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Long-Term Effects of the Rang-Din Nutrition Study Interventions on Maternal and Child Outcomes

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Contents

Abbreviations and Acronyms

AFA	arm fat area
ALA	α-linolenic acid
AMA	arm muscle area
ANCOVA	analysis of covariance
AOR	adjusted odds ratio
BMI	body mass index
CHDP	Community Health and Development Program
CHW	community health worker
CI	confidence interval
cm	centimeter(s)
cm^2	square centimeter(s)
DCCS	dimensional change card sort
FANTA	Food and Nutrition Technical Assistance Project
FCI	Family Care Indicators
g	gram(s)
HAZ	height-for-age z-score
Hb	hemoglobin
HFIAS	Household Food Insecurity Access Scale
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
IFA	iron and folic acid
iLiNS	International Lipid-Based Nutrient Supplement
kcal	kilocalorie(s)
kg	kilogram(s)
km	kilometer(s)
L	liter(s)
LNS	lipid-based nutrient supplement(s)
LNS-C	lipid-based nutrient supplement(s) for children
LNS-PLW	lipid-based nutrient supplement(s) for pregnant and lactating women
m^2	square meter(s)
MAL-ED	Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development
mg	milligram(s)
mm	millimeter(s)

mmHg	millimeter(s) of mercury
MMN	multiple micronutrient(s)
MNP	micronutrient powder(s)
MUAC	mid-upper arm circumference
MUACZ	mid-upper arm circumference-for-age z-score
OR	odds ratio
PCA	principal components analysis
PEDS DM	Parents' Evaluation of Developmental Status Developmental Milestones
RDNS	Rang-Din Nutrition Study
RR	relative risk
SD	standard deviation
SDU	safe delivery unit
SES	socioeconomic status
TSF	triceps skinfold
TSFZ	triceps skinfold-for-age z-score
UCD	University of California, Davis
UNIMMAP	UNICEF/World Health Organization/United Nations University international multiple micronutrient preparation
USAID	U.S. Agency for International Development
VHV	village health volunteer
WAZ	weight-for-age z-score
WHO	World Health Organization
WHZ	weight-for-height z-score

Executive Summary

There is very little evidence regarding the long-term impact of comprehensive nutritional supplementation during pregnancy and the first 2 years of life, the so-called "first 1,000 days," on both the mother's and the child's later growth, health, and development. The Rang-Din Nutrition Study (RDNS) was designed to evaluate the effectiveness, within a community-based program, of home fortification approaches for the prevention of maternal and child undernutrition during the first 1,000 days. Depending on the intervention group, the supplements included small-quantity (20 g/day) lipid-based nutrient supplements (LNS) for enriching home-based foods, one version for pregnant and lactating women (LNS-PLW) and another version for infants and young children (LNS-C), as well as micronutrient powder (MNP) for children. Women who did not receive LNS-PLW were provided with iron and folic acid (IFA). We previously reported on the effects of LNS-PLW on pregnancy outcomes (Dewey et al. 2016) and the postnatal effects of the RDNS interventions on child growth, development, micronutrient status, and health care seeking through 24 months of age (Dewey et al. 2017). This report describes results of a follow-up assessment of the RDNS participants when the children were 40–52 months of age, i.e., 16–28 months after the interventions ended. It includes indicators of child growth and body composition, cognitive development, and food preferences, as well as information on maternal height and body composition, blood pressure, and hemoglobin (Hb).

The RDNS was conducted in 11 rural unions[1] of the Badarganj and Chirirbandar sub-districts in the northwest region of Bangladesh and was carried out by three partners: LAMB (previously known as Lutheran Aid to Medicine in Bangladesh); icddr,b; and the University of California, Davis (UCD). LAMB was responsible for providing the study interventions to the study population via its Community Health and Development Program (CHDP), including the delivery of nutrient supplements. UCD and icddr,b jointly evaluated the interventions.

The study was designed as a researcher-blind, longitudinal, cluster-randomized effectiveness trial with four arms:

- Comprehensive LNS group, in which women received LNS-PLW during pregnancy and the first 6 months postpartum, and their children received LNS-C from 6 to 24 months of age
- Child-only LNS group, in which women received IFA (one tablet of 60 mg of iron and 400 µg of folic acid) daily during pregnancy and every other day during the first 3 months postpartum, and their children received LNS-C from 6 to 24 months of age
- Child-only MNP group, in which women received IFA daily during pregnancy and every other day during the first 3 months postpartum, and their children received MNP containing 15 micronutrients from 6 to 24 months of age
- Control group, in which the women received IFA daily during pregnancy and every other day during the first 3 months postpartum, and their children received no supplements

In the RDNS, we defined a cluster as the supervision area of a community health worker (CHW) of LAMB. The study was implemented in all 64 clusters within the 11 study unions. Each of the four study arms included 16 clusters. The intervention activities of the study were incorporated into the existing CHDP activities of LAMB. Between October 15, 2011 and August 31, 2012, we screened 4,410 pregnant women for eligibility and enrolled 4,011. At baseline, mean maternal age was approximately 22 years, mean education was about 6 years, mean height was 151 cm, mean body mass index (BMI) was about 20

[1] A union is the lowest administrative unit of the local government of Bangladesh, with a population size of about 30,000.

kg/m^2, about a third of the women were thin (BMI < 18.5 kg/m^2), and about 40 percent were nulliparous. The mean gestational age at enrollment was 13 weeks.

Among women who remained in the study, 3,664 live births took place between January 15, 2012 and May 5, 2013. Anthropometric data were collected for 3,516 infants at birth, 3,379 children at 24 months, and 3,400 children at 40–52 months (92.8 percent of live births).

Data analysis was performed based on intention-to-treat (i.e., no women or children were excluded from the analysis based on adherence to the supplements). Maternal outcomes were compared between the comprehensive LNS group and the other three groups combined (in which all women received IFA). Primary analysis of child outcomes was based on a four-group analysis. However, for child anthropometric outcomes, we also report a two-group comparison (comprehensive LNS plus child-only LNS groups combined vs. child MNP and control groups combined), based on the findings at 24 months (Dewey et al. 2017). In addition, we conducted sensitivity analyses for developmental outcomes at 40–52 months by excluding children from the Badarganj sub-district because children in that sub-district were eligible to receive MNP from a government program for children between 6 and 36 months of age, and we had observed effects of MNP on motor and language development at 24 months in the main trial. The government-distributed MNP program may thus have "contaminated" the control group in that sub-district during the intervention period and influenced outcomes for all of the study children in that sub-district because they may have received MNP when they were 24–36 months of age.

In the full sample, there were no significant differences among intervention groups in child growth status at 40–52 months. However, among female children, there were significant differences in weight-for-age and weight-for-height z-scores between the comprehensive LNS group and the control group, approximately 0.2 higher in the former; these differences were not significant among males. In the two-group comparison (combined LNS groups vs. combined non-LNS groups), the difference in height-for-age z-score was also significant among females (+0.09 higher in the combined-LNS group), but not among males. In addition, there was a significant interaction between intervention group and household food insecurity with regard to child stunting at 40–52 months: In households with moderate to severe food insecurity at baseline, stunting rates of children in the combined-LNS group were 5–6 percentage points lower than in the combined non-LNS group. This difference was most evident in female children (a difference of 8–9 percentage points).

We observed no significant differences among groups in the child development z-scores in the separate domains tested (visual-spatial skills, language skills, executive function, and pre-academic skills), but there was a marginal difference in the composite scores: Children in the child-only LNS group had higher scores (+0.12) than those in the control group. When we limited the sample to those in the Chirirbandar sub-district, who were less likely to have received MNP from the government program, this difference was greater (+0.18) and significant. We observed significant differences among groups in the visual-spatial scores, which reflect non-verbal cognitive ability: Children in the child-only LNS group performed better than those in the control group on visual-spatial skills (+0.22). In addition, we found a significant interaction between intervention group and child sex with regard to language skills in the Chirirbandar sub-district: Among females, the two LNS groups had 0.24–0.28 higher z-scores than the control group, whereas among males, the MNP group had 0.23 higher z-scores than the control group.

The RDNS interventions had no effect on child preferences for sweet foods or high-fat foods at 40–52 months of age. LNS-C contains fat and a small amount of sugar and is energy dense (5.9 kcal/g). It is thus reassuring that long-term consumption of LNS-C by children did not alter preferences for sweet or high-fat foods.

For mothers, supplementation with LNS-PLW during pregnancy and the postpartum period was not associated with long-term anthropometric indicators except for a small difference in maternal BMI (+0.19 kg/m^2 higher in the IFA group). These results indicate that maternal LNS-PLW supplementation for about 1 year did not contribute to maternal overweight and/or adiposity in the long term. Among those who were adolescents at enrollment in the trial (and thus may still have been growing), maternal LNS-PLW supplementation was associated with a small but significantly greater increase in height (+0.41 cm in the LNS-PLW group vs. +0.29 cm in the IFA group). There were no significant group differences in maternal blood pressure, Hb, or prevalence of anemia at 40–52 months postpartum.

We conclude that the RDNS intervention had long-term effects on certain outcomes. Although there were no significant group differences in growth status of the children as a whole, the weight of girls was higher in the comprehensive LNS group than in the control group, and among children born into households with moderate to severe food insecurity, the percentage who were stunted was lower among those who had received LNS than among those who had received MNP or no supplement. A similar interaction between intervention group and household food insecurity was observed for newborn stunting (Mridha et al. 2016). This has important implications with regard to the potential for targeting interventions such as LNS, which may be more beneficial for children born to women experiencing food insecurity. Interestingly, the effects of LNS on language development at both 24 months and 3–4 years of age were more evident in girls than in boys, which is consistent with the greater long-term effect on growth among girls than among boys. Further follow-up when the children are school aged will reveal whether these differences translate into school performance.

1 Introduction

Little is known about the long-term impact of comprehensive nutritional supplementation during the "1,000-day window"—the period from conception through a child's second birthday—on later maternal and child growth, health, and human capital (cognitive development, schooling outcomes, employment, income) (Dewey and Begum 2011). Only one of the five cohort studies examined by the Maternal and Child Undernutrition Study Group (Victora et al. 2008) used an experimental design, i.e., the long-term follow-up of a nutritional supplementation trial carried out in Guatemala between 1969 and 1977 (Martorell et al. 1995). That study demonstrated that a pre- and postnatal nutrition intervention that increased height in early life also resulted in significant differences in intellectual functioning at 11–26 years of age and reading comprehension and intelligence scores at 26–42 years of age. Wages earned by men (26–42 years of age) who had received the nutrition supplement during the first 2 years of life were 46 percent higher than among men in the control villages (who had not received the nutrition supplement).

Growth restriction in early life is linked not only to short adult height, but also to certain metabolic disorders and chronic diseases in adulthood (Victora et al. 2008). The "developmental origins of health and disease" hypothesis posits that the intrauterine and early postnatal environment can lead to life-long alterations in metabolic, endocrine, and cardiovascular function (Barker and Thornburg 2013).

The Rang-Din Nutrition Study (RDNS) in rural Bangladesh was designed to evaluate the effectiveness, within a community-based program, of home fortification approaches for the prevention of maternal and child undernutrition during the first 1,000 days. The most common type of home fortification is the use of micronutrient powders (MNP) to enrich complementary foods for infants and young children from 6 to 24 months of age (http://www.hftag.org/). A newer approach is to provide both micronutrients and some key macronutrients, including essential fatty acids, in small quantity (20 g/day) lipid-based nutrient supplements (LNS). LNS have been developed for enriching home-based foods for pregnant and lactating women (LNS-PLW), as well as for infants and young children (LNS-C) (Arimond et al. 2013). The main objective of the RDNS was to evaluate whether provision of LNS-PLW to women during pregnancy and the first 6 months postpartum, and/or provision of LNS-C to their offspring from 6 to 24 months of age, would result in larger positive changes in indicators of maternal and/or child health and nutrition among the study participants than the provision of iron and folic acid (IFA) during pregnancy and the postpartum period[2] and MNP or no supplementation for their children from 6 to 24 months of age.

