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Daily Maternal Lipid-Based Nutrient Supplementation with 20 mg Iron, Compared with Iron and Folic Acid with 60 mg Iron, Resulted in Lower Iron Status in Late Pregnancy but Not at 6 Months Postpartum in Either the Mothers or Their Infants in Bangladesh

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Abstract

Background: Maternal anemia and iron deficiency are prevalent in low- and middle-income countries.

Objective: We aimed to determine the effects of lipid-based nutrient supplements for pregnant and lactating women (LNS-PL) on hemoglobin (Hb), anemia, and iron status (nonprimary outcomes) at 36 weeks of gestation (women) and 6 mo postpartum (women and infants).

Methods: The Rang-Din Nutrition Study, a cluster-randomized effectiveness trial, enrolled 4011 Bangladeshi pregnant women at ≤20 weeks of gestation to receive either daily LNS-PL (20 mg Fe) during pregnancy and the first 6 mo postpartum, or iron and folic acid (IFA, 60 mg Fe + 400 μg folic acid) daily during pregnancy and every other day during the first 3 mo postpartum. Biochemical measurements from a subsample of women ($n = 1128$) and their infants ($n = 1117$) included Hb (g/L), serum ferritin (μg/L), and soluble transferrin receptor (sTfR; mg/L). Anemia was defined as maternal Hb <110 g/L at 36 weeks of gestation, <120 g/L at 6 mo postpartum, or infant Hb <105 g/L; iron deficiency (ID) was defined as ferritin <12 μg/L or elevated sTfR (>8.3 mg/L for women and >11 mg/L for infants).

Results: Compared with the IFA group, women in the LNS-PL group had lower ferritin (–6.2 μg/L; $P < 0.001$) and higher sTfR concentrations (+0.5 mg/L; $P < 0.001$), and higher risk of ID (OR = 1.93; $P < 0.05$) at 36 weeks of gestation but not at 6 mo postpartum, whereas no consistent differences were observed for Hb or anemia. Among infants at 6 mo, there were no group differences except for a lower risk of elevated sTfR (OR = 0.61; $P < 0.05$) in the LNS-PL group than in the IFA group.

Conclusions: Provision of LNS-PL including a lower dose of iron than what is recommended during pregnancy resulted in differences in maternal iron status in late pregnancy that disappeared by 6 mo postpartum, and caused no undesirable effects regarding anemia or iron status of infants. This trial was registered at clinicaltrials.gov as NCT01715038. *J Nutr* 2018;148:1615–1624.

Keywords: lipid-based nutrient supplements, iron and folic acid supplements, hemoglobin, anemia, iron deficiency, pregnant women, lactating women, infants, Bangladesh

Introduction

Anemia, or low hemoglobin (Hb) concentration, is a major global health problem, and iron deficiency (ID) is considered its predominant cause. Global estimates indicate that ∼38% of pregnant women and 29% of nonpregnant women suffer anemia (1). In Bangladesh, nationally representative data from 2011 indicated that 42% of women of reproductive age were anemic (2). Anemia during pregnancy is associated with adverse outcomes including preterm delivery, low birth weight, and perinatal and neonatal mortality (3).

Prenatal iron has been shown to reduce maternal anemia (4), whereas prenatal multiple micronutrient supplementation reduced the risk of low birth weight or small-for-gestational-age births (5). Small-quantity (SQ) lipid-based nutrient supplements

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(LNSs) are a novel home fortification approach, initially developed for young children and then adapted for pregnant and lactating women. SQ-LNSs contain macronutrients (118 kcal, 2.6 g protein, and 10 g fat) and 22 vitamins and minerals. Provision of SQ-LNSs (with 20 mg Fe) for pregnant and lactating women (LNS-PL) has shown positive effects on pregnancy outcomes in Bangladesh (6) and Ghana (7), when compared with iron and folic acid (IFA; 60 mg Fe). However, the same trial in Ghana revealed lower maternal Hb concentration, higher anemia prevalence, and lower iron status in late pregnancy among women who received LNS-PL or multiple micronutrients (MMN, with 20 mg Fe) during pregnancy, when compared with those who received IFA (8). Evidence on the effects of LNS-PL on these indicators during lactation, when the RDA for iron is lower (9 mg/d) than during pregnancy (27 mg/d) (9), or among the infants born to supplemented women, is yet to be published. Using data from the Rang-Din Nutrition Study (RDNS), a community-based cluster-randomized effectiveness trial conducted in rural Bangladesh (6), we aimed to evaluate the effects of maternal LNS-PL supplementation, compared with maternal IFA supplementation, on several nonprimary outcomes, including Hb concentration, anemia, iron biomarkers (ferritin and soluble transferrin receptor—sTfR), and iron deficiency (ID), among women in late pregnancy (36 weeks of gestation) and lactation (6 mo postpartum), and their infants at 6 mo of age.

Methods

Study setting and design. The RDNS was an effectiveness trial conducted in 2 subdistricts of the North-West region of Bangladesh (Badarganj in Rangpur District and Chirirbandar in Dinajpur District), one of the poorest regions of Bangladesh. Further details of the study setting have been described elsewhere (6, 10). LAMB (previously known as Lutheran Aid to Medicine in Bangladesh), a nongovernment organization working in the study area, offered the programmatic platform for conducting this community-based trial through its Community Health and Development Program (CHDP). Briefly, the CHDP provided health services for pregnant women including maternity services at local clinics called Safe Delivery Units (SDU), and home visits and educational group sessions in the villages, delivered by community health workers (CHWs). CHDP staff were responsible for delivering the study interventions described below.

The RDNS was a cluster-randomized trial with 4 equal-size arms: *1)* comprehensive LNS group, in which women received LNS-PL during pregnancy and the first 6 mo postpartum, and their children received LNS for children (LNS-C) from 6 to 24 mo of age; *2)* child-only LNS group, in which women received IFA (1 tablet containing 60 mg Fe and 400 μg folic acid) daily during pregnancy and every other day during the first 3 mo postpartum, and their children received LNS-C from 6 to 24 mo of age; *3)* child-only micronutrient powder (MNP) group, in which women received IFA daily during pregnancy and every other day during the first 3 mo postpartum, and their children received MNP from 6 to 24 mo of age; and *4)* control group, in which the women received IFA daily during pregnancy and every other day during the first 3 mo postpartum, and their children received no supplements. A total of 64 clusters, defined as the supervision area of a CHW, were included in the RDNS and 16 clusters were randomly assigned to each of the 4 arms by the study statistician, after stratification by subdistrict and union (the lowest administrative units in the rural areas of Bangladesh). Randomization procedures included multiple replications and testing for balance across groups. Further aspects of the study design and randomization procedures have been previously described (6). For the purpose of the analyses of the pregnancy and postpartum outcomes, the 3 groups of women who received IFA were combined and compared with the arm that received LNS-PL. Per our clinical trial protocol, maternal anemia and iron status at 6 mo postpartum were secondary outcomes in the RDNS; maternal Hb, anemia, and iron status at 36 weeks of gestation were other predetermined outcomes in the study; child anemia and iron status at 6 mo of age were not prespecified outcomes in the RDNS.

