The Journal of Nutrition
Community and International Nutrition

Low-Dose Iron Supplementation in Infancy Modestly Increases Infant Iron Status at 9 Mo without Decreasing Growth or Increasing Illness in a Randomized Clinical Trial in Rural China[1–3]

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Abstract

Background: Previous trials of iron supplementation in infancy did not consider maternal iron supplementation.

Objective: This study assessed effects of iron supplementation in infancy and/or pregnancy on infant iron status, illnesses, and growth at 9 mo.

Methods: Enrollment occurred from December 2009 to June 2012 in Hebei, China. Infants born to women in a pregnancy iron supplementation trial were randomly assigned 1:1 to iron [~1 mg Fe/(kg · d) as oral iron proteinsuccynilate] or placebo from 6 wk to 9 mo, excluding infants with cord ferritin <35 μg/L. Study groups were pregnancy placebo/infancy placebo (placebo/placebo), pregnancy placebo/infancy iron (placebo/iron), pregnancy iron/infancy placebo (iron/placebo), and pregnancy iron/infancy iron (iron/iron). The primary outcome was 9-mo iron status: iron deficiency (ID) by cutoff (≥2 abnormal iron measures) or body iron <0 mg/kg and ID + anemia (hemoglobin <110 g/L). Secondary outcomes were doctor visits or hospitalizations and weight or length gain from birth to 9 mo. Statistical analysis by intention to treat and dose-response (between number of iron bottles received and outcome) used logistic regression with concomitant RRs and general linear models, with covariate control as applicable.

Results: Of 1482 infants randomly allocated, 1276 had 9-mo data (n = 312–327/group). Iron supplementation in infancy, but not pregnancy, reduced ID risk: RRs (95% CIs) were 0.89 (0.79, 0.998) for placebo/iron compared to placebo/placebo, 0.79 (0.63, 0.98) for placebo/iron compared to iron/placebo, 0.87 (0.77, 0.98) for iron/iron compared to placebo/placebo, and 0.86 (0.77, 0.97) for iron/iron compared to iron/placebo. However, >60% of infants still had ID at 9 mo. Receiving more bottles of iron in infancy was associated with better infant iron status at 9 mo but only among iron-supplemented infants whose mothers were also iron supplemented (i.e., the iron/iron group). There were no group differences in hospitalizations or illnesses and no adverse effects on growth overall or among infants who were iron sufficient at birth.

Conclusions: Iron supplementation in Chinese infants reduced ID at 9 mo without adverse effects on growth or illness. Effects of iron supplementation in pregnancy were observed only when higher amounts of iron were distributed in infancy. This trial was registered at clinicaltrials.gov as NCT00613717. *J Nutr* 2016;146:612–21.

Keywords: iron supplementation, iron deficiency, iron deficiency anemia, infancy, pregnancy, growth, randomized clinical trial

Introduction

Anemia and iron deficiency (ID)[10] in pregnant women and young children are global public health problems. In 2011, 38% of pregnant women and 43% of children aged <5 y were anemic, about half due to ID (1). These percentages mean that 32 million pregnant women and 273 million young children were affected. Pregnancy and infancy are peak periods for ID because of high iron needs to support rapid growth and limited dietary sources of bioavailable iron. ID is of concern not only as a cause of anemia; ID during infancy is also associated with poorer

Manuscript received September 10, 2015. Initial review completed October 10, 2015. Revision accepted December 9, 2015.
First published online January 20, 2016; doi:10.3945/jn.115.223917.

cognitive, motor, and social-emotional development in the short and long term (2, 3).

This study was a randomized clinical trial (RCT) of iron supplementation in infancy that was linked to an RCT of iron supplementation during pregnancy (4). The project was conducted in China at a time when iron supplements for pregnant women and infants were not routinely recommended. The study was designed to set the stage for research on neurodevelopmental outcome depending on timing and duration of iron supplementation. Because iron treatment in infancy has generally not corrected the cognitive, motor, and social-emotional deficits observed in iron-deficient infants (2, 3), the study focused on routine iron supplementation as a preventive approach.

In the pregnancy RCT, iron supplementation significantly reduced maternal anemia, ID, and iron deficiency anemia (IDA), but, unexpectedly, more than half of the women were still iron deficient at or near term, and 45% of neonates had evidence of fetal-neonatal ID, regardless of maternal iron supplementation (4). The infancy RCT thus posed an unforeseen question about the effectiveness of postnatal iron supplementation in improving iron status later in infancy in a setting where many infants had poor iron status at birth. Therefore, this initial report of the linked RCT focuses on infant iron status and adverse effects.

To our knowledge, there is no other study with a similar design. The closest is a Nepal study that added an RCT of micronutrient supplementation at 12–36 mo onto one arm of a prenatal RCT (5). However, iron status effects in numerous RCTs of iron supplementation in either pregnancy or infancy have been summarized in meta-analyses or systematic reviews (6–14). Regarding RCTs of iron supplementation in young children, a meta-analysis published in the *Lancet* in 2013 is most pertinent (9). It focused on children aged 4–23 mo; most studies included were in low- to middle-income countries and provided iron supplements for ≥3 mo. Despite heterogeneity in type and duration of supplementation and enrollment age, the meta-analysis found robust effects of iron supplementation on reducing the risk of anemia, ID, and IDA. However, some individual studies have observed little response to iron supplementation or a high prevalence of ID/anemia postsupplementation [e.g., Olney et al. (15) and Black et al. (16)]. Although the poor response may be due to a heavy burden of infectious disease, such findings also raise questions about the role of poor iron status before birth and whether earlier iron supplementation (i.e., in the first postnatal months or during pregnancy) would result in better iron status later in infancy. Our study addresses these questions. We predicted that iron supplementation beginning in pregnancy and continuing in early infancy would improve infant iron status at 9 mo more than iron supplementation in infancy alone.

Despite unresolved challenges in improving iron status in pregnant women and infants, there is debate about risks of routine iron supplementation. Concerns about potential adverse effects relate to infection and growth, especially in iron-sufficient infants (17, 18). The current study was conducted in a region where serious infectious diseases and generalized undernutrition were virtually absent, which makes minor illnesses and growth the most relevant potential adverse effects. The *Lancet* meta-analysis found that children randomly allocated to iron supplementation had slightly lower gains in length and weight and more vomiting and fever than children randomly allocated to placebo (9). In light of these findings, other important outcomes in this initial report of the linked RCTs are illness and growth.

Methods

Study setting and design. Data were obtained in the course of a study of neurodevelopmental effects of iron supplementation in early life. The study, conducted in rural Sanhe County, Hebei Province, China, involved collaboration between the University of Michigan, Peking University First Hospital, and Sanhe Maternity and Child Health Care Center.

The study design was a RCT of iron supplementation in infancy that built on a RCT of iron supplementation in pregnancy (4). Infants whose mothers were randomly assigned to receive supplemental iron/folate or placebo/folate in pregnancy were randomly allocated to supplemental iron or placebo from 6 wk to 9 mo. The linked RCTs resulted in 4 study groups based on placebo or iron supplementation in pregnancy and infancy: pregnancy placebo/infancy placebo (placebo/placebo), pregnancy placebo/infancy iron (placebo/iron), pregnancy iron/infancy placebo (iron/placebo), and pregnancy iron/infancy iron (iron/iron). Outcomes were assessed at 9 mo. The University of Michigan and Peking University First Hospital ethics committees approved the study.