Results to date indicate a significant impact of the intervention on birth size (Mridha et al. 2016) and on child growth and development at 18–24 months of age (Dewey et al. 2017; Matias et al. 2017). To our knowledge, the RDNS is the first study to report an effect of a prenatal nutrient or food supplement containing multiple micronutrients (MMN) on the prevalence of stunting at birth. In the sample as a whole, newborn stunting was reduced by 18 percent; among infants born before the 10-week disruption in supply of LNS-PLW,[3] the reduction was 30 percent. Similar reductions were seen in the prevalence of small head size at birth. The effects on head size may have critical implications for brain development and

[2] The World Health Organization and the Government of Bangladesh recommend providing IFA daily for at least 3 months postpartum, but we provided IFA (60 mg) every other day during the postpartum period to the control group because the recommended daily allowance for iron during lactation is only 9 mg and the tolerable upper-intake level is 45 mg (Arimond et al. 2013).

[3] During the RDNS, there was a 10-week disruption in the distribution of LNS in order to comply with a new quality control criterion for ready-to-use supplementary foods implemented by the World Food Program i.e., absence of *Cronobacter sakazakii*. During this time, PLW participants were provided with the same dose of IFA as participants in the three IFA treatment arms.

cognitive function. The differences in linear growth and head size were sustained through 24 months of age in the group in which both mothers and children received LNS. In all three intervention groups (comprehensive LNS, child-only LNS, and child-only MNP), there were significant positive effects on child development during the first 24 months of life, particularly for motor and language development.

This report describes results of a follow-up of the RDNS participants when the children were 40–52 months of age. It includes indicators of child growth and body composition, cognitive development, and food preferences, as well as information on maternal height and body composition, blood pressure, and hemoglobin (Hb).

2 Methods

2.1 Study Site, Design, and Ethics Statement

2.1.1 Study Setting and Population

The study was conducted in 11 rural unions[4] of the Badarganj and Chirirbandar sub-districts in the northwest region of Bangladesh, approximately 340 km northwest of Dhaka.

The study was carried out by three partners: LAMB; icddr,b; and the University of California, Davis (UCD). LAMB was responsible for providing the study interventions to the study population, including delivery of nutrient supplements. UCD and icddr,b jointly evaluated the interventions. The health services from LAMB were provided through its Community Health and Development Program (CHDP). For pregnant women, these health services include maternity services at a safe delivery unit (SDU) in each union and regular home visits for antenatal, postnatal, and child care by village health volunteers (VHVs) and community health workers (CHWs).

2.1.2 Study Design and Randomization

The overall aim of the RDNS was to evaluate the impact of nutrient supplementation during the first 1,000 days on the nutritional status of pregnant and lactating women and on the growth, nutritional status, and development of their children. The trial was designed as a researcher-blind, longitudinal, cluster-randomized effectiveness trial with four arms in the ratio of 1:1:1:1 (**Table 1**). The study arms were:

- Comprehensive LNS group, in which women received LNS-PLW during pregnancy and the first 6 months postpartum, and their children received LNS-C from 6 to 24 months of age
- Child-only LNS group, in which women received IFA (one tablet of 60 mg of iron and 400 µg of folic acid) daily during pregnancy (the standard of care) and every other day during the first 3 months postpartum, and their children received LNS-C from 6 to 24 months of age
- Child-only MNP group, in which women received IFA daily during pregnancy and every other day during the first 3 months postpartum, and their children received MNP containing 15 micronutrients from 6 to 24 months of age
- Control group, in which the women received IFA daily during pregnancy and every other day during the first 3 months postpartum, and their children received no supplements

[4] A union is the lowest administrative unit of the local government of Bangladesh.

Table 1. RDNS Interventions for Pregnant and Lactating Women and Their Children by Study Arm

Arm	Interventions for Pregnant and Lactating Women	Interventions for Children
1 – Comprehensive LNS	LNS-PLW during pregnancy and 6 months postpartum	LNS-C from 6 to 24 months
2 – Child-only LNS	IFA during pregnancy and every other day during the first 3 months postpartum	LNS-C from 6 to 24 months
3 – Child-only MNP	IFA during pregnancy and every other day during the first 3 months postpartum	MNP from 6 to 24 months
4 – Control	IFA during pregnancy and every other day during the first 3 months postpartum	None

As seasonality (time interval) was reported to be associated with some of the key outcomes of the study (e.g., birth weight) (Sebayang et al. 2012), we planned to recruit the women over a 1-year period so that all seasons would be represented.

We defined a cluster as the supervision area of a LAMB CHW. Each cluster covered a population of approximately 2,500 to 6,000 people and had three to six VHVs to assist the CHW. The study was implemented in all 64 clusters within the 11 study unions. Each study arm included 16 clusters. We chose a cluster-randomized design because it would have been difficult for a CHW to manage distribution of more than one type of supplement to the households in her or his cluster.

For the randomization, the study statistician at UCD first stratified the 64 clusters by sub-district and union, and then assigned each cluster to one of four sets containing 16 clusters each. This procedure was then replicated several thousand times and each randomization was tested for balance across groups with respect to mean cluster population, number of health facilities and health workers per 1,000 people, number of health- or nutrition-related nongovernmental organizations in the cluster, and source of funding for the CHDP, as well as the standard deviation (SD) of the cluster population size. The final randomization to the four arms was then chosen at random from the acceptable potential randomizations; the letters A, B, C, and D were assigned to the four sets, randomly permuting them by sorting on a randomly generated uniformly distributed number (using SAS for Windows release 9.2) and assigning them to control, child-only MNP, child-only LNS, and comprehensive LNS treatments.

For the follow-up at preschool age, all of the index children born to women who participated in the main trial, as well as the women themselves, were eligible to participate. We initially planned to assess children at approximately 48 months of age, but because of timing constraints with regard to funding, an age range of 40–52 months was chosen. A schedule for recontacting participants was developed to ensure that the average child age at the time of assessment was similar across treatment arms and regional unions and during each month of data collection (January through August 2016). For each week of follow-up data collection, the maximum number of children assessable during that week and the children's ages at that week were calculated. Then the appropriate number of age-eligible children were randomly selected for each week, without replacement, stratified by arm and union.

2.1.3 Ethical Review

The study protocol was approved by the institutional review boards of UCD, icddr,b, and LAMB. The study was registered at ClinicalTrials.gov [NCT01715038]. A modification of the protocol to include the follow-up study was approved by icddr,b and UCD in December 2015. All follow-up participants provided written consent for the follow-up activities.

2.2 Study Interventions

Table 2 shows the composition of the supplements used in the study. The LNS-PLW (20 g/day, 118 kcal/day, one sachet/day) was modeled on the UNICEF/World Health Organization (WHO)/United Nations University international multiple micronutrient preparation (UNIMMAP) for pregnant and lactating women and similar products used in Ghana and Malawi (Arimond et al., 2013). Ingredients included soybean oil, powdered milk, peanut paste, sugar, and MMN. LNS-C (locally called "Sonamoni," with 20 g/day, 118 kcal/day) was very similar to the small quantity LNS used in the International Lipid-Based Nutrient Supplements (iLiNS) Project, except that the iron content was 9 mg instead of 6 mg and the levels of folate, niacin, pantothenic acid, riboflavin, thiamine, vitamin B12, and vitamin B6 were slightly higher to cover the wider age range of 6–24 months. LNS-C was provided as two 10 g sachets per day. Because production of LNS in Bangladesh has not yet been established, LNS-PLW and LNS-C were produced by Nutriset SA in Malaunay, France. MNP (locally called "Pushtikona") was produced by Renata Ltd. in Bangladesh. The dose of IFA was based on WHO recommendations (WHO 2012). IFA tablets were produced by Hudson Pharmaceuticals Ltd. in Bangladesh.

Table 2. Composition of Supplements Used in the Study

Nutrient	LNS-PLW	IFA Tablet	LNS-C	MNP
Ration (g/day)	20	1 tablet	20	1 sachet
Total energy (kcal)	118	0	118	0
Protein (g)	2.6	0	2.6	0
Fat (g)	10	0	9.6	0
Linoleic acid (g)	4.59	0	4.46	0
α-Linolenic acid (g)	0.59	0	0.58	0
Vitamin A (µg Retinol Equivalents- RE)	800	0	400	400
Vitamin C (mg)	100	0	30	30
Vitamin B1(mg)	2.8	0	0.5	0.5
Vitamin B2 (mg)	2.8	0	0.5	0.5
Niacin (mg)	36	0	6	6
Folic acid (µg)	400	400	150	150
Pantothenic acid (mg)	7	0	2.0	0
Vitamin B6 (mg)	3.8	0	0.5	0.5
Vitamin B12 (µg)	5.2	0	0.9	0.9
Vitamin D (IU)	400	0	200	200
Vitamin E (mg)	20	0	6	5
Vitamin K (µg)	45	0	30	0
Iron (mg)	20	60	9	10
Zinc (mg)	30	0	8	4.1
Cu (mg)	4	0	0.34	0.56
Calcium (mg)	280	0	280	0
Phosphorus (mg)	190	0	190	0
Potassium (mg)	200	0	200	0
Magnesium (mg)	65	0	40	0
Selenium (µg)	130	0	20	17
Iodine (µg)	250	0	90	90
Manganese (mg)	2.6	0	1.2	0

LAMB CHDP staff carried out the delivery of the supplements in accordance with the randomization plan developed by the statistician at UCD, which was shared with CHDP staff members. The study evaluation staff received the randomization plan coded only as A, B, C, and D. None of the evaluation staff members was involved in supplement delivery.

The intervention activities, including training of the CHWs and VHVs, storage and distribution of supplements, nutrition education and counseling, and record keeping and reporting, were incorporated into the existing CHDP activities of LAMB staff and are described elsewhere (Dewey et al. 2016).

2.3 Data Collection

In the main trial, the CHWs and VHVs identified pregnant women as part of LAMB's pregnancy surveillance system, as described elsewhere (Dewey et al. 2016). Women were eligible to participate in the evaluation study if gestational age was ≤ 20 weeks and they had no plans to move out of the study area during pregnancy or the following 3 years. Evaluation study staff from icddr,b obtained informed consent and collected all baseline and follow-up data.

Data collection was performed by two separate teams: the "SDU team," who collected clinical and anthropometric data at the SDU, and the "home visit team," who enrolled mothers and collected baseline and follow-up data at participants' homes. For the follow-up at 40–52 months, the SDU team assessed child growth and cognitive development and maternal health outcomes (anthropometric indices, blood pressure, and Hb), and the home visit team collected data on child food preferences and the home environment (Family Care Indicators), as well as updated information on household socioeconomic status (SES), food security and other relevant variables. **Table 3** shows the data collection domains and outcome variables included in this report.

Table 3. Domains and Specific Measurements for Preschool Follow-Up

Data Collection Domain	Outcome Variables
Maternal Outcomes	
Maternal health	Attained height, body mass index (BMI), skinfold thickness, mid-upper arm circumference (MUAC), arm fat, and muscle area Hb, anemia, blood pressure
Index Child Outcomes	
Growth and anthropometric indicators	Height, height-for-age, stunting, weight, weight-for-age, weight-for-height, wasting, skinfold thickness, MUAC, arm fat, and muscle area
Cognitive development	Verbal ability (language), non-verbal ability (visual-spatial), executive function (e.g., attention, working memory, inhibition, cognitive flexibility); pre-academic skills
Food preferences	Preferences for sweet and fatty foods

2.4 Collection of Biological Samples

Blood samples were collected from a randomly selected subsample (n=1,078) of mothers during the SDU visits. Capillary blood was collected using a system specifically designed to collect capillary blood (finger pricks). The first drop of blood was wiped with dry cotton, and light pressure was applied to the end of the finger if needed to re-stimulate blood flow.