The study protocol was approved by the IRBs of the University of California, Davis (UCD), icddr,b (the local research partner), and LAMB. The study was registered at clinicaltrials.gov (NCT01715038). Participants provided individual written consent before the implementation of data collection procedures.

Maternal nutrient supplements. The nutritional composition of the maternal supplements used in the RDNS is presented in **Table 1**. The LNS-PL was produced in Malaunay, France by Nutriset SAS and was packed in individual 20-g sachets for daily consumption. The content of some of the micronutrients was modeled on the UNICEF/WHO/UNU international multiple micronutrient preparation (UNIMMAP) for pregnant and lactating women, whereas for others (i.e., thiamin, riboflavin, niacin, vitamin B-6, vitamin B-12, vitamin D, vitamin E, zinc, copper, and selenium) the content was twice the amount included in the UNIMMAP formulation, based on improved pregnancy outcomes in Guinea-Bissau when those amounts were used (11). The iron content of the LNS-PL (20 mg) was lower than the 30 mg included in the UNIMMAP formulation for several reasons. First, there was evidence that a lower dose may be adequate to prevent ID during pregnancy while lessening side effects (12). When developing the LNS-PL it was estimated that the iron content in the product plus that coming from the women's usual diet would meet the RDA during pregnancy (27 mg/d) (13). We estimated that women in Bangladesh consume ~9 mg Fe/d (14). If absorption is ~10% (which is an underestimate for pregnant women), women would receive ~1 mg of absorbed iron from the diet and would need another ~5 mg absorbed iron from the supplement during pregnancy (13). Assuming ~25% absorption of iron from LNS during pregnancy (13), the 20-mg Fe dose would meet their needs. Second, LNS-PL was designed for both pregnant and lactating women, so the iron content was intended to meet the needs of pregnancy without significantly exceeding the RDA during lactation (9 mg/d). Third, there were technical constraints on exceeding 20 mg Fe in a 20-g sachet of LNS without adversely affecting organoleptic properties.

The daily dose of IFA during pregnancy (60 mg Fe and 400 μg folic acid) was based on WHO recommendations (15), considered the standard of care in Bangladesh. In areas with high rates of anemia during pregnancy (such as Bangladesh), the recommendation is to continue the pregnancy supplementation dose to 3 mo postpartum (16). However, given the lower RDA for iron during lactation, we provided IFA on alternate days during the first 3 mo postpartum in the RDNS. The IFA tablets were produced by Hudson Pharmaceuticals Ltd. in Bangladesh.

Delivery of the supplements (carried out by LAMB CHDP staff) was done according to the randomization plan developed by UCD. The

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Abbreviations used: AGP, α-1 glycoprotein; CHDP, Community Health and Development Program; CRP, C-reactive protein; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; IFA, iron and folic acid supplement; LNS, lipid-based nutrient supplement; LNS-PL, lipid-based nutrient supplement for pregnant and lactating women; MMN, multiple micronutrients; MNP, micronutrient powder; RDNS, Rang-Din Nutrition Study; SDU, safe delivery unit; SQ, small quantity; sTfR, soluble transferrin receptor; UCD, University of California, Davis; UNIMMAP, UNICEF/WHO/United Nations University international multiple micronutrient preparation.

TABLE 1 Nutrient content of maternal supplements by LNS-PL or IFA supplementation group[1]

Nutrient (daily dose)	IFA[2]	LNS-PL[3] (20-g sachet)
Energy, kcal	0	118
Protein, g	0	2.6
Fat, g	0	10
Linoleic acid (18:2n-6), g	0	4.59
α-Linolenic acid (18:3n-3), g	0	0.59
Vitamin A (retinol equivalents), μg	0	800
Thiamin, mg	0	2.8
Riboflavin, mg	0	2.8
Niacin, mg	0	36
Folic acid, μg	400	400
Pantothenic acid, mg	0	7
Vitamin B-6, mg	0	3.8
Vitamin B-12, μg	0	5.2
Vitamin C, mg	0	100
Vitamin D (cholecalciferol), μg	0	10
Vitamin E (d,l-α-tocopherol acetate), mg	0	20
Vitamin K (phylloquinone 5%), μg	0	45
Calcium, mg	0	280
Copper, mg	0	4
Iodine, μg	0	250
Iron, mg	60	20
Magnesium, mg	0	65
Manganese, mg	0	2.6
Phosphorus, mg	0	190
Potassium, mg	0	200
Selenium, μg	0	130
Zinc, mg	0	30

[1] IFA, iron and folic acid tablets; LNS-PL, lipid-based nutrient supplement for pregnant and lactating women.
[2] Nutrient content based on current WHO recommendation (15).
[3] Nutrient content the same as LNS-PL used in other trials (7).

distribution format and messages on how to use LNS-PL have been published elsewhere (6). Distribution of LNS-PL was interrupted from 8 August to 20 October 2012 to comply with a new quality control criterion for ready-to-use supplementary foods, during which time all women received IFA. The study staff collecting the evaluation data were not involved in supplement delivery.

Data collection procedures. Recently identified pregnant women participating in the CHDP were contacted by the evaluation staff for eligibility screening. Eligibility criteria included gestational age ≤20 wk and no plans to move out of the study area during pregnancy or the following 3 y. Eligible women were informed about the study and invited to participate, along with their infants.

Data collection was performed by 2 separate teams, the "home visit team" which enrolled mothers and collected baseline and follow-up data at participants' homes and the "SDU visit team" which collected anthropometric data and bio-specimen samples at the SDU. Data collected at home included socioeconomic variables [used to create a household asset index calculated via principal component analysis (17)] and food security. We also measured the iron content of tube-well water for a subsample of women as previously described (18). Bio-specimens (i.e., urine and blood) were collected from individually randomized subsamples of women and children, which were randomized independently to reduce participant burden. At each time point of SDU assessment, predefined criteria were used to refer participants with certain conditions (e.g., severe anemia, defined as Hb ≤70 g/L) for treatment.