Participants. The pregnancy RCT enrolled women with uncomplicated singleton pregnancies at 16 wk of gestation, on average. Exclusion criteria included maternal age <18 y, not living in Sanhe, not mentally competent, chronic health problem, and hemoglobin <100 g/L or having taken medicinal iron for any duration (4). Neonates born to participating mothers were enrolled between December 2009 and June 2012. All live births were included, except 87 infants with cord-blood serum ferritin (SF) <35 μg/L (**Figure 1**). They were excluded to prevent randomly allocating infants with iron stores indicating brain ID (19) to placebo; they received supplemental iron. Perinatal conditions that may interfere with fetal iron status or behavior/development were uncommon (4). Enrollment totaled 1482 infants.

Enrollment and informed consent. Women in the pregnancy RCT received information about the infancy study at routine prenatal visits. Sanhe Maternity and Child Health Care Center staff provided further information after delivery, answered questions, and obtained signed informed consent from those agreeing to participate.

Randomization and masking. Participants were assigned a unique 4-digit identification number (same for mother and infant). Infants were randomly allocated (1:1 ratio) to iron or placebo using SAS Proc Surveyselect (SAS Institute). Each identification number was assigned according to a random number chart prepared by a University of Michigan statistician (NK) who had no contact with participants or study personnel in China. The supplementation code was not broken until the study and primary analyses were completed. Lee's Pharmaceutical

[1] Supported by a grant from the NIH (R01 HD052069; principal investigator: BL), which included funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the Office of Dietary Supplements; by a grant from Vifor Pharma Ltd. (principal investigator: GZ); and by the São Paulo Research Foundation–Brazil (2014/00018-0) and Methodist University of Piracicaba–Brazil (to DCCS). Lee's Pharmaceutical Holdings Limited, Hong Kong, provided iron proteinsuccynilate oral solution and placebo at cost.
[2] Author disclosures: B Lozoff was an unpaid speaker at 2 seminars supported by Lee's Pharmaceutical Holdings Limited. The topic was iron deficiency and child development (Shanghai, 11 April 2010, and Beijing, 15 May 2011). The company covered hotel accommodations and, for the 2011 seminar, internal airfare between Hangzhou and Beijing. Y Jiang, X Li, M Zhou, B Richards, G Xu, KM Clark, F Liang, N Kaciroti, G Zhao, DCC Santos, Z Zhang, T Tardif, and M Li, no conflicts of interest.
[3] The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding sources. Authors had full control of primary data and did not have an agreement with the funders that limited their ability to complete the research as planned.
[10] Abbreviations used: BI, body iron; ID, iron deficiency; IDA, iron deficiency anemia; RCT, randomized clinical trial; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP, zinc protoporphyrin.
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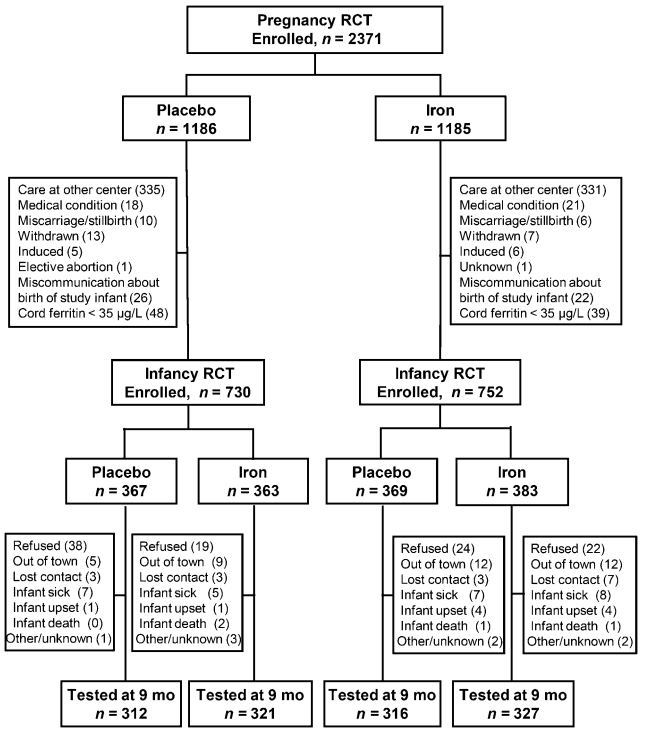

FIGURE 1 Flowchart of participants in an RCT in rural China, 2009–2012, by maternal randomization to placebo or iron (300 mg ferrous sulfate/d) and infant randomization to placebo or iron [~1 mg Fe/(kg · d) as oral iron proteinsuccynilate], resulting in 4 study groups: placebo in pregnancy/placebo in infancy, placebo in pregnancy/iron in infancy, iron in pregnancy/placebo in infancy, and iron in pregnancy/iron in infancy. RCT, randomized clinical trial.

Holdings Limited prepared liquid iron or placebo in identical dark-colored bottles with a dropper marked with the appropriate volume. To help maintain masking, all families were informed that supplements might alter stool color and/or consistency. Study participants, personnel, and investigators were unaware of group assignment in either RCT. The only personal identifying information used in data analysis was birth date (needed to calculate exact age at assessments).

Intervention. In the pregnancy RCT, women were randomly assigned to daily iron (300 mg ferrous sulfate) or placebo + 0.40 mg folate from enrollment to delivery (4). In the infancy RCT, infants were randomly assigned to a single daily dose of ~1 mg Fe/(kg · d) of elemental iron as iron proteinsuccinylate oral solution (Ferplex, Italfarmico, S.p.A., Milan, Italy) from 6 wk to 9 mo or an equal volume of carrier alone. Each milliliter of Ferplex contained 2.7 mg elemental iron. To simplify dosing, the volume was 2 mL/d for infants aged <6 mo and 3 mL/d for infants aged ≥6 mo. Supplementation was not started until 6 wk due to concern about immune compromise in neonates. Families were initially provided with 1 box of supplement (ten 15-mL bottles/box) and instructed to return for more as needed and bring back unused or expired bottles. Staff recorded the number of boxes distributed and bottles returned. Each box provided enough daily supplements for ~10 wk for infants aged <6 mo and 5 wk for infants aged ≥6 mo. We intended to monitor adherence by having parents mark a calendar each day they gave supplement, but only 126 calendars were returned with usable data. Therefore, we estimated adherence by the number of bottles received, calculated as bottles distributed minus bottles returned. Infants with IDA at 9 mo were prescribed therapeutic doses of iron per local practice.