2.5 Quality Assurance

To the extent possible, both study evaluation teams were kept blind to group assignment. Quality control procedures included having the data collection supervisors re-interview 10 percent of randomly selected participants. During the revisit, selected questions were asked again and the responses were compared to

the original data collected. If there was less than 75 percent agreement, the data collector repeated the interview in the presence of the supervisor. The home visit and SDU team leaders and the study investigators made scheduled and unscheduled visits at homes and SDUs, respectively, to ensure the quality of the work and to respond to problems and issues.

2.6 Measurement of Outcome Variables

2.6.1 Maternal and Child Anthropometrics

All anthropometrists were trained and methods were standardized at the beginning of data collection and thereafter using methods described by WHO (WHO 2006). Calibration height rods and weights were used daily to check the accuracy of measurements, and tapes were routinely checked for signs of wear and stretching. Maternal and child heights were measured using a portable stadiometer (ShorrBoard®, Weigh and Measure LLC, Olney, MD, USA; ± 0.1 cm). Maternal and child weights were measured using a portable digital scale (Seca 874, Chino, CA, USA; ± 0.01 kg). Maternal and child mid-upper arm circumferences (MUACs) were measured to the nearest 0.1 cm using a non-stretchable tape (Shorrtape©, Weigh and Measure LLC, Chino, CA, USA). Maternal and child triceps skinfold (TSF) thicknesses were measured using Lange skinfold calipers (Beta Technology, Houston, TX, USA). Measurements were taken in duplicate, and a third measurement was done if the first two measurements differed by more than 0.5 cm for MUAC and height and by more than 0.1 cm for skinfold thickness. The mean of the two closest measurements was used in analysis.

2.6.2 Child Development

We measured several developmental domains, including visual-spatial ability, language, and executive function, and, because the children were close to school age, we also assessed pre-academic skills. Below we include descriptions of the child development tests used to assess each of these domains. Each outcome was defined as the sum of scores from individual items. If eight or more items were involved and the distribution of the total raw scores passed normality checks, then the raw scores were standardized to z-scores within smaller age bands (1–5 months wide) and then aggregated and treated continuously. If the outcome was assessed using fewer items, then it was treated as a count outcome and analyzed in negative binomial models categorized into three age band subsets and also aggregated.

Non-Verbal Ability

Block Design Test: This test was used to assess non-verbal (visual-spatial) ability by asking children to copy increasingly complex patterns using wooden blocks. The test is based on the Wechsler Primary and Preschool Scale of Intelligence (Wechsler 2002). For each item, the tester builds a model using wooden blocks and instructs the child to copy the model with a separate set of wooden blocks. This test included a total of 20 items (models). Rules were put in place that allowed the test to be stopped if the child was unable to replicate three consecutive models. Before being used in the RDNS follow-up, this test had been piloted with Ghanaian children between 4 and 6 years of age, showing sufficient internal and test-retest reliability (Ocansey and Prado 2015). The total score was calculated as the total number of designs correctly replicated by the child.

Language

To assess language development we used subtests of the NEPSY-II (Korkman et al. 2007). These subtests had been piloted with Ghanaian children between 4 and 6 years of age before implementation in the RDNS follow-up; they showed good internal and test-retest reliability (Ocansey and Prado 2015).

NEPSY-II Body Part Naming and Identification Test: This subtest assesses the child's basic language abilities in recognizing and naming body parts. For naming items, the child was asked to name the parts of the body on a figure of a child or on his/her own body if he/she is unable to recognize it on the figure. For identification items, the child was asked to point to the part of the body on the figure as the tester names them aloud. Two scores were calculated: the total number of items correctly named and the total number of items correctly identified. Psychometric analysis of pilot data from children in the study area indicated adequate internal consistency (Cronbach's Alpha coefficients were 0.73 for naming and 0.61 for identification).

NEPSY-II Comprehension of Instructions Test: This subtest is designed to assess the child's ability to understand oral instructions ranging from simple to complex instructions. For each item, the child was asked to point to the appropriate picture in response to the oral instruction given by the tester. Data from pilot testing conducted in the study area with non-RDNS children indicated adequate internal consistency for this test (Cronbach's Alpha coefficient was 0.60). The total score for this subtest was the total number of instructions correctly understood.

Executive Function

In the RDNS follow-up study, we attempted to assess several aspects of executive function, including cognitive flexibility, inhibition, and self-control, using the tests described below.

Separated Dimensional Change Card Sort (DCCS): This test is a measure of cognitive flexibility that assesses the child's ability to switch attention between two different dimensions, such as color and shape (Carlson 2005). Two target pictures that vary along two dimensions were presented. During the test, children were asked to complete six color trials, and then, after a rule switch, to complete six shape trials. The percentage of correct pre-switch (color) trials was used to evaluate comprehension of the task. The score was calculated only for children who were able to correctly sort at least 67 percent of pre-switch cards. An adapted version of this test was implemented in Bangladesh as part of the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study with 5-year-old children (Tofail 2015), and because it showed ceiling effects at that age, it was determined to be suitable for our younger RDNS sample. Psychometric analysis of RDNS pilot data indicated adequate internal consistency (Cronbach's Alpha coefficient was 0.72).

Fruit Stroop: This is an inhibitory control task that assesses whether the child can focus on a subdominant rather than on a dominant characteristic of an image (Kochanska et al. 2000). The tester shows the child three fruits (both a large and a small version of each). Then the child is shown three new pictures, each one showing a little fruit embedded in a drawing of a large, different fruit, and s/he is asked to point to the small fruit, which requires the child to suppress the inclination to choose the large fruit instead. Analysis of data from piloting testing conducted in the study area with non-RDNS children indicated adequate internal consistency (Cronbach's Alpha coefficient was 0.63).

Delayed Gratification Test: This test was designed to measure willpower or self-control in children. It is based on work conducted by Mischel and Ebbesen (1970) known as the "Marshmallow experiment." We tested whether or not the child can delay gratification from a tempting candy (of her or his preference) in between testing sessions. The test included three trials in between the testing sessions for the day. In each of the three trials, the child was shown the candy and asked to make the choice of having one candy immediately or having more candies later, specifically two, three, or four at the end of the second, third, and fourth tasks, respectively. Scoring was based on the number of candies the child chose to have. Before being used in the RDNS follow-up, this test was piloted with Ghanaian children between 4 and 6

years of age, and although it did correlate with age, it did not show sufficient test-retest reliability (Ocansey and Prado 2015).

Pre-Academic Skills

To measure pre-academic skills (literacy and numeracy) in the RDNS follow-up we used the Parents' Evaluation of Developmental Status Developmental Milestones (PEDS: DM) test.

Parents' Evaluation of Developmental Status Developmental Milestones: The PEDS: DM test was developed to screen development and behavior in children from less than a month old to 7 years 11 months of age based on chronological age (Glascoe and Robertshaw 2008). We used the PEDS: DM assessment version of the academic/pre-academic domain to assess early academic skills. Although the PEDS: DM can be administered by parental report, we tested the children directly by asking them to count, read aloud some words, or show some letters of the alphabet. Based on the age of the child, we selected eight items: four numeracy items and four pre-literacy items. Criteria for giving credit to answers were based on the tool's manual (Glascoe and Robertshaw 2008), slightly adapted to our sample. The total score for this pre-academic test was the number of items the child received credit for. Analysis of pilot data indicated inadequate internal consistency (Cronbach's Alpha coefficient was < 0.60), but adequate level of item discrimination (correlations item-test > 0.20).

In addition to the psychometric indicators described above, development scores from all these tests correlated positively and significantly with child's age and home stimulation scores in the RDNS follow-up sample.

2.6.3 Child Food Preferences

The objective of this study component was to assess whether there was any long-term effect of LNS supplementation on child food preferences. Because LNS is high in fat and has a slightly sweet taste, we focused on preferences for sweet and deep-fried or high-fat foods. We developed a list of eight such foods that were available locally and pilot-tested the initial questionnaire. Data on child food preferences were collected by interviewing mothers or primary caregivers of the index children at home, using standardized questionnaires administered by trained field staff. Respondents were asked to rate the child's preference for each kind of sweet or high-fat food using a 5-point Likert scale with ordinal responses ("my child prefers the food a lot," "my child prefers the food a little," "my child is neutral to the food," "my child dislikes the food a little," "my child dislikes the food a lot"). In addition, the respondent was asked two general questions about the overall preference of the child for sweet foods and high-fat foods.

2.6.4 Maternal Hemoglobin and Blood Pressure

The concentration of Hb was assessed using a portable photoreflectrometer (HemoCue America, Brea, CA, USA) approximately 45 seconds after blood sample collection. Blood pressure was measured twice in the right arm in a sitting position using portable sphygmomanometers. Calibration of the sphygmomanometers was done each day before data collection. An initial reading was taken at least 5 minutes after the subject was made comfortable, a second reading was taken after an interval of 10 minutes, and a third reading was taken (10 minutes after the second one) if the difference between the first two measurements was ≥ 10 mmHg for either systolic or diastolic blood pressure. The average systolic and diastolic blood pressure in mmHg was calculated.

2.7 Data Review, Entry, and Management

Data collectors manually checked all forms for completeness before leaving the participant's home or SDU. The data collection supervisors manually checked all the forms for both completeness and consistency before submitting the forms to the data management center for data entry. A reviewer at the data management center reviewed each form again for completeness and consistency before the data entry began. All reviewers recorded findings in a data query log, and the team leaders or supervisors corrected mistakes in the forms after contacting the data collectors and participants.

Data from all data collection forms were double entered in a database created using an Oracle® platform. Discrepancies between the first and second entry for data from all visits were corrected by checking the original data collection forms. Logic checks for different data domains were performed using STATA (version 12.0) to further clean the data. The data were subsequently subjected to case-by-case consistency and accuracy examination using STATA (version 12.0). Generated queries were resolved by consulting the original forms, with the help of the data collector or data collection supervisor, or by a repeat home or SDU visit whenever possible or appropriate.

2.8 Statistical Analyses

2.8.1 Sample Size and Power

For the main trial, we calculated a minimum required sample size of 788 per arm (total of 3,152 in four arms), based on detecting an effect size of > 0.2 (difference between groups, divided by pooled SD) for each continuous outcome with one-sided hypotheses, power=80 percent and α=0.05, assuming an intra-cluster correlation=0.01, and allowing for up to 20 percent attrition by the end of the study (i.e., when the children reached 24 months). Because we exceeded the target sample size during enrollment, we subsequently decided to conduct our analyses using a more conservative two-sided hypothesis approach, to be consistent with other recent trials.

During the first phase of the RDNS, 4,011 pregnant women were enrolled and there were 3,664 live births (to those who remained in the study at the end of pregnancy). Though a total of 3,383 children completed the study at 24 months, we expected that approximately 3,400 children would be available at about 4 years (roughly 850 per group). If we assumed that attrition (non-participation) by about 4 years of age would be 10 percent, the sample size would be approximately 3,060 (roughly 765 per group). Assuming 80 percent power, 95 percent level of significance, and 0.01 intra-cluster correlation, we estimated that we would be able to detect an effect size of $\geq$ 0.205 in continuous outcomes among the four arms. If attrition was 20 percent, the sample size would be approximately 2,720 (roughly 680 per arm), and, by keeping the same assumptions above, we would be able to detect an effect size of $\geq$ 0.213 among the four arms.

Maternal Hb was measured in a subsample. Under the assumptions listed above and adjusting for a 10 percent refusal rate, we calculated a sample size of n=564 per group (LNS and IFA; n=1,128 total). This sample size allows us to detect a minimum effect size of 0.20 in this outcome.