Capillary blood was collected by finger (women) or heel (infants) prick. Hb was measured through the use of the HemoCue Hb 301 System (HemoCue America, Brea, CA), ~45 s after collection. A microvette CB 300 Z was used for sample collection, kept in a rack for ~15–20 min, and then put in the cool bag. Thereafter, serum and red blood cells were separated, and a 0.2 mL PCR tube was used for serum storage and kept at –20°C until shipment to an external laboratory for analysis of iron and inflammation biomarkers.

Serum ferritin, sTfR, C-reactive protein (CRP), and α-1 glycoprotein (AGP) were analyzed by a combined sandwich ELISA method (19). This technique uses a small amount of serum (~30 µL) and an ELISA with different capture and detection antibodies and different solutions of the sample. The interassay CV for the indicators reported in this analysis were: 3.0% (ferritin), 4.6% (sTfR), 6.6% (CRP), and 6.0% (AGP).

Self-reported data on adherence to LNS-PL and IFA at different points in time (i.e., pregnancy, 42 d and 6 mo postpartum) were also collected by asking women how often they had consumed the supplements during the pregnancy or since the last visit. Possible answers included: *1)* not at all, *2)* sometimes (1–3 d/wk), *3)* almost every day (4–6 d/wk), or *4)* regularly/every day.

Definition of outcomes. Hb concentration was analyzed as g/L. We defined maternal anemia as Hb <110 g/L during pregnancy and as Hb <120 g/L at 6 mo postpartum (20). Anemia among 6-mo-old infants was defined as Hb <105 g/L (21). Ferritin (micrograms per liter) and sTfR (milligrams per liter) concentrations were measured as indicators of iron status. CRP (milligrams per liter) and AGP (grams per liter) concentrations were measured as indicators of inflammatory response to correct ferritin values for presence of inflammation, as explained in the statistical analysis section. Dichotomous variables for ferritin and sTfR were defined as follows: low ferritin was defined as ferritin concentration <12 µg/L (22) and high sTfR was defined as sTfR concentration >8.3 mg/L for women (19) and as sTfR concentration >11 mg/L for 6-mo-old infants (21). ID was defined as low ferritin (inflammation-corrected values) or high sTfR, and iron deficiency anemia (IDA) was defined as ID and anemia.

Sample size calculations. For the RDNS biochemical substudy a 10-percentage-point difference between groups in the prevalence of anemia (~45% compared with 35%) and IDA (~25% compared with 15%) was the basis for calculating the size of the subsample for the biochemical outcomes. Assuming 80% power, 95% level of significance, 1-sided hypothesis testing, 0.01 intracluster correlation, and 20% attrition, we needed 494 subjects in the LNS-PL group and 420 subjects in the IFA group ($n = 914$ total target sample size for the biochemical subsample) to detect such differences in the biochemical outcomes. This sample size also allowed us to detect an effect size (i.e., the difference between means of 2 groups divided by the average of the SDs of the 2 groups) of ≥0.2 in biomarker concentrations (continuous outcome variables).

The target sample size for the overall RDNS was $n = 3152$ with an anticipated enrollment period of >1 y, but we reached our target sample size after only 8 mo of enrollment (6). Because we wanted to enroll participants throughout all seasons in a year, we continued enrollment for another 2.5 mo, which resulted in a larger than anticipated sample size for both the overall RDNS sample ($n = 4011$) and the biochemical subsample ($n = 1189$).

Although our original sample size targets were based on 1-sided hypotheses, after becoming aware of evidence linking MNP (one of the study child supplements) to diarrhea and possibly other negative morbidity outcomes (23) and in light of our larger than anticipated sample size, we subsequently decided to use a 2-sided hypothesis testing approach for all RDNS analyses.

Statistical analysis. A data analysis plan was developed before starting the analysis and revealing group assignment. All analyses were conducted with the use of SAS software version 9.4 (SAS Institute Inc., Cary, NC). Primary analysis was performed based on a modified intention-to-treat analysis (i.e., no women were excluded from the analysis based on adherence to the supplementation) with the use of a complete case approach. Effects of the intervention on maternal outcomes were first analyzed through the use of a time-by-treatment interaction term in a linear mixed model ANCOVA for continuous

outcomes, and logistic regression with robust SEs for dichotomous outcomes. This model also included random effects for participant (to account for the repeated measurements on an individual) and for cluster nested within treatment group and for union nested within subdistrict (to account for the randomization scheme). The equation for the analytic models is presented below:

$$Y_{ijklmn} = \mu_{.....} + \rho_{i(j)} + \alpha_i + \beta_k + (\alpha\beta)_{jk} + \gamma_{l(i)} + \delta_m + \theta_{n(m)} + \varepsilon_{ijklmn}$$

where $\mu.....$ is a constant; $\rho_{i(j)}$: represents participant, as a random variable, nested within treatment; α_i: represents treatment assignment; i = IFA, LNS; β_k: represents time, as a fixed effect; k = 20 wk (baseline), 36 wk, 6 mo; $(\alpha\beta)_{jk}$: represents the interaction between treatment and time; $\gamma_{l(i)}$: represents cluster nested within treatment group as a random variable; δ_m: represents subdistrict; and $\theta_n(m)$: represents union nested within subdistrict as a random variable.

When the time-by-treatment interaction term was significant ($P < 0.05$), we conducted separate (cross-sectional) analysis for the outcome at each measurement time (late pregnancy and postpartum), using the same statistical modeling approaches. Analysis of child outcomes did not include repeated-measurements analysis, because these were measured only at 6 mo. All analyses were done first with only the maternal baseline biochemical value as a covariate (minimally adjusted models) and then repeated adjusting for covariates associated with the outcome at $P < 0.10$ (adjusted models). We transformed continuous outcome variables when residuals in the model did not follow a normal distribution. In those cases, the baseline value for that outcome underwent the same data transformation when included in the models and the reported statistics are back-transformed.

