure 1.
Attrition rates based on completed blood specimen collection.

Table 1.

Sociodemographic characteristics of the study population at baseline per study group

Demographic Characteristic	Treatment (n=160)		Control (n=138)	
	No.	(%)	No.	(%)
Interviewee is the mother of participant				
No	14	(9)	4	(3)
Yes	146	(91)	134	(97)
Interviewee is the child's primary caregiver				
No	1	(1)	0	(0)
Yes	159	(99)	138	(100)
Age of primary caregiver (years)				
≤22	47	(29)	46	(33)
23–30	61	(38)	54	(39)
≥31	52	(33)	38	(28)
Maximum education level of primary caregiver				
Did not attend school	13	(8)	6	(4)
Primary school (1^{st}–6^{th} grade)	131	(82)	112	(81)
Secondary school (7^{th}–9^{th} grade)	6	(4)	7	(5)
Career/diversified	10	(6)	12	(9)
University	0	(0)	1	(1)
Marital status of primary caregiver				
Cohabiting	63	(39)	70	(51)
Married	42	(26)	42	(30)
Single/Divorced/Widowed/Separated	21	(13)	26	(19)
Age at first birth of primary caregiver/mother (years)				
≤22	132	(84)	113	(82)
23–30	24	(15)	21	(15)
≥31	1	(1)	3	(2)
Missing	3		1	
Total number of pregnancies				
1	45	(28)	44	(32)
2	29	(18)	32	(23)
≥3	86	(54)	62	(45)
Total number of live children				
1	49	(31)	50	(36)
2	31	(19)	33	(24)
≥3	79	(50)	54	(39)
Missing	1		1	
Number of people living in home				
≤4	33	(21)	26	(19)
5–8	82	(51)	74	(54)

Demographic Characteristic	Treatment (n=160)		Control (n=138)	
	No.	(%)	No.	(%)
≥9	45	(28)	38	(28)
Number of adults living in home				
≤2	86	(54)	64	(46)
3–5	62	(39)	62	(45)
≥6	12	(8)	12	(9)
Other people's children live/eat in interviewee's home				
No	116	(73)	87	(63)
Yes	44	(27)	51	(37)
Primary caregiver was employed at study entry				
No	143	(90)	125	(91)
Yes	16	(10)	13	(9)
Missing	1		0	
Age of Child				
≤12 months	86	(54)	72	(52)
> 12 months	74	(46)	66	(48)

Table 2.

Adherence and grams of *Plumpy'doz* consumed daily at four time points among intervention children.

Time Point (Sample size)	Protocol dose adherence[b]		Any Plumpy'doz consumption	
	%	Mean intake[a] (SD)	%	Mean intake[a] (SD)
Month 3 (149)	4.7	88.3 (42.4)	72.5	34.7 (23.8)
Month 6 (150)	8.7	102.2 (40.5)	72.7	50.5 (28.3)
Month 9 (128)	5.5	105.3 (43.6)	72.7	48.5 (26.9)
Month 12 (129)	1.6	81.6 (10.5)	69.0	50.0 (16.7)

[a] Mean intake in grams, SD=standard deviations among consumers only

[b] Protocol adherence: 46.3+ daily grams for children aged ≤12 months; 70+ daily grams for children aged >12 months

Table 3.