2.8.2 Outcome and Covariate Variable Definitions

For child anthropometric outcomes, we used WHO 2006 Child Growth Standards to determine weight-for-age z-score (WAZ), height-for-age z-score (HAZ), weight-for-height z-score (WHZ), mid-upper arm circumference-for-age z-score (MUACZ), and triceps skinfold-for-age z-score (TSFZ) (WHO Child Growth Standards 2011). We defined stunting as HAZ < −2, underweight as WAZ < −2, and wasting as WHZ < −2; we also defined < −2 SD as low values for MUACZ and TSFZ. We defined being in the

lowest 10 percent of the study sample as low values for arm muscle area (AMA) ([MUAC − (π x TSF)^2] ÷ 4π) (Gibson 2005) and arm fat area (AFA) (MUAC x TSF ÷ 2) (Rolland-Cachera et al. 1997). Extreme observations for z-scores were truncated at 4 units from the sample median.

For child development outcomes, total scores were determined as described above (Section 2.6.2). We calculated z-scores of the developmental outcomes based on the distribution of the RDNS sample. Out of the three language scores available, we chose to analyze the body part identification score due to higher numbers of missing data for the other two language scores. Besides the scores from each test, we used factor analysis to calculate a development composite score as the first identified factor. The factor analysis indicated that all the tests were measuring the same underlying variable and supported the use of this single composite score. The score was calculated as the weighted sum of all development tests reported, with the exception of the DCCS score (due to a large number of missing data from children who did not pass the "understanding" criterion). Roughly equal weighting was given to the fruit stroop test, the pre-academic test, and the block design test. Slightly higher weighting was given to the body identification test and lower weighting to the delayed gratification test.

The two key outcome variables for food preferences were the caregiver's overall ratings of the child's preference for sweet foods and for high-fat foods. We initially conducted descriptive analysis of the preferences for each type of sweet and high-fat food and found that the combined scores for each of these categories were in alignment with the ratings for overall preferences. Therefore, we decided to use the overall preference ratings. Because preferences for both categories of foods were generally highly positive, we created two dichotomous variables: yes/no for "prefers sweet foods a lot" and yes/no for "prefers high-fat foods a lot."

Maternal anthropometric outcomes include weight (kg), body mass index (BMI) (kg/m^2), MUAC (cm), TSF (mm), AFA, and AMA. Because the RDNS cohort included adolescent women who may still have been in active growth in height during (and possibly after) receiving LNS-PLW (Riley et al. 1989), change in height (cm) from baseline to follow-up was also analyzed as an outcome in this follow-up study, but only among women who were ≤ 19 years old at enrollment in the RDNS main trial.

We defined maternal anemia as Hb < 120 g/L. For currently pregnant women, we added 10 g/L to the value prior to data analysis because of hemodilution during pregnancy (Koller 1982). We defined high systolic blood pressure as ≥ 140 mmHg and high diastolic blood pressure as ≥ 90 mmHg. We then defined high blood pressure as high systolic or diastolic blood pressure.

From several SES variables, we calculated several composite variables, in which higher values represented higher SES. These variables include ownership index, air quality quintile, housing category, toilet type category, garbage disposal category, and a composite SES index. The ownership index was constructed using principal components analysis (PCA) from a set of 19 yes/no questions about whether or not a household owned a particular item. These items included televisions, irrigation pumps, tables, bicycles, sewing machines, and other goods. The air quality quintile was constructed from PCA on five questions about cooking methods and smoking, and then quintiles were constructed. Housing, toilet type, and garbage disposal categories were constructed based on the relative quality or reported construction. The composite SES index was constructed using PCA on the combined individual questions used above. The Household Food Insecurity Access Scale (HFIAS) (Coates and Swindale 2007) was used to calculate the HFIAS score, and participants were categorized into four levels of household food insecurity: severe food insecurity, moderate food insecurity, mild food insecurity, and food security.

We assessed home stimulation at follow-up using the Family Care Indicators (FCI) scale, developed by UNICEF (Kariger et al. 2012) and validated in Bangladesh (Hamadani et al. 2010). The FCI scale

consists of items (scored yes=1 and no=0) about play materials, activities with the child, and availability of reading materials at home. The total score was calculated as the sum of scores of all individual items.

2.8.3 Hypothesis Testing

A detailed data analysis plan was developed before starting each analysis and revealing group assignment. Primary analysis was performed based on intention-to-treat (i.e., no children or mothers were excluded from the analysis based on adherence to the supplements). All analyses adjusted for the randomization by accounting for the effect of union (nested within sub-district) and the random effect of cluster.

In the main trial, data collection was timed to the age of the target child. For the 4-year follow-up, data collection took place between the ages of 40 and 52 months. To account for this change in study design, all follow-up modeling controlled for child age at measurement as a fixed effect in addition to the existing study design covariates of cluster, union, and sub-district. Study design covariates were included in both unadjusted and covariate adjusted models.

Pregnancy status at measurement was expected to alter the maternal outcomes of weight, BMI, AFA, AMA, MUAC, and TSF, so models for these outcomes also included pregnancy status at measurement in the same way as study design covariates. We also expected that maternal Hb (after adjustment for hemodilution) and blood pressure may be influenced by pregnancy and thus included pregnancy status at the time of follow-up measurement as a potential adjustment covariate alongside the pre-specified baseline covariates.

For child and maternal anthropometry, development, Hb, and food preferences, effects of the intervention were analyzed using mixed model analysis of covariance (ANCOVA) for continuous outcomes, mixed model logistic regression for dichotomous outcomes, and mixed model negative binomial regression for count outcomes. For all analyses, if the global null hypothesis was rejected at the 0.05 level, then we performed post-hoc pairwise comparisons of groups correcting for multiple-comparisons using the Tukey-Kramer method. All models first evaluated the unadjusted effect of intervention before repeating those analyses with adjustments for covariates previously specified in our statistical plan. Those covariates were tested for association with each outcome ($p<0.10$) in a bivariate analysis, and only the covariates that met that criterion were included in adjusted models. In the analysis of count and continuous outcomes, we first calculated unadjusted group means before repeating those analyses with adjustments for covariates. In the analysis of dichotomous outcomes, we calculated unadjusted group percentages and 95 percent confidence intervals (CIs); statistical comparisons in unadjusted and covariate adjusted models were based on the log odds of the outcome occurring. Risk ratios for dichotomous outcomes controlling for the cluster randomization were calculated using log-binomial model estimations (McNutt et al. 2003).

In predefined subgroup analyses, we tested for interactions between intervention group and selected covariates listed by including each interaction term in the adjusted models. For significant effect modifiers ($p<0.10$), we assessed the adjusted group effect at different levels of the effect modifier (SAS LSMEANS option). Based on this assessment, selected effect modifiers were further examined in analyses stratified by the categorical covariate. When assessing the group effect at different levels of any significant effect modifier, we adjusted for multiple comparisons using the Tukey-Kramer approach. The covariates and effect modifiers examined are detailed in **Table 4**.

Maternal outcomes were compared between the LNS-LNS group and the combined IFA groups (IFA-LNS, IFA-MNP, and IFA-control). Primary analysis of child outcomes was based on a four-group analysis. For many child outcomes at 24 months of age, we observed similar results for certain

intervention groups (e.g., LNS-LNS and IFA-LNS). Because the 4-year follow-up occurred approximately 2 years since their last exposure to the interventions, we expected that this convergence of similar intervention groups may have continued. To investigate this, we combined intervention groups in secondary rounds of analyses of child outcomes. For each outcome, if no pairwise difference was found between IFA-LNS and LNS-LNS groups or between IFA-control and IFA-MNP groups, then they were combined. This resulted in two or three group comparisons in the secondary round of analyses, which followed the same analysis principles as the primary four-group comparisons.

In the Badarganj sub-district, the government of Bangladesh distributed MNP (containing vitamin A, vitamin C, folic acid, iron, and zinc) for children between 6 and 36 months of age. For study participants receiving LNS-C or MNP from the RDNS (through 24 months of age), the study team told caregivers not to feed any other vitamin and mineral tablets, capsules, or MNP sachets, but the control group was free to participate in the government MNP program. Moreover, since RDNS supplementation ended after 24 months, we expected that children in all groups in that sub-district might have received MNP supplementation from the government. Because we observed effects of MNP on developmental outcomes (but not growth) at 24 months, we conducted sensitivity analyses for developmental outcomes at approximately 4 years by excluding children from the Badarganj sub-district, i.e., only children in the Chirirbandar sub-district were included.

Table 4. Variables Tested as Potential Covariates and Effect Modifiers, by Outcome Category[a]

	Child Growth	Child Development	Child Food Preferences	Maternal Height	Maternal Weight	Maternal Hb and Blood Pressure
Maternal (at enrollment)						
Age	CE	CE		CE	CE	CE
Education	CE	CE	CE	CE	CE	CE
BMI	CE	CE		CE	CE	CE
Height	CE	CE		CE		
Primiparity	CE	CE		CE	CE	CE
Years since menarche				CE		
Duration of gestation	CE	CE		CE	CE	CE
Household (at enrollment)						
Food security	CE	CE	CE	CE	CE	CE
Children under 5 years		CE	CE			
SES index				C	C	
Air quality	CE	CE				
Garbage disposal	CE	CE				
Housing quality	CE	CE	C			CE
Asset ownership	CE	CE	CE			CE
Toilet type	CE	CE				
Religion		CE				
Nuclear family			C			
Child characteristics						
Sex	CE	CE	CE			
Age at measurement	CE	CE	CE	CE	CE	CE
School attendance			CE			
Post-enrollment						
Anthropometrist	C			C	C	
FCI score		CE				
Mother pregnant at measurement					C	CE
Pregnancy after study child			CE			

[a] "C" indicates potential covariate, "E" indicates tested effect modifier.

3 Results

3.1 General Context

3.1.1 Number of Women and Children Included in the Follow-up

Between October 15, 2011 and August 31, 2012, we screened 4,410 pregnant women for eligibility and enrolled 4,011 (1,047 in the LNS group and 2,964 in the three IFA groups combined). Of these, 3,664 live births took place between January 15, 2012 and May 5, 2013 to women who remained in the study. Thirty sets of twins were born (including stillbirths) and one twin from each pair was randomly selected for analyses. Anthropometric data were collected for 3,516 infants at birth, 3,379 children at 24 months (92.2 percent of live births), and 3,400 children at follow-up (92.8 percent of live births).