In addition, we conducted per protocol analyses by confining the analysis to those who reported acceptable adherence to supplement consumption. During pregnancy this was defined as self-reported consumption of the assigned supplement ≥4 times during the past week. Acceptable adherence for maternal postpartum and child outcomes was evaluated with the use of a weighted average of self-reported adherence recalled for 3 time periods: pregnancy (weight equals 26 wk), 42-d postpartum (weight equals 6 wk), and postpartum endpoint (weights equal 20 wk for the LNS-PL group and 7 wk for the IFA group). Adherence questions coding ranged from "0" (no consumption) to "4" (daily consumption); we defined acceptable adherence as a weighted average of ≥2. Further exploratory analysis of maternal and infant outcomes was also done to examine the effect of the intervention on participants who were not affected by the interruption of LNS-PL during pregnancy.

Ferritin values were corrected for inflammation via an adaptation of the approach used by Thurnham and collaborators (24) by mathematically adjusting individual values for the presence of inflammation with the use of the following inflammation categories: reference (if CRP ≤ 5.0 mg/L and AGP ≤ 1.0 g/L), incubation (if CRP > 5.0 mg/L and AGP ≤ 1.0 g/L), and convalescence (if AGP > 1.0 g/L, regardless of CRP values). We combined the 2 convalescence categories proposed by Thurnman et al. (24) (i.e., early and late convalescence) into 1 (i.e., convalescence) owing to the small number of cases in these categories. We computed adjustment factors via the ratio of the geometric mean log of the ferritin concentration in the reference category to the geometric mean log of the ferritin concentration in each of the other 2 categories and applied the resulting correction factors to create corrected individual values for this variable.

Results

In total, 4011 pregnant women were enrolled in the RDNS between 15 October 2011 and 31 August 2012. Biochemical data were obtained from 1128 women at baseline, 875 women at 36 weeks of gestation, 1041 women at 6 mo postpartum (Figure 1), and 1117 children at 6 mo. Based on these sample sizes, we had 78%, 84%, and 78% power to detect an effect size (25) of 0.2 in the difference between groups for any of the continuous outcomes at 36 weeks of gestation, 6 mo lactation, or 6 mo of age (child), respectively.

Effects of maternal intervention on late pregnancy and postpartum outcomes. A total of 1175 participants had biochemical data either at baseline (n = 1128; 507 in the LNS-PL and 621 in the IFA group), 36 weeks of gestation (n = 875; 396 in the LNS and 479 in the IFA group), or 6 mo postpartum (n = 1041; 464 in the LNS and 577 in the IFA group), and therefore were included in the repeated-measurements analysis. This sample represented 29.3% of those enrolled in the RDNS; women included in the repeated-measurements analysis were similar to those not included in terms of maternal and sociodemographic characteristics (data not shown).

Overall, at baseline women had a mean ± SD age of 22.0 ± 5.1 y, had 6.3 ± 3.3 y of education, and were enrolled at 12.9 ± 3.7 weeks of gestation. About one-third of them (32.2%) had a BMI (kg/m^2) <18.5, 40.7% were nulliparous, and 37.8% reported to have experienced moderate or severe food insecurity. Mean ± SD Hb concentration at baseline was 116.1 ± 12.8 g/L, with 29.2% categorized as anemic and 16.7% with inflammation (i.e., CRP > 5.0 mg/L or AGP > 1.0 g/L). Table 2 presents the sample characteristics at baseline of women with biochemical data at 36 weeks of gestation and 6 mo postpartum, by intervention group. The percentage of women with inflammation was 14.7% at 36 weeks of gestation and 15.6% at 6 mo postpartum.

Results for continuous and dichotomous outcomes by intervention group are shown in Tables 3 and 4, respectively. There were significant differences between intervention groups (P value for the interaction term time-by-treatment <0.05) in Hb concentration in unadjusted analysis (P = 0.048), but not after adjusting for covariates (P = 0.152). In unadjusted cross-sectional analysis, a trend towards a significant difference in Hb concentration was detected at 36 weeks of gestation (P = 0.080), but no differences were observed at 6 mo postpartum (P = 0.649). No significant differences in prevalence of anemia were observed (P = 0.114 and P = 0.351 in unadjusted and adjusted analyses, respectively).

There were significant group differences in both uncorrected and inflammation-corrected ferritin ($P < 0.001$ for the interaction term time-by-treatment for both outcomes in both unadjusted and adjusted analyses). Before correcting for inflammation, ferritin concentration was higher in the IFA group than in the LNS-PL group at 36 weeks of gestation ($P < 0.001$, unadjusted and adjusted analyses) and 6 mo postpartum (P = 0.031, unadjusted and adjusted analyses). Similar results were observed when ferritin was corrected for inflammation, but the difference at 6 mo postpartum did not reach significance (36 weeks of gestation: $P < 0.001$ unadjusted and P = 0.002 adjusted analysis; 6 mo postpartum: P = 0.066 unadjusted and P = 0.059 adjusted analysis). The proportion of women with low ferritin (with or without correction for inflammation) tended to be higher in the LNS-PL group than in the IFA group (P value for the interaction term time-by-treatment = 0.093 based on uncorrected and P = 0.071 based on inflammation-corrected values). However, the differences were not significant after adjusting for covariates (P value for the interaction term time-by-treatment = 0.237 based on uncorrected and P = 0.177 based on inflammation-corrected values).

There was a significant group difference in sTfR concentration ($P < 0.001$ for the interaction term time-by-treatment in both unadjusted and adjusted analyses), which was driven by the difference at 36 weeks of gestation, when the mean sTfR