Study outcomes. The primary outcome was infant iron status (ID and IDA) at 9 mo. Secondary outcomes were illnesses and weight/length gain from birth to 9 mo. All blood samples were collected for research purposes. Cord-blood samples were obtained in the pregnancy RCT by sterile needle puncture immediately after cord clamping, which generally occurred within 60 s of delivery. Finger stick samples (~1.0 mL) at 9 mo were obtained by trained staff members using contact-activated lancets (BD Microtainer) and techniques to facilitate free blood flow, such as warming and gravity. A complete blood count, including hemoglobin and mean corpuscular volume, was performed by using a Sysmex KX-21N Auto Hematology Analyzer (SYSMEX Corporation). In Sanhe, whole blood for zinc protoporphyrin (ZPP)/heme was stored at 4°C and protected from light; serum for other iron measures was stored at −20°C. Samples were transferred weekly to Peking University First Hospital where ZPP/heme was analyzed by using a hematofluorometer (AVIV Biomedical), SF and serum transferrin receptor (sTfR) by chemiluminescent immunoassay (Beckman Coulter Access 2 Immunoassay System; Beckman Coulter), and serum C-reactive protein by rate nephelometry (Hitachi 7600; Hitachi). Both laboratories maintained standard quality control procedures.

We defined anemia at 9 mo per WHO guidelines as hemoglobin <110 g/L (1). ID was defined in 2 ways: ≥2 abnormal iron measures by cutoff (20) [mean corpuscular volume <74 fL (21), ZPP/heme >69 μmol heme/mol (22), SF <12 μg/L (23)] or body iron (BI) <0 mg/kg, calculated from SF and sTfR (4, 24–27). IDA was defined as anemia plus ID. Fetal-neonatal ID, drawn from the pregnancy RCT, was defined as cord SF <75 μg/L or ZPP/heme >118 μmol heme/mol (4, 28–31).

Infant weight, length, and head circumference were measured by trained obstetrical staff at birth and pediatric staff at 9 mo. On the basis of parental report, project staff recorded reasons for hospitalizations and doctor visits between birth and 9 mo.

Sample size. The targeted n was 500/group (total = 2000). The pregnancy RCT did not reach this target due to budgetary constraints and higher than expected attrition. The actual number of neonates enrolled was 363–383/group, with 9-mo outcome data of 305–318/group for iron status, 312–327/group for anthropometric measurements, and 350–368/group for doctor visits and hospitalizations. Using n values for iron status/anthropometry and $\alpha = 0.05$, we had 80% power to detect a 16% or larger reduction in ID/IDA at 9 mo (RR = 0.84) and 0.23-SD differences in weight/length gain across groups. The 0.23-SD difference also applied to group differences in other continuous variables.

Statistical analysis. The primary analysis was based on intention to treat. Logistic regression models were used to test for group differences in categorical background characteristics and iron status outcomes. Where pairwise differences were significant, we calculated RR. General linear models with pairwise comparisons were used to test for group differences in continuous background characteristics and individual iron and anthropometric measurements. Differences in adverse events were compared with logistic regression, controlling for number of doctor visits to adjust for multiple illnesses in the same child. Subgroup analyses used similar statistical models and χ^2/Fisher's exact tests to consider differential effects depending on iron status at birth. In addition, we used general linear models or logistic regression to analyze dose-response relations within infancy RCT iron-supplemented groups (i.e., between number of bottles of iron received and infant outcomes at 9 mo). Because the number of bottles received corresponded closely to the number of boxes distributed, we categorized the number of bottles relative to the 40 bottles planned as very low (≤10), low (11–20), or medium (>20). For dose-response analysis, we evaluated potential confounding factors and considered any that were significantly or marginally related to both independent and dependent variables. Statistical significance was set at $P < 0.05$.

Descriptive data are expressed as means ± SDs and n and/or %. Results for group comparisons of outcomes are expressed as means (95% CIs) for continuous variables and RR (95% CI) for categorical ones.

Results

A total of 2371 women were enrolled in the pregnancy RCT: 1186 placebo and 1185 iron supplemented. A total of 1482 infants were enrolled in the infancy RCT: 730 born to mothers in the placebo group and 752 born to mothers in the

iron-supplemented group. As previously reported (4), attrition in the pregnancy RCT groups was similar in proportion and reason, mostly due to delivery at a nonparticipating health center (Figure 1). There were 2 additional reasons for attrition before infant enrollment. In 48 instances, there was miscommunication (e.g., infancy RCT staff was not notified when a mother in the pregnancy RCT had given birth). Another 87 infants with cord-blood SF <35 μg/L were excluded per protocol. All other infants were randomly assigned to placebo or iron supplement, resulting in 4 study groups approximately equal in number (Figure 1).

A total of 1276 infants were assessed at 9 mo. The number of infants per group was 312 placebo/placebo, 321 placebo/iron, 316 iron/placebo, and 327 iron/iron (Figure 1). Attrition (206/1482, 13.9%) affected the groups similarly in proportion and reason. The most common reasons were refusal (103/206, 50.0%), family out of town or not reached (54/206, 26.2%), and infant sick at the scheduled assessment or too tired/upset for testing (37/206, 18.0%).

Background/baseline characteristics

The tables show information by group for infants with data at 9 mo. With sole exception of head circumference at birth, there were no statistically significant overall group differences in background characteristics (**Table 1**). Slightly more than half the infants were male. More than three-fourths were first-born, all but a few born at term (37–41 wk). Birth by cesarean section was common, as is customary in China. Birth weight was 3.36 ± 0.37 kg. Around one-third of mothers completed high school, and most families had household income below the county cutoff for public assistance in housing (32). Age at assessment after supplementation in the infancy RCT was 9.3 ± 0.4 mo. More than 80% of infants were still breastfeeding, with >50% receiving breast milk as the sole milk source. Solids were introduced to ~20% of infants by 4 mo and ~75% by 6 mo; small amounts of egg yolk or rice porridge were common first solids. Regarding differences in head circumference at birth, group contrasts showed smaller head circumference in iron/placebo compared with iron/iron and placebo/placebo groups; absolute differences were only 0.3 cm. There was no indication of worrisome bias due to attrition. The only significant differences were that infants with 9-mo outcome data were born with a mean gestational age 1.2 d higher than that of infants without data ($P = 0.04$), and more were born by cesarean section (68.5% compared with 59.4%, $P = 0.01$).

Supplement distributed and adverse events

Information on number of supplement bottles distributed/returned was available for 962 infants. For the remaining 314 infants, project staff were confident that 1 box was distributed at 6 wk per protocol but simply not recorded. We therefore used this value in further analyses. There were no group differences in number of bottles received (Table 1) (overall: 13.1 ± 6.2 bottles). This was far below the 40 bottles of supplement needed to cover the entire planned supplementation period. Returned bottles were recorded for only 45 infants. Thus, number of bottles received corresponded closely to number of boxes. Most families (945/1276, 74.1%) did not request additional boxes beyond the initial one; 254/1276 (19.9%) requested 1 additional box (total 20 bottles), and 77/1276 (6.0%) requested >2 boxes. Only 15/1276 (1.2%) received ≥40 bottles as planned. The few available calendars indicated that many families did not give supplements daily, with a wide range between first and last dose regardless of number of bottles received. The age at last dose was 5.6 ± 2.5 mo (≤6 mo for >50%). If these data can be extrapolated to the full sample, more than half of the infants received supplements only before 6 mo.