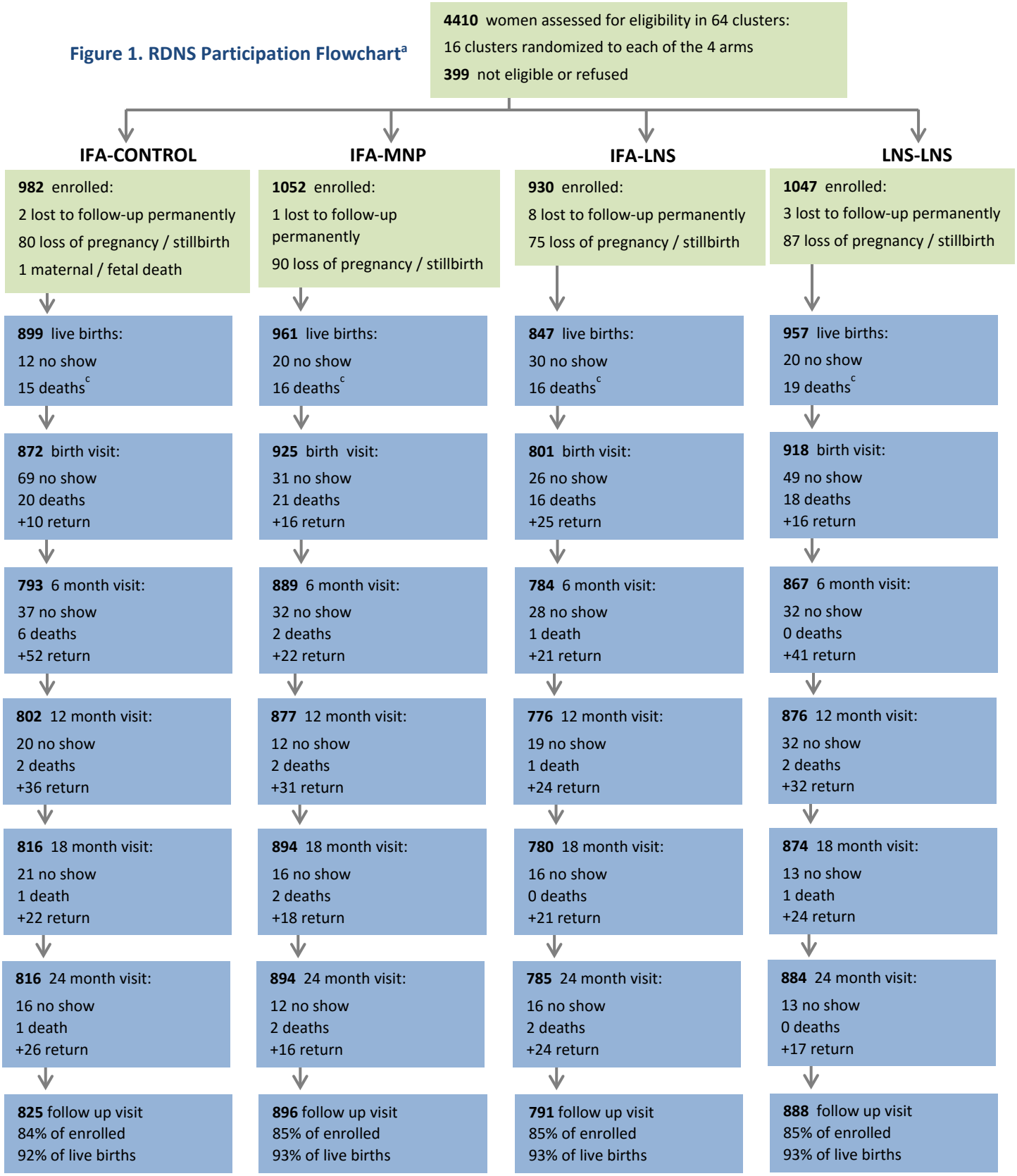
Data of any kind were collected for 3,459 mother-child dyads. The maximum number of mother-child dyads that we hoped to include in the follow-up was 3,499. Thus, the study team was able to gather data for 98.9 percent of the maximum potential number of participants. The primary reason for non-participation was moving out of the study area. **Table 5** shows the sample size for the main outcomes in the follow-up.

Table 5. Sample Sizes for Main Outcomes in Preschool Follow-Up

Outcome	N
Maternal anthropometric status	3,310 (82.5% of enrolled)
Child growth and anthropometric indicators	3,400 (92.8% of live births)
Child development	3,378 (92.2% of live births)
Food preferences	3,440 (93.9% of live births)

Figure 1 shows the RDNS participation flowchart.

Figure 1. RDNS Participation Flowchart[a]



[a] Includes only target children. Attendance to visit determined by anthropometric data

[b] 366 gestational age > 140 days; 22 planning to leave the study site; 8 refused to consent; 3 husbands refused to consent

[c] 1 death of infant at least 14 days old in IFA-Control, 1 in IFA-MNP, 1 in IFA-LNS, 2 in LNS-LNS

3.1.2 Characteristics of the Study Sample

At baseline, sociodemographic, anthropometric, and obstetric characteristics of participants were similar across intervention groups (**Table 6**). On average, the women were about 22 years of age. Mean maternal height was around 151 cm, mean BMI was about 20 kg/m^2, about a third of the women were thin (BMI < 18.5 kg/m^2), and about 40 percent were nulliparous. The mean gestational age at enrollment was around 13 weeks.

Table 6. Maternal Baseline Characteristics[a]

	LNS-LNS n=899	IFA-LNS n=803	IFA-MNP n=903	IFA-Control n=835
Age, years	21.8 ± 4.9	21.8 ± 4.8	22.0 ± 4.9	22.0 ± 5.2
Years of formal education	6.5 ± 3.1	6.3 ± 3.4	6.1 ± 3.2	6.1 ± 3.3
Household SES index	0.10 ± 2.3	0.07 ± 2.4	-0.04 ± 2.2	0.08 ± 2.2
HFIAS severe insecurity	69 (7.7)	60 (7.5)	87 (9.6)	84 (10.1)
HFIAS moderate insecurity	238 (26.5)	247 (30.8)	264 (29.2)	242 (29.0)
HFIAS mild insecurity	143 (15.9)	107 (13.3)	123 (13.6)	127 (15.2)
HFIAS secure	449 (49.9)	389 (48.4)	429 (47.5)	382 (45.7)
Height, cm	150.8 ± 5.3	150.5 ± 5.4	150.6 ± 5.4	150.7 ± 5.5
BMI (adjusted to 96 days of gestation) [b], kg/m^2	19.9 ± 2.7	20.1 ± 2.6	20.0 ± 2.6	20.0 ± 2.8
Low BMI (< 18.5 kg/m^2)	280 (31.1)	253 (31.5)	259 (28.7)	255 (30.5)
Nulliparous	372 (41.4)	336 (41.9)	336 (37.3)	314 (37.6)
Gestational age at enrollment, weeks	13.1 ± 3.8	13.2 ± 3.9	13.2 ± 3.9	13.1 ± 3.8

[a] All values are mean ± SD or n (%).
[b] Adjusted for 96th day of gestation via polynomial regression with the gestational age at measurement.

3.2 Impact on Child Growth at 40-52 Months of Age

Table 7 shows that there were no significant differences between intervention groups in HAZ, WAZ, WHZ, MUACZ, TSFZ, AFA, or AMA at approximately 4 years of age. Similar results were found for the dichotomous outcomes (**Table 8**). Adjustment for predetermined covariates did not change these results (data not shown). When the combined child LNS group (LNS-LNS and IFA-LNS) was compared with the combined non-LNS group (IFA-MNP and IFA-control), there was a marginally significant difference in WAZ: −1.58 ± 0.86 vs. −1.64 ± 0.89, respectively, p=0.064.

Table 7. Continuous Outcomes – Full Sample[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
HAZ	−1.52 (−1.60, −1.44)	−1.55 (−1.64, −1.46)	−1.61 (−1.69, −1.52)	−1.56 (−1.64, −1.47)	0.303
WAZ	−1.57 (−1.65, −1.49)	−1.60 (−1.68, −1.51)	−1.66 (−1.73, −1.58)	−1.63 (−1.71, −1.55)	0.241
WHZ	−1.03 (−1.11, −0.94)	−1.03 (−1.12, −0.95)	−1.08 (−1.16, −1.00)	−1.07 (−1.15, −0.99)	0.539
MUACZ	−0.65 (−0.72, −0.58)	−0.65 (−0.72, −0.58)	−0.70 (−0.77, −0.63)	−0.65 (−0.72, −0.58)	0.549
TSFZ	−0.38 (−0.47, −0.29)	−0.40 (−0.50, −0.31)	−0.41 (−0.50, −0.32)	−0.36 (−0.45, −0.27)	0.835
AFA (cm^2)	5.89 (5.75, 6.04)	5.83 (5.68, 5.98)	5.85 (5.70, 6.00)	5.91 (5.76, 6.07)	0.791
AMA (cm^2)	13.06 (12.90, 13.22)	13.07 (12.91, 13.24)	12.97 (12.81, 13.14)	13.02 (12.85, 13.19)	0.735

[a] All values are mean (95% CI).

Table 8. Dichotomous Outcomes – Full Sample[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
Stunting	29.4%	30.1%	32.0%	29.6%	0.713
	1.02 (0.76, 1.36)	1.03 (0.76, 1.39)	1.12 (0.84, 1.50)	–	
	1.01	1.02	1.08	–	
Underweight	30.9%	31.4%	32.4%	32.1%	0.858
	0.93 (0.70, 1.23)	0.98 (0.73, 1.30)	1.01 (0.77, 1.34)	–	
	0.95	0.98	1.01	–	
Wasting	13.6%	12.2%	13.1%	15.0%	0.467
	0.84 (0.57, 1.26)	0.80 (0.53, 1.20)	0.83 (0.56, 1.24)	–	
	0.86	0.82	0.86	–	
High WHZ[b] (≥ 1)	0.8%	1.5%	1.1%	0.9%	–
Low MUACZ	4.8%	3.2%	4.0%	4.1%	0.517
	1.18 (0.57, 2.43)	0.76 (0.34, 1.69)	0.97 (0.46, 2.04)	–	
	1.17	0.77	0.97	–	
Low TSFZ	8.0%	6.7%	7.4%	6.4%	0.761
	1.28 (0.67, 2.45)	1.07 (0.54, 2.10)	1.19 (0.61, 2.29)	–	
	1.25	1.06	1.17	–	
High TSFZ (≥ 1)	9.9%	8.6%	9.5%	10.7%	0.440
	0.98 (0.64, 1.50)	0.78 (0.49, 1.22)	0.88 (0.57, 1.36)	–	
	0.98	0.79	0.89	–	
Low AFA	11.3%	8.9%	10.4%	9.3%	0.680
	1.19 (0.72, 1.96)	0.96 (0.57, 1.63)	1.11 (0.67, 1.84)	–	
	1.16	0.96	1.10	–	
Low AMA	9.2%	9.9%	10.5%	10.4%	0.707
	0.88 (0.57, 1.36)	0.93 (0.60, 1.44)	1.05 (0.69, 1.60)	–	
	0.89	0.93	1.04	–	

[a] Results presented as prevalence, odds ratio (OR) (95% CI), relative risk (RR).
[b] Prevalence too low for analysis.

Results of tests for interactions with potential effect modifiers were significant for child sex (p for interaction 0.002–0.033). **Table 9** and **Table 10** show the continuous outcomes for females and males separately. Among females, there were significant differences in WAZ and WHZ and marginally significant differences in HAZ, MUACZ, and AMA in the 4-group comparison: mean values for WAZ and WHZ were about 0.2 z-score higher in the LNS-LNS group compared to the IFA-control group.

Among males, there was a significant group difference in WHZ and a marginally significant difference in MUACZ, but none of the pairwise group differences was significant.

Table 11 and **Table 12** show the dichotomous outcomes for females and males, respectively. There were marginally significant differences in underweight in both females and males, and for low AFA in males, but none of the pairwise group differences was significant.

When the combined child LNS group (LNS-LNS and IFA-LNS) was compared with the combined non-LNS group (IFA-MNP and IFA-control), there were significant group differences in HAZ and WAZ among females: −1.54 ± 0.88 vs. −1.63 ± 0.87 for HAZ, respectively, p=0.046, and −1.63 ± 0.87 vs. −1.73 ± 0.87 for WAZ, respectively, p=0.019. There were no significant differences among males, and no significant differences in the dichotomous outcomes among either females or males.

Table 9. Continuous Outcomes – Female Children[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
HAZ	−1.50 (−1.60, −1.39)	−1.59 (−1.70, −1.49)	−1.65 (−1.75, −1.55)	−1.61 (−1.71, −1.50)	0.075
WAZ[b]	−1.56 (−1.66, −1.46)[d]	−1.71 (−1.81, −1.61)[d,e]	−1.71 (−1.81, −1.61)[d,e]	−1.76 (−1.86, −1.66)[e]	0.008
WHZ[c]	−0.99 (−1.09, −0.89)[d]	−1.12 (−1.23, −1.02)[d,e]	−1.07 (−1.17, −0.97)[d,e]	−1.18 (−1.28, −1.07)[e]	0.028
MUACZ	−0.62 (−0.72, −0.53)	−0.73 (−0.83, −0.63)	−0.72 (−0.82, −0.63)	−0.74 (−0.84, −0.65)	0.099
TSFZ	−0.41 (−0.51, −0.30)	−0.49 (−0.60, −0.38)	−0.44 (−0.55, −0.34)	−0.45 (−0.56, −0.34)	0.715
AFA (cm^2)	6.09 (5.90, 6.28)	5.89 (5.70, 6.09)	6.01 (5.82, 6.20)	5.97 (5.78, 6.17)	0.434
AMA (cm^2)	12.93 (12.73, 13.14)	12.76 (12.54, 12.97)	12.69 (12.48, 12.90)	12.67 (12.46, 12.89)	0.090

[a] All values are mean (95% CI).
[b] LNS-LNS vs. IFA-control, p=0.007.
[c] LNS-LNS vs. IFA-control, p=0.020.
[d,e] Groups that do not share a common superscript differ from each other (p<0.05).