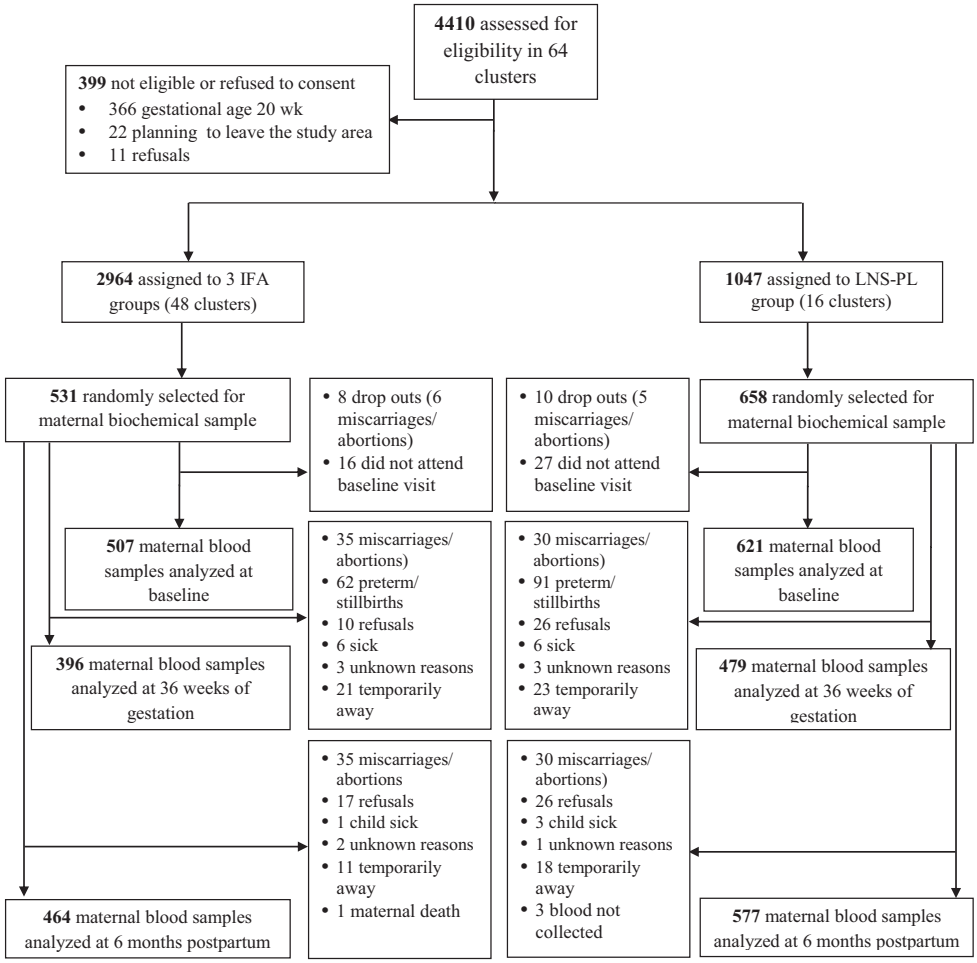

FIGURE 1 Study participation flow diagram. Women in the IFA groups received IFA daily during pregnancy and every other day during the first 3 mo postpartum. Women in the LNS-PL group received LNS-PL during pregnancy and the first 6 mo postpartum. IFA, iron and folic acid; LNS-PL, lipid-based nutrient supplements for pregnant and lactating women.

concentration was lower in the IFA group than in the LNS-PL group ($P = 0.005$ and $P = 0.007$ in unadjusted and adjusted analyses, respectively), whereas no significant difference was observed at 6 mo postpartum ($P = 0.672$ in unadjusted and $P = 0.853$ in adjusted analysis). A trend towards a higher proportion of women with high sTfR in the LNS-PL group compared with the IFA group was observed (P value for the interaction term time-by-treatment = 0.091 in unadjusted and $P = 0.087$ in adjusted analysis).

Prevalence of ID also differed by group ($P = 0.011$ and $P = 0.024$ for the interaction term time-by-treatment in unadjusted and adjusted analyses, respectively), which was

TABLE 2 Baseline characteristics of women with biochemical data at any time point (36 weeks of gestation and/or 6 mo postpartum; $n = 1175$) and of women whose children had biochemical data at 6 mo of age ($n = 1117$), by LNS-PL or IFA supplementation group[1]

Variable	Women in the maternal biochemical sample		Women in the child biochemical sample	
	LNS-PL ($n = 648$)	IFA ($n = 527$)	LNS-PL ($n = 295$)	IFA ($n = 822$)
GA at enrollment, wk	13.2 ± 3.8	13.0 ± 3.8	12.7 ± 3.7	13.0 ± 3.7
Age, y	21.9 ± 5.0	22.2 ± 5.2	22.0 ± 4.8	22.2 ± 5.1
Years of education[2]	6.5 ± 3.2	6.0 ± 3.3	6.6 ± 3.2	6.1 ± 3.3
Asset index	0.0 ± 2.2	–0.1 ± 2.2	0.1 ± 2.2	0.0 ± 2.1
Food insecure, *n* (%)	316 (48.8)	246 (46.7)	140 (47.5)	393 (47.8)
Nulliparous, *n* (%)	273 (42.2)	204 (38.8)	117 (39.7)	312 (38.0)
Height, cm	150.7 ± 5.2	150.7 ± 5.4	151.0 ± 5.3	150.8 ± 5.4
BMI, kg/m^2	20.0 ± 2.8	19.9 ± 2.7	19.9 ± 2.6	20.0 ± 2.6
Hemoglobin, g/L	116.2 ± 12.7	116.0 ± 13.0	116.1 ± 13.0	115.5 ± 13.0
Serum ferritin, μg/L	60.7 (57.2, 64.0)	62.5 (58.8, 66.4)	63.7 (55.3, 65.3)	62.7 (58.9, 66.7)
Serum sTfR, mg/L	5.0 (4.8, 5.1)	5.0 (4.8, 5.1)	4.9 (4.8, 5.1)	5.0 (4.8, 5.0)

[1] Values are arithmetic means ± SDs or geometric means (95% CIs) unless otherwise indicated. GA, gestational age; IFA, iron and folic acid supplement; LNS-PL, lipid-based nutrient supplement for pregnant and lactating women; sTfR, soluble transferrin receptor.
[2] Means were significantly different by intervention group in both maternal and child biochemical subsamples.

TABLE 3 Maternal and child hemoglobin and iron indicators at 36 weeks of gestation and 6 mo after birth, by LNS-PL or IFA supplementation group[1]