Some parent report data on hospitalizations and doctor visits were available for 96.7% of infants enrolled (1433/1482); information through to 9 mo was available for 73.6% (939/1276). There no statistically significant group differences in

TABLE 1 Infant and family characteristics by group in the combined pregnancy and infancy RCT[1]

Group	Placebo/placebo (PP)	Placebo/iron (PI)	Iron/placebo (IP)	Iron/iron (II)	*P* value[2]	Significant contrasts[3]
n	312	321	316	327		
Infant characteristics						
Male, *n*/total *n* (%)	165/311 (53.1)	150/321 (46.7)	162/316 (51.3)	171/327 (52.3)	0.38	
Birth weight, g	3375 ± 375	3366 ± 383	3328 ± 379	3373 ± 353	0.35	
Gestational age, wk	39.7 ± 1.1	39.7 ± 1.1	39.7 ± 1.1	39.6 ± 1.1	0.70	
Birth length, cm	49.8 ± 1.4	49.6 ± 1.4	49.7 ± 1.5	49.6 ± 1.5	0.12	II < PP
Head circumference, cm	34.2 ± 1.2	34.1 ± 1.2	33.9 ± 1.3	34.2 ± 1.3	0.03	IP < PP, II
First born, *n*/total *n* (%)	233/305 (76.4)	246/313 (78.6)	254/310 (81.9)	247/321 (77.0)	0.32	
Birth by cesarean section, *n*/total *n* (%)	213/308 (69.2)	198/315 (62.9)	228/314 (72.6)	226/325 (69.5)	0.06	PI < IP
Age at 9-mo testing, mo	9.29 ± 0.42	9.27 ± 0.46	9.34 ± 0.57	9.27 ± 0.46	0.18	II < IP
Breastfed at 9 mo,[4] *n*/total *n* (%)	189/226 (83.6)	197/244 (80.7)	190/236 (80.5)	212/247 (85.8)	0.34	
Family characteristics						
Maternal age, y	24.2 ± 3.6	24.6 ± 3.6	24.5 ± 3.6	25.0 ± 4.0	0.09	PP < II
Maternal education, ≥high school, *n*/total *n* (%)	96/309 (31.1)	117/314 (37.3)	105/314 (33.4)	104/322 (32.3)	0.39	
Net family income ≤50,000 yuan/y,[5] *n*/total *n* (%)	262/306 (85.6)	259/315 (82.2)	251/306 (82.0)	270/316 (85.4)	0.45	
Supplement bottles received, *n*	12.7 ± 5.7	13.4 ± 5.7	13.1 ± 6.6	13.3 ± 6.8	0.59	

[1] Values are means ± SDs for continuous variables and *n*/total *n* (%) for categorical ones. Numbers (*n*) vary slightly due to missing data. II, iron in pregnancy/iron in infancy; IP, iron in pregnancy/placebo in infancy; PI, placebo in pregnancy/iron in infancy; PP, placebo in pregnancy/placebo in infancy; RCT, randomized clinical trial.
[2] Based on general linear models for continuous variables and logistic regression for categorical variables.
[3] Statistically significant pairwise comparison(s) between groups, $P < 0.05$.
[4] Breast milk as the sole source of milk or any breast milk in addition to formula or another source of milk. Feeding data, collected by questionnaire, were available for ~75% of the sample.
[5] Family income level for Sanhe County that qualified for public assistance in housing (32).

hospitalizations or doctor visits (data not shown). There were only 3–7 hospitalizations/group for any reason between birth and 9 mo. Many infants had doctor visits for upper respiratory symptoms (41–47%/group); a visit for pneumonia was reported in 3–5%/group. Doctor visits for gastrointestinal symptoms were reported in 16–21%/group.

Impact of iron supplementation on infant iron status. The impact of iron supplementation on infant iron status at birth is summarized in **Table 2**. As expected, the groups differed in maternal ID at or near term. As previously reported (4), maternal iron supplementation reduced the prevalence of ID, but many women still had ID (BI <0 mg/kg in 41% of

TABLE 2 Fetal iron status indicators and infant iron status at the 9-mo assessment by group in the combined pregnancy and infancy RCT[1]

	Placebo/placebo (PP)	Placebo/iron (PI)	Iron/placebo (IP)	Iron/iron (II)	*P* value[2]	Significant contrasts[3]	RR (95% CI)
n	305	311	305	318			
Fetal iron status indicators							
Maternal ID,[4] *n*/total *n* (%)	183/300 (61.0)	205/309 (66.3)	121/297 (40.7)	129/310 (41.6)	<0.01	II < PI	0.63 (0.53, 0.73)
						II < PP	0.68 (0.58, 0.80)
						IP < PI	0.61 (0.52, 0.72)
						IP < PP	0.67 (0.57, 0.79)
ID at birth,[5] *n*/total *n* (%)	123/300 (41.0)	131/310 (42.3)	131/301 (43.5)	132/313 (42.2)	0.94		
Iron measures: birth and 9 mo							
Hb, g/L							
Birth	149 (147, 151)	153 (151, 155)	151 (149, 153)	154 (152, 155)	<0.01	IP, PP < II	
						PP < PI	
9 mo	111 (110, 112)	113 (112, 114)	111 (110, 113)	113 (111, 114)	0.15		
MCV, fL							
Birth	103 (102, 103)	102 (102, 103)	102 (102, 103)	102 (102, 103)	0.54		
9 mo	68.9 (68.5, 69.4)	69.1 (68.7, 69.6)	69.1 (68.6, 69.5)	68.8 (68.3, 69.2)	0.62		
SF, μg/L							
Birth	113 (107, 121)	114 (107, 121)	116 (109, 123)	113 (106, 120)	0.96		
9 mo	11.2 (10.1, 12.4)	12.2 (11.1, 13.5)	11.5 (10.4, 12.8)	11.7 (10.6, 13.0)	0.65		
ZPP/H, μmol/mol heme							
Birth	97.8 (94.2, 101.5)	95.8 (92.4, 99.4)	95.0 (91.5, 98.6)	94.3 (90.9, 97.8)	0.55		
9 mo	80.6 (76.1, 85.3)	70.8 (67.0, 74.9)	75.7 (71.6, 80.2)	72.6 (68.6, 76.7)	<0.01	PI, II < PP	
sTfR, nmol/L							
Birth	31.0 (29.9, 32.1)	29.5 (28.5, 30.5)	29.9 (28.9, 30.9)	29.9 (28.9, 30.9)	0.21	PI < PP	
9 mo	24.0 (23.1, 24.9)	23.8 (23.0, 24.7)	23.1 (22.3, 24.0)	24.6 (23.7, 25.5)	0.13	IP < II	
BI, mg/kg							
Birth	7.31 (7.05, 7.58)	7.42 (7.16, 7.67)	7.40 (7.14, 7.66)	7.41 (7.15, 7.66)	0.95		
9 mo	−0.09 (−0.53, 0.35)	0.27 (−0.17, 0.71)	0.16 (−0.28, 0.61)	0.00 (−0.43, 0.44)	0.68		
9-mo iron status outcomes							
Anemia, Hb <110 g/L, *n*/total *n* (%)	138/305 (45.3)	108/311 (34.7)	121/305 (39.7)	125/318 (39.3)	0.07	PI < PP	0.77 (0.63, 0.93)
ID,[6] *n*/total *n* (%)							
Cutoff (≥2 abnormal iron measures)	208/305 (68.2)	188/311 (60.5)	210/305 (68.9)	189/318 (59.4)	0.02	PI < PP	0.89 (0.79, 1.00)
						PI < IP	0.79 (0.63, 0.98)
						II < PP	0.87 (0.77, 0.98)
						II < IP	0.86 (0.77, 0.97)
BI <0 mg/kg	137/305 (44.9)	135/311 (43.4)	141/305 (46.2)	147/318 (46.2)	0.88		
IDA,[6] *n*/total *n* (%)							
Hb <110 g/L + ID by cutoff	117/305 (38.4)	87/311 (28.0)	101/305 (33.1)	106/318 (33.3)	0.06	PI < PP	0.73 (0.58, 0.92)
Hb <110 g/L + ID by BI	90/305 (29.5)	70/311 (22.5)	83/305 (27.2)	92/318 (28.9)	0.18	PI < PP	0.76 (0.58, 1.00)
Serum CRP, mg/L	0.11 (0.099, 0.14)	0.15 (0.12, 0.18)	0.09 (0.08, 0.12)	0.14 (0.11, 0.17)	0.02	IP < PI, II	