Table 10. Continuous Outcomes – Male Children[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
HAZ	−1.56 (−1.67, −1.44)	−1.52 (−1.63, −1.40)	−1.57 (−1.68, −1.46)	−1.50 (−1.62, −1.39)	0.784
WAZ	−1.60 (−1.70, −1.50)	−1.49 (−1.60, −1.38)	−1.61 (−1.71, −1.51)	−1.49 (−1.60, −1.39)	0.112
WHZ[b]	−1.08 (−1.18, −0.98)	−0.95 (−1.05, −0.84)	−1.08 (−1.18, −0.98)	−0.96 (−1.07, −0.86)	0.045
MUACZ	−0.69 (−0.77, −0.59)	−0.57 (−0.66, −0.48)	−0.67 (−0.75, −0.58)	−0.56 (−0.64, −0.47)	0.074
TSFZ	−0.38 (−0.50, −0.26)	−0.30 (−0.42, −0.17)	−0.37 (−0.48, −0.25)	−0.26 (−0.38, −0.14)	0.449
AFA (cm^2)	5.68 (5.49, 5.86)	5.80 (5.61, 5.99)	5.70 (5.52, 5.88)	5.87 (5.68, 6.06)	0.426
AMA (cm^2)	13.16 (12.97, 13.36)	13.37 (13.17, 13.58)	13.24 (13.04, 13.43)	13.38 (13.18, 13.58)	0.330

[a] All values are mean (95% CI).
[b] No significant Tukey-Kramer adjusted pairwise differences analysis.

Table 11. Dichotomous Outcomes – Female Children[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
Stunting	27.4%	32.6%	34.6%	30.9%	0.218
	0.88 (0.59, 1.33)	1.09 (0.73, 1.64)	1.20 (0.81, 1.78)	–	
	0.91	1.06	1.12	–	
Underweight	30.3%	37.5%	32.0%	36.3%	0.096
	0.76 (0.51, 1.14)	1.07 (0.72, 1.59)	0.83 (0.56, 1.23)	–	
	0.84	1.04	0.88	–	
Wasting	12.9%	12.9%	12.5%	16.7%	0.999
	0.72 (0.42, 1.22)	0.74 (0.43, 1.28)	0.70 (0.41, 1.19)	–	
	0.76	0.78	0.74	–	
Low MUACZ	4.5%	4.0%	3.9%	4.9%	0.917
	0.92 (0.37, 2.31)	0.81 (0.31, 2.12)	0.81 (0.31, 2.10)	–	
	0.92	0.81	0.81	–	
Low TSFZ	5.7%	6.1%	7.1%	6.3%	0.872
	0.87 (0.37, 2.05)	0.95 (0.40, 2.25)	1.12 (0.49, 2.56)	–	
	0.88	0.96	1.11	–	
Low AFA	7.5%	8.8%	8.9%	8.8%	0.840
	0.82 (0.39, 1.74)	1.02 (0.49, 2.15)	1.02 (0.49, 2.11)	–	
	0.83	1.02	1.01	–	
Low AMA	11.5%	15.1%	13.4%	14.4%	0.511
	0.78 (0.45, 1.35)	1.05 (0.62, 1.79)	0.95 (0.56, 1.62)	–	
	0.81	1.04	0.96	–	

[a] Results presented as prevalence, OR (95% CI), RR.

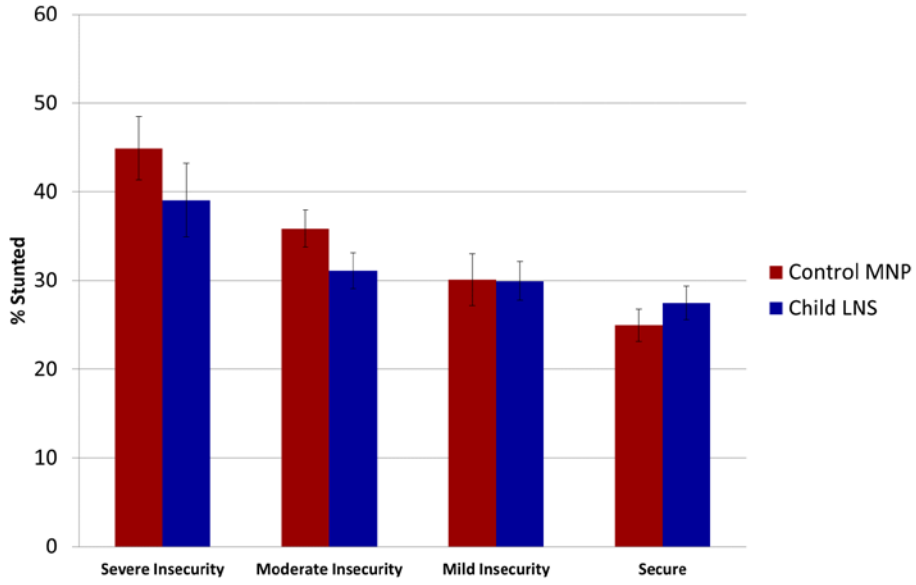
Table 12. Dichotomous Outcomes – Male Children[a]

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
Stunting	31.3%	27.9%	29.4%	28.1%	0.680
	1.18 (0.77, 1.81)	0.98 (0.63, 1.54)	1.05 (0.69, 1.62)	–	
	1.13	0.99	1.04	–	
Underweight	31.5%	25.3%	32.7%	27.8%	0.096
	1.18 (0.79, 1.77)	0.88 (0.57, 1.35)	1.26 (0.85, 1.89)	–	
	1.13	0.91	1.18	–	
Wasting	14.4%	11.5%	13.7%	13.3%	0.846
	1.05 (0.57, 1.95)	0.86 (0.44, 1.65)	1.02 (0.55, 1.91)	–	
	1.04	0.87	1.02	–	
Low MUACZ	5.2%	2.3%	4.2%	3.4%	0.286
	1.57 (0.55, 4.55)	0.67 (0.19, 2.39)	1.22 (0.41, 3.63)	–	
	1.55	0.68	1.21	–	
Low TSFZ	10.4%	7.4%	7.7%	6.5%	0.264
	1.74 (0.81, 3.74)	1.20 (0.53, 2.73)	1.22 (0.55, 2.71)	–	
	1.66	1.19	1.20	–	
Low AFA	15.1%	9.0%	11.9%	9.9%	0.079
	1.60 (0.86, 2.95)	0.90 (0.45, 1.79)	1.24 (0.66, 2.33)	–	
	1.50	0.90	1.22	–	
Low AMA	7.0%	4.6%	7.7%	6.5%	0.329
	1.12 (0.54, 2.31)	0.70 (0.30, 1.60)	1.20 (0.59, 2.42)	–	
	1.11	0.71	1.18	–	

[a] Results presented as prevalence, OR (95% CI), RR.

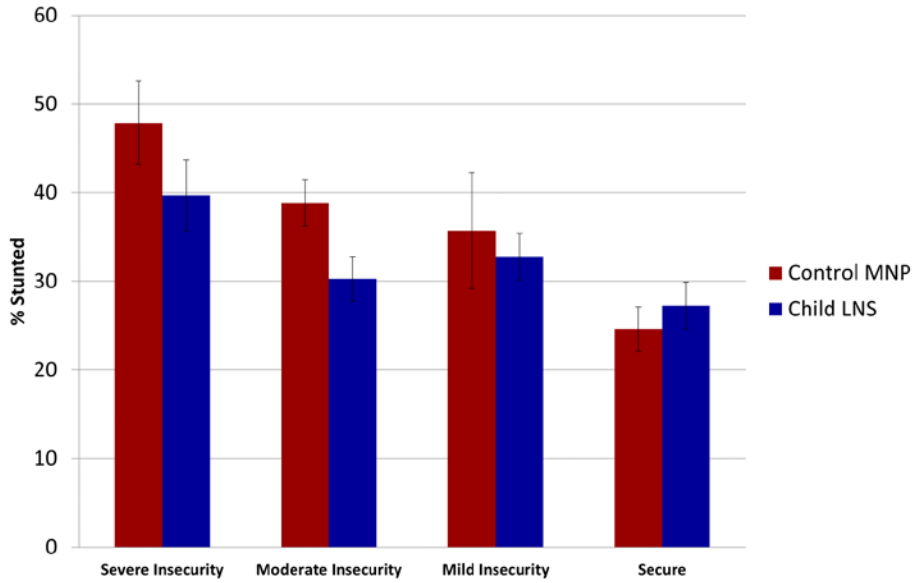
Other potential effect modifiers were generally not significant except for food insecurity. In the two-group comparison (sexes combined), stunting was less prevalent in the combined child LNS group than in the combined non-LNS group among children in households with greater food insecurity, but not in those with mild or no food insecurity (p for interaction 0.0217) (**Figure 2**). This was more evident in females (**Figure 3**) than in males (**Figure 4**).

Figure 2. Stunting at Follow-Up, by Food Security and Treatment Categories – Full Sample



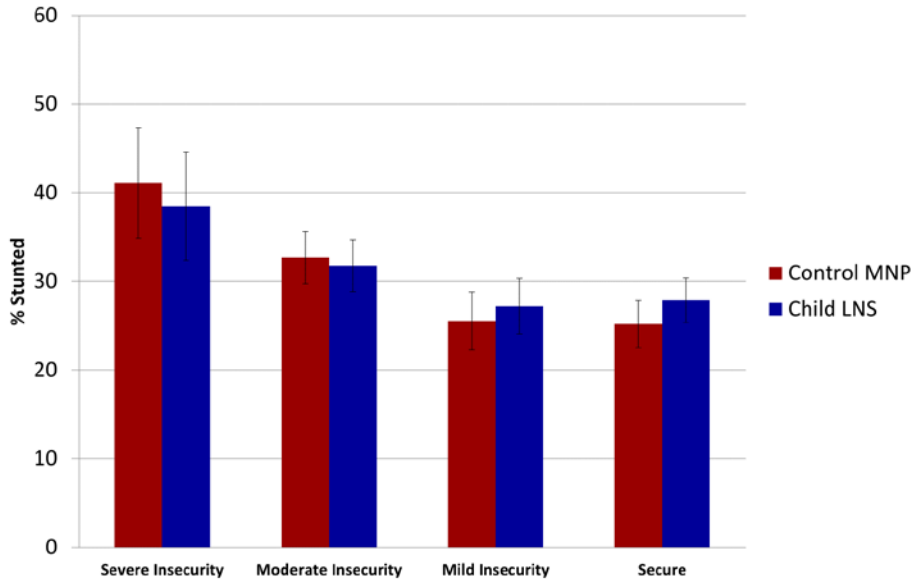
P for interaction between treatment group and continuous food security: p=0.022

Figure 3. Stunting at Follow-Up, by Food Security and Treatment Categories – Female Children



P for interaction between treatment group and continuous food security: p=0.011

Figure 4. Stunting at Follow-Up, by Food Security and Treatment Categories – Male Children



P for interaction between treatment group and continuous food security: p=0.596

3.3 Impact on Child Development

This section presents results on the effect of the interventions on the child development outcomes described in Section 2.6.2. Continuous outcomes were standardized to z-scores based on the distribution of the RDNS sample; standardization was calculated within smaller age bands (1–5 months wide) and then data were aggregated.