Intent to Treat, Comparison of biomarkers at three time points, per study group

Biomarker	Control[a] [n]	Intervention[b] [n]	P-value[c]
Vitamin B_{12} (pg/mL)			
Baseline	177.3±2.3 [93]	226.5±2.1 [105]	0.14
Month 6	189.1±2.0 [97]	248.5±2.0 [110]	0.03
Month 12	227.0±2.2 [99]	285.3±2.0 [122]	0.08
Vitamin B_{12} deficiency[d] (%)			
Baseline	58.1 [93]	47.6 [105]	0.25
Month 6	67.0 [93]	43.6 [105]	0.01
Month 12	61.6 [99]	48.3 [122]	0.09
CRP (mg/L)			
Baseline	0.75±4.2 [97]	1.35±5.2 [115]	0.03
Month 6	0.89±4.7 [96]	0.78±4.4 [114]	0.60
Month 12	0.74±6.2 [99]	0.73±6.8 [121]	0.96
CRP >3 mg/L (%)			
Baseline	18.6 [97]	33.0 [115]	0.02
Month 6	21.9 [96]	16.7 [114]	0.43
Month 12	23.2 [99]	20.7 [121]	0.53
Folate (ng/mL)[e]			
Baseline	17.36±1.4 [93]	17.66±1.5 [110]	0.76
Month 6	16.65±1.4 [97]	18.48±1.5 [115]	0.06
Month 12	16.25±1.4 [99]	18.73±1.4 [122]	0.002
Hemoglobin (g/L)[f]			
Baseline	101.8±1.2 [134]	106.7±1.1 [144]	0.10
Month 6	102.5±1.2 [128]	106.6±1.2 [144]	0.17
Month 12	105.7±1.1 [109]	108.7±1.1 [134]	0.14
Anemia (%)[f]			
Baseline	50.0 [134]	39.6 [144]	0.18
Month 6	65.6 [128]	50.7 [144]	0.07
Month 12	55.1 [109]	51.5 [134]	0.60
Transferrin receptors (μg/mL)			
Baseline	8.11±1.6 [97]	8.70±1.5 [115]	0.26
Month 6	7.81±1.7 [96]	8.20±1.6 [112]	0.55
Month 12	7.62±1.5 [98]	7.11±1.8 [122]	0.63
Transferrin >8.3 μg/mL (%)			
Baseline	49.5 [97]	52.2 [115]	0.68
Month 6	45.8 [96]	49.1 [112]	0.67
Month 12	28.6 [98]	26.2 [122]	0.86
Zinc (μg/dL)			

Biomarker	Control[a] [n]	Intervention[b] [n]	P-value[c]
Baseline	62.4±1.6 [109]	66.2±1.4 [128]	0.35
Month 6	65.6±1.3 [91]	64.9±1.3 [111]	0.84
Month 12	63.4±1.2 [77]	65.1±1.2 [99]	0.55
Zinc <65 μg/dL (%)			
Baseline	47.7 [109]	37.5 [128]	0.42
Month 6	51.7 [91]	54.1 [111]	0.83
Month 12	62.3 [77]	55.6 [99]	0.48
Retinol (μmol/L)[f]			
Baseline	0.75±1.3 [82]	0.80±1.3 [97]	0.006
Month 6	0.82±1.3 [89]	0.92±1.2 [102]	0.03
Month 12	0.82±1.4 [75]	0.88±1.3 [96]	0.34
Vitamin A Deficiency (Retinol <0.70 μmol/L) (%)[g]			
Baseline	45.1 [82]	26.8 [97]	<0.0001
Month 6	25.8 [89]	13.7 [102]	0.19
Month 12	33.3 [75]	17.7 [96]	0.03
Riboflavin (nmol/L)			
Baseline	215.9±1.7 [92]	254.4±1.4 [134]	0.11
Month 6	273.5±1.4 [78]	277.3±1.4 [52]	0.89
Month 12	261.4±1.3 [86]	265.1±1.3 [117]	0.78
Riboflavin <170 nmol/L (%)			
Baseline	20.7 [92]	8.2 [134]	0.18
Month 6	6.4 [78]	11.5 [52]	0.31
Month 12	4.7 [86]	3.4 [117]	0.70

[a,b] Geometric means and geometric standard deviation.

[c] P-values based on GEE analysis using PROC GENMOD, taking into account randomization at village level.

[d] Cut points were <167.9 pg/ml for 5–11 mo old, <266.79 pg/ml for 11–24 mo olds, and <319.6 for >24 mo olds.

[e] There were no cases of folate deficiency defined as <3 ng/mL at any time point.

[f] Hemoglobin values adjusted for altitude. Cut points were <100 g/L for <12 mo olds, <110 g/L for 12 to 24 mo olds, and <111 g/l for 2 to 5 yr olds.

[g] P-values for retinol and vitamin A deficiency based on GEE analysis using PROC GENMOD, taking into account randomization at village level and adjusted for baseline value and time point-specific measured CRP level.