			Unadjusted model		Adjusted model	
Outcome sample time point	LNS-PL	IFA	*P* value for time-by-group interaction term[2]	*P* value for group at specific time point[3]	*P* value for time-by-group interaction term[2]	*P* value for group at specific time point[3]
Hemoglobin, g/L						
Mother ($n = 1174$)	$n = 648$	$n = 526$	0.048		0.152[4]	
36 weeks of gestation	114.1 (113.1, 115.2)	115.6 (114.4, 116.7)		0.080		—
6 mo postpartum	122.5 (121.6, 123.4)	122.2 (121.2, 123.2)		0.649		—
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	106.9 (105.4, 108.4)	105.6 (104.6, 106.5)	—	0.108	—	0.119[5]
Serum ferritin, μg/L[6]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	<0.001		<0.001[7]	
36 weeks of gestation	25.5 [23.4, 27.8]	32.4 [29.7, 35.3]		<0.001		<0.001
6 mo postpartum	41.4 [39.8, 43.1]	47.1 [45.2, 49.0]		0.031		0.031
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	34.8 [30.9, 39.5]	29.4 [25.2, 35.3]	—	0.197	—	0.340[8]
Inflammation-corrected serum ferritin, μg/L[6,9]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	<0.001		<0.001[10]	
36 weeks of gestation	24.4 [22.3, 26.8]	30.6 [28.1, 33.3]		<0.001		0.002
6 mo postpartum	41.1 [38.6, 43.9]	44.8 [42.0, 47.5]		0.066		0.059
Child ($n = 1115$)	$n = 294$	$n = 821$				
6 mo of age	28.8 [25.8, 32.3]	26.8 [24.8, 29.0]	—	0.203	—	0.370[11]
Serum soluble transferrin receptor, mg/L[6]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	<0.001		<0.001[12]	
36 weeks of gestation	6.2 (5.9, 6.4)	5.7 (5.5, 5.9)		0.005		0.007
6 mo postpartum	5.4 (5.2, 5.6)	5.4 (5.2, 5.5)		0.672		0.853
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	8.8 (8.4, 9.3)	9.3 (9.1, 9.6)	—	0.058	—	0.126[13]

[1] Values are arithmetic means (95% CIs) or geometric means [95% CIs] for log-transformed data. AGP, α-1 glycoprotein; CRP, C-reactive protein; IFA, iron and folic acid supplement; LNS-PL, lipid-based nutrient supplement for pregnant and lactating women.
[2] Linear mixed model repeated-measures ANCOVA, with union (nested within subdistrict) and cluster (nested within group) as random effects and participant as a random effect to account for the repeated measures, and the interaction term treatment group × time point.
[3] Linear mixed model ANCOVA, including union (nested within subdistrict) and cluster (nested within group) as random effects.
[4] Adjusted for gestational age, CRP, AGP, BMI, assets index, and tube well iron content at baseline, and season at time of measurement; $n = 1125$.
[5] Adjusted for child's sex, season at time of measurement, and maternal age, height, and BMI, and parity at baseline; $n = 1116$.
[6] Statistical testing conducted with the use of a log-transformed variable. Values presented were back-transformed.
[7] Adjusted for maternal age, gestational age, CRP, AGP, assets index, and tube well iron content at baseline, and season at time of measurement; $n = 1125$.
[8] Adjusted for child's sex, season at time of measurement, and maternal age and BMI, gestational age, parity, asset index, and food security at baseline; $n = 1117$.
[9] Corrected for presence of inflammation following Thurnham et al.'s (24) approach.
[10] Adjusted for maternal age, gestational age, AGP, assets index, and tube well iron content at baseline, and season at time of measurement; $n = 1125$.
[11] Adjusted for child's sex, season at time of measurement, and maternal age and BMI, gestational age, parity, and food security at baseline; $n = 1115$.
[12] Adjusted for season at time of measurement, and maternal age, AGP, parity, and tube well iron content at baseline; $n = 1125$.
[13] Adjusted for child's sex, season at time of measurement, and maternal age, gestational age, and parity at baseline; $n = 1117$.

again driven by differences at 36 weeks of gestation, when the prevalence of ID was lower in the IFA group than in the LNS-PL group ($P = 0.005$ in unadjusted and $P = 0.001$ in adjusted analysis), whereas no difference was observed at 6 mo postpartum ($P = 0.707$ in unadjusted and $P = 0.766$ in adjusted analysis). There were no significant differences in prevalence of IDA ($P = 0.173$ and $P = 0.191$ for the interaction term time-by-treatment in unadjusted and adjusted analyses, respectively).

In the per protocol analysis including only women with acceptable adherence ($n = 874$), results were similar to those observed in the whole sample, except that the differences in Hb concentration (unadjusted analysis) and prevalence of ID (unadjusted and adjusted analyses) detected in the whole sample were no longer significant in the per protocol subsample, and a significant difference between groups was detected for prevalence of IDA in the unadjusted (but not adjusted) analysis (**Supplemental Tables 1 and 2**). Further exploratory analysis including only participants whose pregnancy was not affected by the interruption of LNS-PL ($n = 377$; **Supplemental Tables 3 and 4**) revealed significant differences by group that were not observed in the whole sample. Women in the IFA group had higher Hb concentration, lower rate of anemia, lower rate of low inflammation-corrected ferritin, and lower rate of high sTfR (dichotomous outcomes also listed in Table 4) than those in the LNS-PL group.

Effects of maternal intervention on infant outcomes. In total, 1117 infants with biochemical data at 6 mo of age were included in this analysis, of whom 822 were in the IFA group and 295 in the LNS group. This analytic sample represented 31% of live births in the RDNS sample; maternal sociodemographic characteristics in this subsample were similar to those enrolled but not included in this analysis, with the exception that the former were enrolled 2 d earlier in gestation ($P = 0.022$) and were 0.46 cm taller ($P = 0.016$) than the latter. Table 2 shows that the baseline characteristics of women whose

TABLE 4 Prevalence of maternal and child anemia low ferritin, high sTfR, iron deficiency, and iron deficiency anemia at 36 weeks of gestation and 6 mo postdelivery by LNS-PL or IFA supplementation group[1]

			Unadjusted model		Adjusted model	
Outcome sample time point	LNS-PL, %	IFA, %	*P* value for time-by-group interaction term[2]	*P* value for group at specific time point[3]	*P* value for time-by-group interaction term[2]	*P* value for group at specific time point[3]
Anemia[4]						
Mother ($n = 1174$)	$n = 648$	$n = 526$	0.114		0.351[5]	
36 weeks of gestation	36.0	32.5		—		—
6 mo postpartum	37.6	38.8		—		—
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	44.4	45.6	—	0.716	—	0.781[6]
Low serum ferritin[7]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	0.093		0.237[8]	
36 weeks of gestation	18.4	9.8		—		—
6 mo postpartum	7.3	5.4		—		—
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	14.2	15.6	—	0.593	—	0.620[9]
Low inflammation-corrected serum ferritin[10]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	0.071		0.177[11]	
36 weeks of gestation	20.0	10.9		—		—
6 mo postpartum	7.5	5.4		—		—
Child ($n = 1115$)	$n = 294$	$n = 821$				
6 mo of age	17.3	18.9	—	0.554	—	0.562[12]
High serum sTfR[13]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	0.091		0.087[14]	
36 weeks of gestation	19.0	13.1		—		—
6 mo postpartum	8.3	8.6		—		—
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	21.0	30.3	—	0.005	—	0.012[15]
Iron deficiency[16]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	0.011		0.024[17]	
36 weeks of gestation	29.4	19.4		0.005		0.043[18]
6 mo postpartum	12.7	12.0		0.707		0.766[18]
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	29.5	35.9	—	0.052	—	0.106[19]
Iron deficiency anemia[20]						
Mother ($n = 1172$)	$n = 648$	$n = 524$	0.173		0.191[21]	
36 weeks of gestation	15.0	9.6		—		—
6 mo postpartum	8.0	7.3		—		—
Child ($n = 1117$)	$n = 295$	$n = 822$				
6 mo of age	18.0	21.0	—	0.197	—	0.262[22]