3.3.1 Visual-Spatial Skills

Table 13 shows that there were no significant differences among intervention groups in block design test z-scores. Adjustment for covariates yielded similar results. However, when the analysis was limited to children in Chirirbandar (**Table 14**), significant differences among groups were observed (p=0.031). Pairwise tests between groups in this sub-analysis indicated significant differences between the IFA-LNS and the IFA-control groups in z-scores (p=0.036) and marginally significant differences between the LNS-LNS and IFA-MNP groups and the IFA-control group (p=0.095 and p=0.089, respectively). After adjustment for predetermined covariates, the difference in z-scores between the IFA-MNP and the IFA-control groups became significant (p=0.026), while the difference between the LNS-LNS and the IFA-control groups was attenuated (p=0.127).

3.3.2 Language Skills

There were no significant differences among intervention groups overall in body parts identification z-scores (Table 13). Adjustment for covariates yielded similar results. Age-adjusted analysis of children in Chirirbandar (Table 14) indicated marginally statistically significant differences among groups; further adjustment for baseline covariates provided similar results (p=0.061).

3.3.3 Executive Function

No significant differences among intervention groups in z-scores in any of the three executive function tests were observed (Table 13). Similarly, there were no significant differences among groups in the z-scores of the fruit stroop, DCSS, and delayed gratification tests in Chirirbandar (Table 14). Adjustment for predetermined baseline covariates did not change the results for fruit stroop and delayed gratification (data not shown). DCSS scores were not further analyzed because of significant reduction in sample size as a result of the fact that 65 percent of children were unable to correctly sort at least 67 percent of pre-switch cards (i.e., they did not pass the criterion for understanding the test).

3.3.4 Pre-Academic Skills

No significant differences among intervention groups in pre-academic z-scores were observed (Table 13). However, adjustment for baseline covariates (i.e., having a child under 5 years at enrollment, FCI score, air quality, garbage disposal quality, housing quality, toilet quality, child gender; maternal age, BMI, height, and nulliparity at enrollment; and HFIAS score) resulted in a marginally statistically significant difference among groups (p=0.076). Similar age-adjusted (Table 14) and further-adjusted results were observed when the analysis was limited to children in Chirirbandar.

3.3.5 Development Composite Z-Score

Table 13 shows that marginally significant differences among intervention groups were observed in the composite score (p=0.097). Further adjustment for baseline covariates attenuated this marginal association (p=0.109).

When the analysis was limited to children in Chirirbandar (Table 14), significant differences among groups were observed for age-adjusted (p=0.0498) and further covariate-adjusted results (p=0.013). Pairwise tests between groups in this sub-analysis indicated significant differences between the IFA-LNS and IFA-control groups in z-scores (0.12 vs. −0.06; p=0.032); further adjustment for pre-determined baseline covariates yielded similar results (p=0.016).

3.3.6 Effect Modification for Child Development Outcomes

Tests for interactions with potential effect modifiers were significant ($p<0.05$) for one or more development outcomes with respect to maternal age (development composite z-score), maternal height (body parts identification z-score), maternal BMI (body parts identification z-score), gestational age at enrollment (pre-academic z-score), child's age (body parts identification and development composite z-scores), and child's sex (body parts identification in Chirirbandar). For language scores, differences among groups were more evident among female vs. male children (p-values for the interaction term were 0.067 in the full sample and 0.016 in the Chirirbandar sample; **Figure 5**). Other effect modifiers identified did not exhibit a consistent pattern and therefore were not explored further.

Table 13. Development Outcomes – Full Sample

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
Body parts identification z-score[a]	0.02 (−0.07, 0.10)	0.05 (−0.04, 0.14)	−0.01 (−0.10, 0.07)	−0.04 (−0.13, 0.05)	0.516
Block design z-score[a]	0.02 (−0.09, 0.12)	0.05 (−0.06, 0.15)	0.01 (−0.09, 0.11)	−0.06 (−0.17, 0.04)	0.308
Dimensional change card sort score[b]	3.00 (2.76, 3.24)	3.10 (2.86, 3.35)	3.01 (2.78, 3.26)	2.80 (2.58, 3.04)	0.362
Fruit stroop score[b]	1.82 (1.73, 1.92)	1.88 (1.78, 1.98)	1.82 (1.73, 1.92)	1.81 (1.72, 1.91)	0.773
Delayed gratification score[b]	1.54 (1.48, 1.60)	1.58 (1.52, 1.64)	1.57 (1.51, 1.63)	1.56 (1.50, 1.62)	0.851
Pre-academic z-score[a]	−0.03 (−0.15, 0.09)	0.05 (−0.07, 0.17)	0.06 (−0.06, 0.18)	−0.05 (−0.17, 0.07)	0.172
Composite development z-score[a]	−0.01 (−0.09, 0.07)	0.08 (0.00, 0.16)	0.01 (−0.07, 0.09)	−0.04 (−0.12, 0.04)	0.097

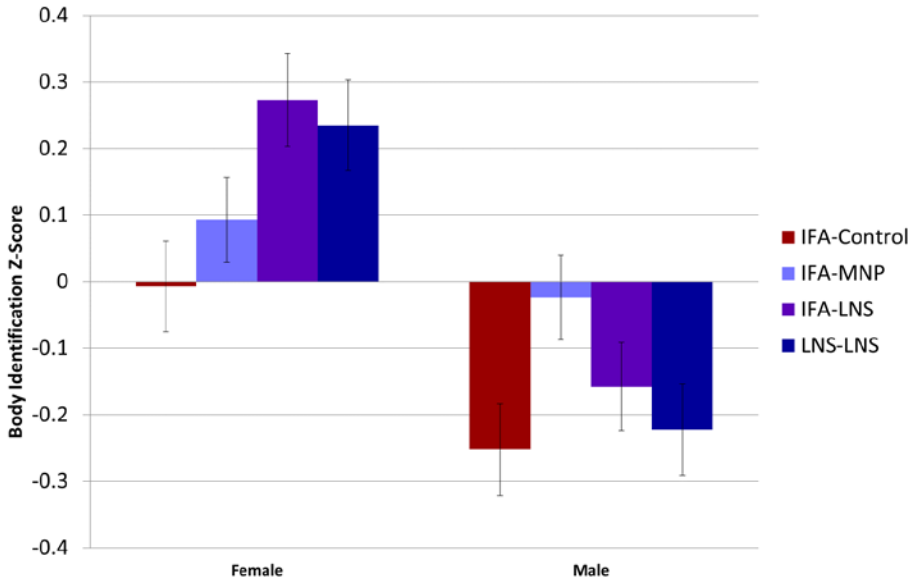
[a] ANCOVA age-adjusted; mean (95% CI).
[b] Negative binomial age-adjusted; expected score (95% CI).

Table 14. Development Outcomes – Chirirbandar Sub-District

	LNS-LNS	IFA-LNS	IFA-MNP	IFA-Control	p-value
Body parts identification z-score[a]	0.04 (−0.08, 0.16)	0.04 (−0.07, 0.16)	0.03 (−0.08, 0.14)	−0.13 (−0.25, −0.02)	0.096
Block design z-score[a,b]	0.10 (−0.03, 0.22)[e,f]	0.13 (0.00, 0.25)[e]	0.09 (−0.03, 0.21)[e,f]	−0.09 (−0.22, 0.03)[f]	0.031
Dimensional change card sort score[c]	2.95 (2.60, 3.34)	3.07 (2.72, 3.45)	3.10 (2.77, 3.47)	2.90 (2.56, 3.29)	0.832
Fruit stroop score[c]	1.81 (1.68, 1.96)	1.88 (1.75, 2.02)	1.83 (1.71, 1.96)	1.79 (1.65, 1.93)	0.787
Delayed gratification score[c]	1.55 (1.45, 1.65)	1.64 (1.54, 1.74)	1.58 (1.50, 1.66)	1.53 (1.45, 1.61)	0.306
Pre-academic z-score[a]	0.04 (−0.08, 0.17)	0.17 (0.05, 0.29)	0.15 (0.04, 0.26)	0.05 (−0.07, 0.17)	0.274
Composite development z-score[a,d]	0.03 (−0.07, 0.13)[e,f]	0.12 (0.02, 0.22)[e]	0.05 (−0.03, 0.13)[e,f]	−0.06 (−0.16, 0.04)[f]	0.0498

[a] ANCOVA age-adjusted; mean (95% CI).
[b] IFA-LNS vs. IFA-control, p=0.036
[c] Negative binomial age-adjusted; expected score (95% CI).
[d] IFA-LNS vs. IFA-control, p=0.032.
[e,f] Groups that do not share a common superscript differ from each other (p<0.05).

Figure 5. Body Parts Identification Z-Score, by Treatment Arm, Chirirbandar Sub-District



P for interaction between treatment arm and child gender: p=0.016

3.4 Impact on Child Food Preferences

As shown in **Table 15**, there were no differences in the prevalences of preference for sweet foods or high-fat foods among the four intervention groups. We also carried out further exploratory analysis by combining the two LNS groups (LNS-LNS and IFA-LNS) and comparing them with the combined non-LNS group (IFA-MNP and IFA-control) and did not find any differences. In the combined LNS group, 65.0 percent and 64.4 percent preferred high-fat and sweet foods, respectively; in the combined non-LNS group, 65.8 percent and 63.9 percent preferred high-fat and sweet foods, respectively.

Table 15. Child Food Preferences, by Intervention Group[a]

	LNS-LNS (n=898) %, OR	IFA-LNS (n=802) %, OR	IFA-MNP (n=902) %, OR	IFA-Control (n=835) %	p-value
Preference for sweet foods					
Unadjusted	64.4% 0.89 (0.67, 1.19)	65.6% 0.96 (0.71, 1.29)	64.0% 0.89 (0.66, 1.18)	67.8% –	0.640
Adjusted[b]	64.5% 0.91 (0.68, 1.22)	65.6% 0.97 (0.72, 1.31)	64.0% 0.87 (0.65, 1.17)	67.8% –	0.609
Preference for high-fat foods					
Unadjusted	65.1% 1.12 (0.86, 1.47)	63.5% 1.11 (0.84, 1.46)	65.5% 1.18 (0.90, 1.55)	62.3% –	0.421
Adjusted[c]	65.1% 1.15 (0.87, 1.51)	63.6% 1.13 (0.86, 1.49)	65.6% 1.19 (0.91, 1.57)	62.2% –	0.359

[a] Results presented as prevalence, OR (95% CI).
[b] Adjusted for number of children in household under age 5, family type (nuclear/joint), housing category, maternal education, household ownership category, and square root of food security.
[c] Adjusted for child's sex, maternal education, household ownership category, and square root of food security.

3.5 Impact on Maternal Health Outcomes

3.5.1 Maternal Anthropometric Status

Table 16 shows that there were no significant differences between groups (IFA vs. LNS-PLW) in maternal weight, BMI, MUAC, TSF, AFA, or AMA in the full sample. When we adjusted for covariates, mean maternal BMI in the IFA group was significantly higher than that observed in the LNS group (21.7 vs. 21.5 kg/m^2; p=0.020); marginally significant differences in the same direction were also observed for maternal weight (p=0.070), MUAC (p=0.099), TSF (p=0.067), and AFA (p=0.088) after adjusting for covariates.

Maternal age (p for interaction term=0.009) and parity (p for interaction term=0.038) at baseline modified the effect of the intervention on maternal weight at follow-up. Differences in maternal weight between intervention groups were more evident among older vs. younger women, and among those who already had a child at baseline vs. those who were nulliparous women (data not shown).

Among women who were adolescents at enrollment in the RDNS main trial (n=1,234 with data at the preschool follow-up), those who received LNS during pregnancy and postpartum had a greater increase in height than those who received IFA (+0.41 vs. +0.29 cm; p=0.025). Adjustment for covariates yielded similar results.