[1] Values are means (95% CIs) for continuous variables and *n*/total *n* (%) for categorical ones. Numbers (*n*) vary slightly due to missing data. BI, body iron; CRP, C-reactive protein; fL, femtoliters; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; II, iron in pregnancy/iron in infancy; IP, iron in pregnancy/placebo in infancy; MCV, mean corpuscular volume; PI, placebo in pregnancy/iron in infancy; PP, placebo in pregnancy/placebo in infancy; RCT, randomized clinical trial; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP/H, zinc protoporphyrin/heme.

[2] Based on general linear models or logistic regression. ZPP/H, SF, sTfR, and CRP (cord blood and 9 mo) were log-transformed to normalize the distribution. Geometric means are shown. Higher values of ZPP/H and sTfR indicate poorer iron status.

[3] Statistically significant pairwise comparison(s) between groups, $P < 0.05$.

[4] Maternal ID defined as BI <0 mg/kg. BI for mothers and infants was calculated by using SF and sTfR according to the formula in Cook et al. (24): body iron (mg/kg) = −[log10 (sTfR · 1000/ferritin) − 2.8229]/0.1207. This formula uses an sTfR assay described in Flowers et al. (25). To convert Beckman Coulter sTfR concentrations for use in the formula, we built on published data for Flowers, Ramco, and Beckman Coulter sTfRs. As reported in Pfeiffer et al. (26), the Ramco assay was similar to Flowers et al. (25). Ramco and Beckman Coulter assays were part of a WHO study that used a standard reference reagent for sTfR (27). The Ramco assay yielded sTfR concentrations 4.3 times higher than Beckman Coulter, so the Flowers sTfR equivalent was calculated by the following formula: Flowers sTfR = 4.3 × Beckman Coulter sTfR.

[5] ID at birth defined as cord SF <75 μg/L or ZPP/H >118 μmol heme/mol. SF <75 μg/L has been used in studies of prenatal ID neurodevelopmental effects (28–30), and ZPP/H >118 μmol/mol heme is the US 90th percentile (31).

[6] ID and IDA results are presented by 2 different criteria for ID: cutoff, ≥2 abnormal iron measures and BI <0 mg/kg.

iron-supplemented mothers and 66% of those randomly allocated to placebo). Consequently, there was more maternal ID in placebo/iron and placebo/placebo compared with iron/placebo and iron/iron groups. The groups showed small but statistically significant differences in cord-blood hemoglobin that did not consistently correspond to the pregnancy RCT. Hemoglobin at birth was somewhat lower in placebo/placebo compared with placebo/iron and iron/iron groups but also in iron/placebo compared with iron/iron groups. Except for higher sTfR in the placebo/placebo compared with the placebo/iron group, groups were similar in other cord-blood iron status measures. Many neonates had evidence of fetal-neonatal ID, as previously reported (4). Even excluding neonates with cord SF <35 μg/L, 41–44% of infants/group had cord SF <75 μg/L or ZPP/heme >118 μmol heme/mol (4).

At 9-mo testing (Table 2), infants showed little infection/inflammation, as indicated by low C-reactive protein concentrations in all groups. ZPP/heme was the only individual 9-mo iron measure showing a significant overall effect of iron supplementation. Groups assigned to iron in infancy (placebo/iron and iron/iron) had lower ZPP/heme concentrations than groups assigned to placebo (iron/placebo and placebo/placebo). There was no overall difference in the prevalence of anemia, but group contrasts showed the risk of anemia was significantly reduced by 23% in the placebo/iron compared with the placebo/placebo group.

ID was common in study infants at 9 mo. However, supplementation effects were statistically significant only for ID defined by cutoff. ID was less common in groups assigned to iron in infancy than those assigned to placebo (placebo/iron, 60.5% and iron/iron, 59.4% compared with placebo/placebo, 68.2% and iron/placebo, 68.9%). Group contrasts showed the following significant reductions in ID risk: for placebo/iron, 11% reduction compared with placebo/placebo and 21% compared with iron/placebo; for iron/iron, 13% reduction compared with placebo/placebo and 14% compared with iron/placebo. There were no statistically significant overall or group differences in ID defined by BI.

IDA was also common at 9 mo. IDA prevalence by cutoff was highest in the placebo/placebo group; placebo/iron, iron/placebo, and iron/iron groups did not differ from each other. There was no overall difference in IDA prevalence for ID defined by BI. However, the only significant group contrast was similar for IDA by cutoff and BI; IDA risk was reduced for placebo/iron compared with placebo/placebo by 27% using cutoff and 24% using BI.

Infant growth at 9 mo

There were no statistically significant overall supplementation effects for 9-mo anthropometric measures (**Table 3**). However, in light of debate about effects of iron supplementation on weight/length gain, we examined individual group contrasts. Weight gain was significantly lower in placebo/iron compared with iron/iron and iron/placebo groups. There were no group differences in length gain.

Subgroup analyses based on infant iron status at birth

To address risks of giving iron to iron-sufficient infants, we conducted subgroup analyses depending on iron status at birth. Among infants who were iron sufficient at birth, more of the few infants taken to the doctor for anatomical concerns (e.g., blocked tear duct, hernia, ptosis) received placebo (9/400) than iron (1/405; Fisher exact $P = 0.01$). There were no other differences in adverse events (doctor visits/hospitalization; data not shown) or anthropometric measurements/growth (**Table 4**). Among infants who were iron deficient at birth, more of those randomly allocated to iron were reported to have visited a doctor for upper respiratory symptoms/fever than those assigned to placebo [145/307 (47.2%) compared with 119/304 (39.1%); $\chi^2 = 4.07$, $P = 0.04$]. There were no other group differences in adverse events, anthropometric measurements, or growth.