[1] Values are percentages. AGP, α-1 glycoprotein; CRP, C-reactive protein; Hb, hemoglobin; IFA, iron and folic acid supplement; LNS-PL, lipid-based nutrient supplement for pregnant and lactating women; sTfR, soluble transferrin receptor.
[2] Repeated-measures logistic regression analyses with robust SEs, including union (nested within subdistrict) and cluster (nested within group) as random effects and participant as a random effect to account for the repeated measures, and the interaction term treatment group × time point.
[3] Logistic regression analyses at specific time point, with union (nested within subdistrict) and cluster (nested within group) as random effects.
[4] Defined as Hb < 110 g/L, gestation; Hb < 120 g/L, 6 mo postpartum; Hb < 105 g/L, children.
[5] Adjusted for season at time of measurement and gestational age, maternal BMI and AGP, and tube well iron content at baseline; $n = 1125$.
[6] Adjusted for child's sex, season at time of measurement, and maternal age and BMI, and parity at baseline; $n = 1116$.
[7] Defined as serum ferritin < 12 μg/L.
[8] Adjusted for season at time of measurement, and maternal age, height, and CRP, and tube well iron content at baseline; $n = 1125$.
[9] Adjusted for child's sex, season at time of measurement, gestational age at enrolment, and maternal age and BMI, parity, food security, and asset index at baseline; $n = 1117$.
[10] Defined as inflammation-corrected serum ferritin < 12 μg/L.
[11] Adjusted for season at 36 weeks of gestation, and maternal age and height, and tube well iron content at baseline; $n = 1125$.
[12] Adjusted for child's sex, gestational age at enrolment, and maternal age, parity, and food security at baseline; $n = 1115$.
[13] Defined as serum sTfR > 8.3 mg/L, women; sTfR > 11 mg/L, children.
[14] Adjusted for maternal AGP, and tube well iron content at baseline; $n = 1125$.
[15] Adjusted for child's sex, season at time of measurement, and maternal age and parity at baseline; $n = 1117$.
[16] Defined as low inflammation-corrected serum ferritin or high serum sTfR.
[17] Adjusted for season at time of measurement, maternal age and AGP, and tube well iron content at baseline; $n = 1125$.
[18] Adjusted for outcome status at baseline, season at time of measurement, and maternal age and AGP, and tube well iron content at baseline; $n = 840$ at 36 weeks of gestation, $n = 997$ at 6 mo postpartum.
[19] Adjusted for child's sex, season at time of measurement, and gestational age, maternal age, and parity at baseline; $n = 1117$.
[20] Defined as low inflammation-corrected serum ferritin or high serum sTfR and Hb < 110 g/L, gestation; Hb < 120 g/L, 6 mo postpartum; Hb < 105 g/L, children.
[21] Adjusted for CRP, and tube well iron content at baseline; $n = 1125$.
[22] Adjusted for child's sex, season at time of measurement, and maternal age and BMI, and parity at baseline; $n = 1115$.

infants were included in this analysis were similar between the intervention groups, except for maternal education; in addition, there was no significant difference in infant sex: 47% and 51% were males in the LNS-PL and IFA groups, respectively ($P = 0.250$). Prevalence of inflammation among infants at 6 mo was 33.8%.

There were no significant differences by group in infant Hb at 6 mo of age ($P = 0.108$) or the proportion of infants with anemia ($P = 0.716$); adjustment for covariates did not change these results (Tables 3 and 4). Overall, infants' uncorrected and inflammation-corrected ferritin concentrations at 6 mo were similar between groups ($P = 0.197$ and $P = 0.203$, respectively) and adjustment for covariates generated similar results (Table 3). Similarly, there were no significant differences by group in the proportion of infants with low ferritin ($P = 0.593$ based on uncorrected and $P = 0.554$ based on inflammation-corrected values), with similar results after adjusting for covariates (Table 4).

There was a trend towards a higher sTfR concentration in the IFA group compared with the LNS-PL group ($P = 0.058$), but this was attenuated after adjustment for covariates (Table 3). However, the proportion of infants with high sTfR was higher in the IFA group compared with the LNS-PL group ($P = 0.005$), which was still statistically significant after adjustment for covariates (Table 4). The proportion of infants with ID tended to be higher in the IFA group than in the LNS-PL group ($P = 0.052$), but this association was attenuated after adjustment for covariates (Table 4). There were no significant group differences in the proportion of infants with IDA ($P = 0.197$); adjustment for covariates did not change this result (Table 4).

Results observed in the per protocol analysis, including only infants whose mothers had acceptable adherence ($n = 874$), indicated several significant differences favoring the LNS-PL group (Supplemental Tables 1 and 2). Besides the group difference in high sTfR observed in the whole subsample, mean infant ferritin concentration was higher and sTfR concentration was lower in the LNS-PL group than in the IFA group, and the proportion with ID was significantly lower among infants in the LNS-PL group (compared with the IFA group). Further exploratory analysis including only infants whose mother's pregnancy was not affected by the interruption of LNS-PL (Supplemental Tables 3 and 4, $n = 376$) revealed significant differences that were not observed in the overall subsample. Infants in the LNS-PL group had higher Hb concentration ($P = 0.035$ in unadjusted and $P = 0.044$ in adjusted analysis) and lower rates of anemia (only in unadjusted analysis, $P = 0.042$) and IDA ($P = 0.035$ in unadjusted and $P = 0.044$ in adjusted analysis) at 6 mo, compared with those in the IFA group (Supplemental Tables 3 and 4).