Table 16. Maternal Anthropometric Outcomes[a]

		Maternal LNS	Maternal IFA	p-value
Weight[b] (kg)	Unadjusted	49.21 (47.95, 50.58)	49.47 (48.32, 50.62)	0.515
	Adjusted[c]	48.96 (48.46, 49.47)	49.42 (49.04, 49.80)	0.070
Height difference[d] (cm)	Unadjusted	0.41 (0.28, 0.55)	0.29 (0.18, 0.40)	0.025
	Adjusted[e]	0.40 (0.27, 0.54)	0.30 (0.18, 0.41)	0.042
BMI[b] (kg/m^2)	Unadjusted	21.55 (21.01, 22.10)	21.74 (21.24, 22.24)	0.241
	Adjusted[f]	21.48 (21.27, 21.69)	21.71 (21.55, 21.88)	0.020
MUAC[b] (cm)	Unadjusted	26.51 (26.05, 26.96)	26.59 (26.17, 27.00)	0.560
	Adjusted[g]	26.42 (26.23, 26.61)	26.57 (26.42, 26.71)	0.099
TSF[b] (cm)	Unadjusted	1.66 (1.57, 1.75)	1.69 (1.61, 7.77)	0.327
	Adjusted[h]	1.64 (1.59, 1.69)	1.69 (1.64, 1.73)	0.067
AFA[b] (cm^2)	Unadjusted	22.91 (21.33, 24.50)	23.34 (21.91, 24.76)	0.406
	Adjusted[i]	22.57 (21.68, 23.45)	23.29 (22.61, 23.96)	0.088
AMA[b] (cm^2)	Unadjusted	36.29 (35.51, 37.08)	36.30 (35.64, 36.95)	0.995
	Adjusted[j]	36.20 (35.85, 36.55)	36.24 (36.03, 36.44)	0.848

[a] All values are mean (95% CI).
[b] Full sample; controlled for pregnancy status at measurement in unadjusted and adjusted models.
[c] Adjustment covariates include SES index, food security score, anthropometrist, gestational age at enrollment, and maternal age, education, BMI, and nulliparity at enrollment.
[d] Adolescent mother subsample (n=1,234).
[e] Adjustment covariates include maternal age, education, and years since menarche at enrollment.
[f] Adjustment covariates include SES index, food security score, gestational age at enrollment, and maternal age, education, BMI, and nulliparity at enrollment.
[g] Adjustment covariates include SES index, food security score, anthropometrist, gestational age at enrollment, and maternal age, education, BMI, and nulliparity at enrollment.
[h] Adjustment covariates include SES index, food security score, anthropometrist, gestational age at enrollment, and maternal age, education, and BMI at enrollment.
[i] Adjustment covariates include SES index, food security score, anthropometrist, gestational age at enrollment, and maternal age, education, BMI, and nulliparity at enrollment.

[j] Adjustment covariates include SES index, food security score, anthropometrist, gestational age at enrollment, and maternal age, education, BMI, and nulliparity at enrollment.

3.5.2 Maternal Blood Pressure

In the full sample, there were no between-group (IFA vs. LNS-PLW) differences with respect to mean diastolic blood pressure, mean systolic blood pressure, prevalence of high diastolic blood pressure, prevalence of high systolic blood pressure, or prevalence of high blood pressure. However, when adjusted for covariates, the prevalence of high diastolic blood pressure tended to be lower in the LNS-PLW group (0.96 percent in the LNS-PLW group, 1.91 percent in the IFA group; adjusted odds ratio [AOR], 0.46, 95 percent CI of AOR, 0.20, 1.07; p=0.071).

3.5.3 Maternal Hemoglobin

In the subsample for Hb assessment (n=946), there were no between-group (IFA vs. LNS-PLW) differences with respect to mean Hb or prevalence of anemia. The mean Hb was 127 g/L in both groups. The prevalence of anemia was 25.2 percent in the LNS-PLW group and 25.4 percent in the IFA group.

4 Discussion

4.1 Child Growth

In the full sample, there were no significant differences among intervention groups in child growth status at 3–4 years of age. However, among female children, there were significant differences in WAZ and WHZ between the LNS-LNS group and the control group, with an approximately 0.2 higher z-score in the former. There were no differences in growth status among males. In a two-group comparison (combined-LNS vs. combined non-LNS), the difference in HAZ also became significant among females (+0.09 in the combined-LNS group), but not among males. It is not too surprising that the intervention group differences in the full sample were not significant, given that the differences at 24 months were modest and there was already evidence of some attenuation in the odds ratios (ORs) for stunting between 18 and 24 months of age (Dewey et al. 2017). However, it is noteworthy that there was a significant interaction between intervention group and household food insecurity with regard to child stunting at 3–4 years of age: In households with moderate to severe food insecurity at baseline, stunting rates of children in the combined-LNS group were 5–6 percentage points lower than in the combined non-LNS group. This difference was most evident in female children (a difference of 8–9 percentage points). A similar interaction between intervention group and household food insecurity was observed for newborn stunting (Mridha et al. 2016).

4.2 Child Development

We observed no significant differences among groups in the child development scores in each domain, but there was a marginal difference in the composite scores: Children who received LNS postnatally (only) had higher z-scores (+0.12) than those in the control group. When we limited the sample to those in Chirirbandar, who were less likely to have received MNP from a government program, this difference in z-scores increased (+18) and reached statistical significance. We also observed significant differences among groups in the visual-spatial scores (block design test), which reflect non-verbal cognitive ability. In this sub-analysis, children in the postnatal LNS group performed better than those in the control group on visual-spatial skills (+0.22 z-score).

The lack of significant group differences in language skills in this follow-up study would suggest that the beneficial effects on language at 24 months that we previously reported (in all three intervention groups) (Matias et al. 2017) were not sustained at around 3–4 years of age. However, we observed a trend toward higher language scores in the intervention vs. the control groups at 3–4 years when the analysis was limited to Chirirbandar, where the control group was less likely to have received MNP. It is notable that the mean language scores (body part identification) in the three intervention groups did not differ much between the full sample and the subsample in Chirirbandar, whereas the scores for the control group were lower (by 0.09 z-score) in Chirirbandar than in the full sample. This is consistent with the possibility that the MNP received by children in the control group in Badarganj may have contributed to better language development, thereby attenuating group differences in the full sample.

In addition, we found a significant interaction between intervention group and child sex with regard to language skills in Chirirbandar: Among females, the two LNS groups had 0.24–0.28 higher z-scores than the control group, whereas among males the MNP group had 0.23 higher z-scores than the control group. A trend toward a sex difference in how language development responded to the intervention was also observed in the full sample at 24 months of age.

4.3 Child Food Preferences

The RDNS interventions had no effect on the preferences for sweet foods or high-fat foods at 3–4 years of age. LNS-C contains fat and a small amount of sugar, and is energy dense (5.9 kcal/g). Since acceptance of and preference for a particular food depends not only on its intrinsic, e.g., nutritional, properties but also on recent food experience (Freidin et al. 2012), it is reassuring that long-term consumption of LNS-C by children did not alter preferences for sweet or high-fat foods. There is evidence from animal studies that maternal high-fat food supplementation, e.g., with margarine, appears to "program" the offspring for increased leptin sensitivity and a lower preference for high-fat foods (Sanchez et al. 2012). In our case, the mothers of the children in the LNS-LNS group consumed LNS for about 1 year. Though we did not measure leptin sensitivity in the children, we did not find any evidence of a decline in the preference for high-fat foods among children in the LNS-LNS group. Therefore, our results suggest that neither LNS-PLW nor LNS-C influences child preferences for sweet or high-fat foods.

4.4 Maternal Health

In the full sample, maternal supplementation with LNS-PLW during pregnancy and the postpartum period was not associated with long-term anthropometric indicators, except for a difference in maternal BMI in the adjusted results (+0.23 kg/m^2 higher in the control group), a small difference that is not likely to have any clinical implications. These results indicate that maternal LNS-PLW supplementation for about 1 year did not contribute to maternal overweight and/or adiposity in the long term.

Among those who were adolescents at enrollment into the trial (and thus may still have been growing), maternal LNS-PLW supplementation was associated with a greater increase in height, but again, the difference between groups was very small (+0.10 cm higher in the LNS-PLW group). In girls, growth ceases at a median of 4.8 years after the onset of menarche and, although the total gain in stature varies considerably, most girls can grow up to 5–7 cm during that time (Spear 2002). Estimations from mostly never-pregnant Bangladeshi adolescent women suggest growth of about 4 cm during the first 3 postmenstrual years (Riley et al. 1989). However, pregnancy during this period may affect this process. In another longitudinal study also conducted in northern Bangladesh, no change in height from the first trimester of pregnancy to months postpartum was observed among adolescents (Rah et al 2008). Thus, although the gains in stature observed in the RDNS were small, maternal LNS-PLW supplementation may have the potential to positively affect growth in young mothers.

We did not observe any differences in systolic blood pressure, diastolic blood pressure, or the proportion of women with high systolic and/or diastolic blood pressure between the LNS-PLW and IFA groups. Although LNS contains ALA and ALA is known to lower blood pressure, we did not find any such effect in our study either at 6 months postpartum or at the time of the longer-term follow-up. One reason could be the quantity of ALA. LNS-PLW contains 0.59 g of ALA whereas 2.6 to 8 g/d is needed to lower systolic or diastolic blood pressure (Takeuchi et al., 2007). Therefore, LNS-PLW can be considered as a supplement that has no effect on the blood pressure of the women who consume it.

We also found no differences in mean Hb or prevalence of anemia between the LNS-PLW and IFA groups. This finding is not surprising since the follow-up occurred 34 to 49 months after the end of LNS-PLW or IFA consumption by the women. Moreover, LNS-PLW vs. IFA supplementation did not have an effect on mean Hb concentration or prevalence of anemia at 36 weeks gestation (Dewey et al. 2016) or at 6 months postpartum (data not yet published), although there was an effect on iron status of the women. However, it is expected that the differences in body iron stores would not persist up to 34–49 months after the end of consumption of the supplements.

4.5 Strengths and Limitations

This follow-up study has several strengths and limitations. Strengths include a very low rate of attrition (due mostly to travel out of the study area rather than refusal to participate), resulting in data collection at approximately 4 years of age for 92.7 percent of the children who were born alive; use of well-trained anthropometrists who performed measurements according to WHO standards that were standardized; and careful selection of developmental tests appropriate for the age of the children and thorough training of the child assessment team in administering the tests.

The main limitation was the government MNP distribution in one of the study sub-districts, which was beyond our control but may have affected outcomes in the "control" group in that sub-district. In addition, although we attempted to maintain blinding of data collectors conducting growth and development assessments to intervention group assignment, the home visit team that interviewed caregivers regarding child food preferences may have had prior knowledge of intervention group. Finally, we examined effects within several targeted subgroups, and these effect modification results need to be interpreted with caution due to the number of hypotheses being tested.

4.6 Conclusions

Several long-term effects of the RDNS intervention were observed when the index children were 3–4 years of age. Although there were no significant group differences in growth status of the children as a whole, among girls we found that WAZ and WHZ were higher in the LNS-LNS group than in the control group, and among children born into households with moderate to severe food insecurity, the percentage who were stunted was lower in those who had received LNS than in those who had received MNP or no supplement. This has important implications with regard to the potential for targeting interventions such as LNS, which may be more beneficial for children born to women experiencing food insecurity. Interestingly, the effects of LNS on language development at both 24 months and 3–4 years of age were more evident in girls than in boys, which is consistent with the greater long-term effect on growth among girls than among boys. Further follow-up when the children are school-aged could reveal whether these differences translate into school performance.

It is reassuring that there were no differences among intervention groups with regard to child food preferences, and no evidence of any increase in maternal overweight or blood pressure associated with consumption of LNS during pregnancy and for the first 6 months postpartum. It is intriguing that maternal consumption of LNS resulted in a small but significantly greater increase in maternal height among those who were adolescents at enrollment, suggesting that nutritional interventions long after the 1,000-day window have some potential to increase maternal stature in vulnerable subgroups—in this case, pregnant adolescents. Further research is needed to evaluate this potential effect in other populations.

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