Regarding iron outcomes (Table 4), ZPP/heme concentrations at 9 mo were lower in infants assigned to iron than placebo regardless of iron status at birth. Iron supplementation reduced the 9-mo ID risk (by cutoff) by roughly the same degree for infants born iron sufficient (RR = 0.87; 95% CI: 0.77, 0.99) or iron deficient (RR = 0.86; 95% CI: 0.77, 0.96). Among infants who were iron deficient at birth, iron supplementation also reduced risk of anemia (RR = 0.80; 95% CI: 0.65, 0.97) and IDA by cutoff (RR = 0.78; 95% CI: 0.63, 0.97). There were no significant differences using BI to define ID or IDA.

Dose-response effects

We analyzed dose-response relations between number of bottles of iron received in placebo/iron and iron/iron groups and infant outcomes, considering potential confounding factors. For iron status and illness outcomes, we examined maternal age, education, and parity; family income; gestational age at birth; birth weight/length; cord-blood iron status; weight/length gain between birth and 9 mo; and breastfeeding as potential confounding variables. Iron status at birth met the criterion of association

TABLE 3 Infant anthropometric measurements at the 9-mo assessment by group in the combined pregnancy and infancy RCT[1]

Group	Placebo/placebo (PP)	Placebo/iron (PI)	Iron/placebo (IP)	Iron/iron (II)	*P* value[2]	Significant contrasts[3]
n	312	321	316	327		
Weight-for-age *z* score[4]	0.89 (0.77, 1.00)	0.80 (0.69, 0.91)	0.92 (0.81, 1.03)	0.97 (0.86, 1.08)	0.20	PI < II
Height-for-age *z* score	0.33 (0.21, 0.45)	0.24 (0.13, 0.36)	0.30 (0.18, 0.41)	0.33 (0.22, 0.45)	0.70	
Weight-for-height *z* score	1.02 (0.90, 1.14)	0.96 (0.84, 1.08)	1.07 (0.95, 1.19)	1.12 (1.00, 1.24)	0.30	
Head circumference *z* score	0.10 (−0.02, 0.23)	0.01 (−0.11, 0.14)	0.04 (−0.08, 0.17)	0.13 (0.01, 0.26)	0.53	
Weight gain, birth to 9 mo,[5] g	6270 (6146, 6395)	6161 (6039, 6283)	6354 (6231, 6477)	6356 (6235, 6477)	0.09	PI < II, IP
Length gain, birth to 9 mo,[5] cm	22.4 (22.1, 22.7)	22.3 (22.1, 22.6)	22.4 (22.1, 22.7)	22.7 (22.4, 23.0)	0.30	

[1] Values are means (95% CIs). Numbers (*n*) vary slightly due to missing data. II, iron in pregnancy/iron in infancy; IP, iron in pregnancy/placebo in infancy; PI, placebo in pregnancy/iron in infancy; PP, placebo in pregnancy/placebo in infancy; RCT, randomized clinical trial.
[2] Overall *P* value based on general linear models.
[3] Statistically significant pairwise comparison(s) between groups, $P < 0.05$.
[4] *z* scores based on WHO growth curves (33).
[5] Weight and length gains adjusted by age in days at 9-mo testing.

TABLE 4 Infant iron status and anthropometry at 9 mo depending on iron status at birth and supplementation in infancy[1]

	Iron sufficient at birth			Iron deficient at birth		
	Placebo	Iron	*P* value[2]	Placebo	Iron	*P* value[2]
n	347	360		254	263	
Iron outcomes						
Hb, g/L	113 (112, 114)	113 (112, 114)	0.54	109 (107, 111)	112 (111, 114)	<0.01
MCV, fL	69.5 (69.1, 69.9)	69.3 (68.9, 69.6)	0.39	68.3 (67.7, 68.8)	68.6 (68.1, 69.1)	0.40
SF,[3] μg/L	12.6 (11.5, 13.8)	12.8 (11.7, 14.1)	0.76	9.72 (8.72, 10.8)	11.0 (9.84, 12.3)	0.12
ZPP/H,[3] μmol/mol heme	71.6 (68.3, 75.1)	65.7 (62.8, 68.7)	0.01	88.5 (82.5, 95.1)	80.3 (75.2, 85.8)	<0.05
sTfR,[3] nmol/L	22.6 (21.8, 23.4)	23.8 (23.1, 24.5)	0.03	25.1 (24.1, 26.2)	24.7 (23.7, 25.6)	0.53
Anemia, Hb < 110 g/L, *n*/total *n* (%)	130/347 (37.5)	128/360 (35.6)	0.60	125/254 (49.2)	103/263 (39.2)	0.02
ID[4]	219/347 (63.1)	198/360 (55.0)	0.03	195/254 (76.8)	173/263 (65.8)	<0.01
IDA[5]	100/347 (28.8)	98/360 (27.2)	0.64	115/254 (45.3)	93/263 (35.4)	0.02
Anthropometric measurements[6]						
Weight-for-age *z* score	0.95 (0.84, 1.06)	0.86 (0.75, 0.96)	0.23	0.84 (0.72, 0.96)	0.93 (0.81, 1.06)	0.28
Height-for-age *z* score	0.33 (0.22, 0.43)	0.25 (0.14, 0.36)	0.32	0.29 (0.16, 0.42)	0.34 (0.21, 0.47)	0.61
Weight-for-height *z* score	1.10 (0.98, 1.22)	1.03 (0.91, 1.14)	0.38	0.97 (0.85, 1.10)	1.07 (0.94, 1.20)	0.29
Head circumference *z* score	0.06 (−0.08, 0.19)	0.06 (−0.05, 0.16)	1.00	0.13 (−0.01, 0.26)	0.11 (−0.03, 0.25)	0.87
Weight gain, birth to 9 mo, g	6335 (6214, 6455)	6204 (6088, 6321)	0.13	6289 (6170, 6409)	6330 (6190, 6470)	0.66
Length gain, birth to 9 mo, cm	22.4 (22.1, 22.7)	22.4 (22.1, 22.7)	0.92	22.5 (22.2, 22.8)	22.5 (22.2, 22.8)	0.78

[1] Values are means (95% CIs) for continuous variables and *n*/total *n* (%) for categorical ones. Numbers (*n*) vary slightly due to missing data. Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; MCV, mean corpuscular volume; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP/H, zinc protoporphyrin/heme.
[2] Overall *P* value based on general linear models or logistic regression.
[3] Values are geometric means (95% CIs). Statistical analyses were conducted on log-transformed SF, ZPP/H, and sTfR.
[4] ID defined as ≥2 abnormal iron measures (MCV <74 fL, ZPP/H >69 μmol heme/mol, SF <12 μg/L).
[5] IDA defined as Hb <110 g/ and ≥2 abnormal iron measures.
[6] *z* scores based on WHO growth curves (33).

with both number of bottles received and outcome and contributed significantly to models for ZPP/heme and ID by cutoff. For growth, potential confounding variables were the same except for weight/length gains, which were outcomes. Birth length, the only variable to meet the criterion, contributed significantly to models for weight-for-age and weight-for-length *z* scores.