Discussion

We found that women who received LNS-PL with 20 mg Fe had lower Hb, ferritin, and sTfR concentrations and higher risk of ID during late pregnancy compared with those who received IFA with 60 mg Fe, but there was no significant difference in risk of anemia or IDA, and the differences in mean maternal Hb and biomarkers of iron status disappeared by 6 mo postpartum after correcting for inflammation. There were no group differences in Hb or iron status indicators among the infants at 6 mo of age, except that those in the LNS-PL group were less likely to have elevated sTfR (i.e., they had a lower risk of ID based on that biomarker) compared with those in the IFA group.

Similar results were reported from an efficacy trial conducted in Ghana in which similar IFA (with 60 mg Fe) and LNS-PL (with 20 mg Fe) products were also delivered to pregnant women, and a third intervention group received MMN (with 20 mg Fe) (7). In that trial, women who received LNS-PL (and generally also those in the MMN group) had lower Hb concentration, higher sTfR, and higher risk of anemia and IDA in late pregnancy than those in the IFA group (8). The most likely explanation for these findings is the much larger dose of iron in the IFA group (60 compared with 20 mg/d) during pregnancy. It is possible that the amount of iron in the LNS-PL was too low for pregnancy. The formulations used in the RDNS and in the Ghana trial were developed to meet the nutrient needs of both pregnant and lactating women, given that the recommended intakes for most nutrients (other than iron) are similar for both groups (13). For iron, it was estimated that the daily dose of 20 mg plus iron coming from the diet would meet the RDA of 27 mg Fe during pregnancy, while not greatly exceeding the RDA (9 mg/d) for iron during lactation. Previous evidence had suggested that 20 mg Fe/d was adequate to prevent ID anemia during pregnancy, while causing fewer gastrointestinal side effects, compared with higher doses of iron (12). That evidence, however, was from a high-income country, and it is possible that dietary iron needs during pregnancy are higher among women in Bangladesh because they consume a largely plant-based diet from which iron absorption would be relatively low.

A key consideration in interpreting these results is whether or not the higher risk of maternal ID in late pregnancy observed in the LNS-PL group had any negative functional consequences, given that there is debate about the most appropriate cutoffs to use for both Hb and markers of iron status during pregnancy. Although the standard cutoff for identifying anemia during pregnancy is Hb <110 g/L (20), the lowest risk of adverse birth outcomes has been seen in women with Hb ~95–105 g/L (26, 27), with a higher risk of adverse outcomes observed when Hb is above that range. A recent review confirmed the existence of a U-shaped curve for the risk of adverse birth outcomes with maternal Hb concentrations (27). In the RDNS we observed less fetal growth restriction in the LNS-PL group than in the IFA group (6) despite the fact that standard cutoffs indicated a higher risk of maternal ID during pregnancy in the former group. Therefore, future decisions regarding the iron content of LNS-PL should be aimed at identifying the most effective dosage for improving both maternal iron status and birth outcomes.

During the postpartum period, our study was designed so that women in the LNS-PL group would receive a daily dose of 20 mg Fe during the first 6 mo and those in the IFA group would receive the equivalent of 30 mg Fe/d during the first 3 mo postpartum. Both of these daily iron amounts are higher than the RDA (9 mg/d) for iron during lactation (9). The total dose of iron to be delivered during the first 6 mo postpartum was ~3600 mg for the LNS-PL group and ~2700 mg for the IFA group. This likely explains why we did not see differences between groups in maternal Hb or iron status indicators at 6 mo postpartum. The only exception was in uncorrected ferritin concentration which was lower among those who received LNS-PL than among those who received IFA.

The results for infants at 6 mo of age, who were exposed to the supplements in utero and while breastfeeding (99% of women were still breastfeeding at 6 mo postpartum), were mostly consistent with those observed among the women at

6 mo postpartum. Surprisingly, infants whose mothers received LNS-PL were less likely to have elevated sTfR than those whose mothers received IFA, even though the LNS-PL mothers received much less iron during pregnancy. The iron received by mothers during lactation should not have affected the amount of iron that infants received after birth, because maternal iron intake does not affect breast-milk iron concentration (28). Iron status of breastfed infants during the first 6 mo of life is largely determined by their iron stores at birth, which are related not only to maternal iron status during pregnancy but also to birth weight (29). In the RDNS, women in the IFA group had higher iron status during pregnancy, but women in the LNS-PL gave birth to heavier children (6). However, in a mediation analysis (data not shown), the higher birth weight in the LNS-PL group did not appear to explain the difference in percentage of infants with elevated sTfR. An alternative explanation is that other nutrients provided by LNS-PL had an impact on infant iron status by altering nutrient stores at birth or breast-milk composition. Vitamin A is one such candidate, because it is involved in iron metabolism (30) and maternal intake of vitamin A during lactation can affect milk vitamin A content (28).

Because this study was a randomized trial, our main findings are based on the intention-to-treat analyses. However, given that adherence in an effectiveness trial (such as the RDNS) is not as closely monitored as in an efficacy trial, findings among those who consumed the supplement at acceptable levels are also informative. Results from such analyses indicated stronger positive effects of LNS-PL compared with IFA on infant iron status at 6 mo.

The study we conducted had some limitations. The main limitation was our inability to blind the women to the type of supplement provided, because the supplements were very different in appearance and taste. Nonetheless, researchers responsible for collection of outcome data were kept blind to treatment groups. A second limitation was a disruption of the LNS-PL supply for a period of 10 wk, which was beyond our control and compromised our ability to investigate the full potential of LNS-PL as an intervention. Third, we assessed adherence to supplement consumption recommendations by women's retrospective recall, instead of direct observation, so the adherence data could be inaccurate. This study also had some strengths, including the use of 2 independent teams: 1 to conduct the intervention (led by LAMB) and another to evaluate impact (led by icddr,b and UCD), a large and representative sample, and a relatively low attrition rate (mostly due to delivery before the late pregnancy follow-up visit or travel outside the study area).

We conclude that pre- and postnatal provision of LNSs containing a lower amount of iron than what is currently recommended during pregnancy resulted in significant differences in maternal iron indicators in late pregnancy but had no negative effects among their infants and may have improved infant iron status at 6 mo of age. These findings, along with evidence that LNS-PL resulted in better birth outcomes in our study population, suggest the need for further research and evaluation of iron supplementation recommendations for pregnant and lactating women.

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