Dose-response analyses uniformly indicated better infant iron status with a greater number of bottles of iron received—but only with iron supplementation in both pregnancy and infancy (iron/iron group). Although few families received >20 bottles of iron, there was a significant linear relation between number of bottles and outcome for every single iron measure except ZPP/heme (**Table 5**). In group contrasts, the medium group (>20 bottles) had significantly higher hemoglobin, SF, and BI and lower sTfR than the very low group (≤10 bottles) and lower sTfR than the low group (11–20 bottles). SF and BI were significantly higher in low compared with very low groups, and there were suggestive trends for higher hemoglobin and BI ($P = 0.06$) in medium compared with low groups. Risk of ID and IDA, whether by cutoff or BI, was significantly reduced in medium compared with very low groups and, for IDA by cutoff, in low compared with very low groups (Table 5). Effects were most dramatic for BI: the relative risk of IDA was reduced 86% in medium compared with very low groups ($P = 0.03$). However, iron status was not good even in the medium group, as indicated by a mean BI of 2.43 mg/kg, only slightly above the ID cutoff of 0 mg/kg.

In dose-response analyses for growth, there were no significant effects in the iron/iron group. In the group with supplementation in infancy but not pregnancy (placebo/iron), there were linear effects for 9-mo weight-for-age and weight-for-length *z* scores: the more bottles of iron received, the heavier the infant for age and length. Mean weight-for-age and weight-for-length *z* scores were 0.72 (95% CI: 0.58, 0.86) and 0.84 (95% CI: 0.69, 0.99) in the very low group, 0.97 (95% CI: 0.74, 1.20) and 1.21 (95% CI: 0.95, 1.46) in the low group, and 1.10 (95% CI: 0.61, 1.58) and 1.39 (95% CI: 0.86, 1.93) in the medium group, respectively. Group contrasts were significant for weight-for-length but not weight-for-age; weight-for-length in both medium and low groups was greater than the very low group ($P = 0.05$ and 0.01, respectively). In dose-response analyses for illnesses, there were no statistically significant relations.

Discussion

In this double RCT, we found that iron supplementation in infancy improved infant iron status at 9 mo. The reduction in ID risk we observed was lower than the mean in a recent meta-analysis (9) (RR = 0.87 in our study compared with 0.30 in the meta-analysis). Because ID and IDA were common in study infants at 9 mo regardless of supplementation group, the overall outcome of the RCT was less than optimal despite statistically significant effects. To consider possible explanations for this unexpected finding, we use a physiologic framework for iron status in infancy (34), specifically, iron status at birth, iron needs, and iron sources (iron losses are unlikely to be a major factor in this setting). There seems little doubt that an inadequate amount of supplemental iron played a role. For >74% of families, only 1 box of supplements (10 bottles) was received. It appears from the limited available calendar data that many families did not give supplements daily and stopped around 6 mo. Thus, it is likely that many infants did not receive supplemental iron from 6–9 mo, when iron needs are exceptionally high. Furthermore, iron needs before 6 mo were probably higher in this sample than often considered for breastfed infants (35, 36). Almost half had indications of fetal-neonatal ID, meaning that they entered the rapid infancy growth period with a substantial iron deficit. Iron needs to support growth and red blood cell mass expansion were

TABLE 5 Dose-response results in iron-supplemented infants whose mothers received iron in pregnancy: Relations between the number of bottles of iron received and infant iron status at 9 mo[1]

	Number of bottles of iron			*P* value[2]	Significant contrasts[3]	RR (95% CI)[4]
	≤10 (VL)	11–20 (L)	>20 (M)			
n	229	66	21			
Iron measures						
Hb, g/L	112 (110, 113)	114 (111, 117)	120 (114, 125)	<0.01	M > VL, L[5]	
MCV, fL	68.5 (67.9, 69.0)	69.3 (68.3, 70.2)	70.1 (68.3, 71.8)	0.03	M[5] > VL	
SF,[6] μg/L	10.7 (9.6, 12.0)	13.9 (11.2, 17.1)	18.3 (12.6, 26.6)	<0.01	M, L > VL	
ZPP/H,[6,7] μmol/mol heme	74.3 (69.3, 79.6)	70.7 (62.2, 80.3)	68.3 (54.8, 85.1)	0.36		
sTfR,[6] nmol/L	25.3 (24.3, 26.3)	24.1 (22.4, 26.0)	19.5 (17.1, 22.3)	<0.001	M < VL, L	
BI	−0.42 (−0.91, 0.07)	0.67 (−0.24, 1.58)	2.43 (0.81, 4.04)	<0.001	M, L > VL M > L[5]	
Iron status indicators						
ID (cutoff)[7]	142/229 (62.0)	38/66 (57.6)	8/21 (38.1)	0.05	M vs. VL	0.61 (0.35, 1.07)
ID (BI)	115/229 (50.2)	26/66 (39.4)	5/16 (23.8)	0.01	M vs. VL	0.47 (0.22, 1.03)
IDA (cutoff)	87/229 (38.0)	16/66 (24.2)	2/21 (9.5)	<0.01	L vs. VL	0.64 (0.40, 1.01)
					M vs. VL	0.25 (0.07, 0.95)
IDA (BI)	76/229 (33.2)	14/66 (21.2)	1/21 (4.8)	<0.001	M vs. VL	0.14 (0.02, 0.98)
					L[5] vs. VL	0.64 (0.39, 1.05)

[1] Values are means (95% CIs) for continuous variables and *n* (%) for categorical ones. BI, body iron; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; L, low; M, medium; MCV, mean corpuscular volume; SF, serum ferritin; sTfR, serum transferrin receptor; VL, very low; ZPP/H, zinc protoporphyrin/heme.
[2] *P* values are for the linear trend.
[3] Significant contrasts ($P < 0.05$) are based on general linear models for continuous measures and logistic regression for iron status indicators.
[4] RR is shown for the significant corresponding contrasts for iron status indicators.
[5] Suggestive trend ($P < 0.10$) is shown to indicate the pattern of findings.
[6] Values are geometric means (95% CIs). Statistical analyses were conducted on log-transformed SF, ZPP/H, and sTfR.
[7] Iron status at birth was a significant covariate for ZPP/H and ID (cutoff). Adjusted values are shown.

also high in this population, both pre- and postnatally. Pregnancy weight gain was high, fetal growth was good (4), and, at 9 mo, the mean weight-for-age *z* score was 0.89. Taken together, this was a sample with poor iron status at birth, high iron needs for growth, and insufficient supplemental iron intake.

Conclusions about the impact of maternal iron supplementation on infant iron status differed for the RCT intention-to-treat analysis and the dose-response analysis. In the primary analysis by RCT group, there was no added benefit of maternal iron supplementation over that with iron supplementation in infancy, contrary to prediction, and no benefit of iron supplementation in pregnancy in the absence of iron in infancy. These findings seem to make sense in terms of the little or no impact of maternal iron supplementation on cord-blood iron measures in the prenatal RCT, despite excellent maternal response to iron supplementation (4). However, in dose-response analyses, it was only in the group that received iron supplementation in both pregnancy and infancy that a higher number of bottles of iron related to better infant iron status at 9 mo. One might speculate that iron supplementation during fetal development altered regulatory processes or set points that facilitated erythropoiesis and repletion of iron stores when supplemental iron was provided above some minimum in infancy. This concept would be consistent with a recent report of developmental plasticity of red blood cell homeostasis during the fetal period: iron availability to the fetus exerted a long-lasting influence on red blood cell clearance and turnover in both monkey and human infants (37). However, our findings require replication before further interpretation, because few infants received even close to the targeted amount of supplemental iron.

Reasons for the families' minimal use of study supplements are unclear, although some parents reported hesitation to give supplements to infants who appeared healthy. Nonetheless, our dose-response analyses strongly suggest that infant iron status could improve with more supplemental iron. Consistent findings across almost all iron status measures show that number of bottles of iron received corresponded to something biologically meaningful, likely reflecting adherence at least to some degree. When >20 bottles of iron were received, IDA by BI was essentially eliminated, and means for all iron measures were normal (although several were close to ID cutoffs). The findings demonstrate that infants responded to iron proteinsuccynilate at a dose suitable for routine supplementation.

We found different responses to supplementation for several iron measures. ZPP/heme showed more effect of iron supplementation than any other individual iron measure or BI in the RCT analysis, whereas it was the only measure that did not show an effect in dose-response analyses. In contrast, BI showed a clear relation with number of bottles of iron received (negative with ≤10 bottles but positive, albeit minimally, with more bottles). BI is an increasingly popular iron status index, combining ferritin, a measure of iron stores, and sTfR, a measure of the demand for iron to support erythropoiesis. Our results indicate that using ZPP/heme or BI alone might miss functionally important effects. This issue warrants further investigation.

Growth and adverse events were other outcomes of interest. There were no effects of supplementation in either infancy or pregnancy on linear growth from birth to 9 mo, indicating the absence of an adverse effect of iron supplementation on infant length gain. Small RCT group differences in weight gain are hard to interpret in terms of iron supplementation. Weight gain was highest in the group that was iron supplemented in both

pregnancy and infancy—higher than in the group receiving iron only in infancy. But the placebo/iron group had lower weight gain than the group that received iron only during gestation. However, in dose-response analyses, placebo/iron group infants with a greater number of bottles of iron received had higher weight-for-age and weight-for-length *z* scores. The inconsistent pattern of weight and weight gain does not support interpretation as a benefit or risk of iron supplementation in infancy. In our subgroup analysis of infants who were iron sufficient at birth, there were no growth differences at 9 mo between those randomly allocated to iron or placebo in infancy.

There was no impact of iron supplementation in infancy and/or pregnancy on doctor visits or hospitalization. In subgroup analyses of infants who were iron sufficient at birth, the only difference was fewer doctor visits for minor anatomical concerns (e.g., blocked tear duct, hernia) in the iron-supplemented group. It is hard to see how this difference could relate to iron supplementation in infancy. However, among infants who were iron deficient at birth, parents reported more doctor visits for upper respiratory symptoms for infants receiving iron than placebo. These results may be pertinent to meta-analysis findings of more fever and vomiting in iron-supplemented infants (9).

The minimal supplement consumption is a major study limitation, although it confirms the voluntary nature of study participation (e.g., parents could choose not to request additional supplements but still participate in other aspects of the study). It may also inadvertently inform the debate about iron supplementation in breastfed infants <6 mo of age (38), who are often considered to need little or no iron from external sources other than breast milk (35, 36). Most study families took only the initial box of supplements at 6 wk, which generally seemed to have been used by 6 mo. Even this minimal amount of iron reduced the prevalence of ID at 9 mo compared with placebo, suggesting that these breastfed infants benefited from iron before 6 mo of age. However, the study design did not systematically vary age of starting supplements and cannot determine if 9-mo iron status would be as good or better with supplementation starting after 6 mo, as suggested by a Honduran and Swedish study (39). However, 2 small North American studies also reported hematologic improvements in term breastfed infants who received iron supplements from 1 to 6 mo of age (40, 41). One assessed developmental outcomes as well and observed benefits at 13 mo (40).

In any case, our findings suggest that current estimates of iron needs in healthy term breastfed infants (35, 36) may not apply to infants in much of the world. Many infants are not born under conditions that are optimal for iron status, such as having a mother with good iron status in pregnancy, being born iron sufficient, and having delayed cord clamping. The field needs better ways to determine how much iron breastfed infants need in settings where many have poor iron status at birth and are doubling or tripling their birth weight younger than previously. New approaches that improve iron delivery to the fetus and young infant are also urgently needed in such settings.

Other limitations: we could not calculate how many doses or how much iron each infant actually received or when supplements were given. Nonetheless, it is clear the amount for most infants was insufficient to meet iron needs between 6 wk and 9 mo. The limited intake of supplemental iron may limit the study's ability to detect effects (positive or negative). It is also important to note that tissue iron needs and effects of iron supplementation for other developing organs, such as the brain, may not parallel those for blood. Our dose-response analyses were limited by the relatively small number of infants for whom a more adequate amount of iron was distributed. Because the number of bottles of iron for these few infants was sufficient to last beyond 6 mo, we cannot determine whether their better iron status at 9 mo was due to a greater total amount of supplemental iron or intake after 6 mo. Reliance on parental report regarding doctor visits and hospitalizations is another limitation. Such data were also missing for ~25% of infants at 9 mo. The study was not powered to detect statistically significant differences in infrequent adverse events, such as infant death or major illness.

In sum, we found that iron supplementation in infancy reduced the risk of ID at 9 mo, whereas iron supplementation in pregnancy had no impact, based on the RCT design. The reduced risk was observed regardless of iron status at birth. However, close to two-thirds of infants had ID at 9 mo and one-third had IDA, despite supplementation. Among infants whose mothers were iron supplemented who were themselves randomly allocated to iron, the more bottles of iron received, the better iron status at 9 mo. Nonetheless, infants with the highest number of bottles still had iron measures that were close to cutoffs indicating ID. These results point to challenges in preventing ID and IDA among breastfed infants in at-risk populations, even when their health is otherwise excellent. There were no adverse effects of iron supplementation on infant health or growth overall or among infants who were iron sufficient at birth.

Acknowledgments

We appreciate the guidance of the Independent Data Monitoring Committee: Sean Lynch, Zuguo Mei, Christine McLaren, and Zu-Pei Chen. BL, GX, ZZ, TT, and ML contributed to the study conception and design; YJ, XL, MZ, GX, FL, GZ, ZZ, and ML conducted the research; BR and NK performed the statistical analysis; BL, KMC, and DCCS wrote the article; and BL had primary responsibility for final content. All authors critically revised the manuscript and read and approved the final manuscript.

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