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Contribution of iron status at birth to infant iron status at 9 months: data from a prospective maternal-infant birth cohort in China

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Abstract

BACKGROUND/OBJECTIVES—The contribution of iron status at birth to iron status in infancy is not known. We used a physiologic framework to evaluate how iron status at birth related to iron status at 9 months, taking iron needs and sources into account.

SUBJECTS/METHODS—In a longitudinal birth cohort in China, iron status measures in cord blood and venous blood in infancy (9 months) and clinical data were prospectively collected in 545 healthy term maternal–infant dyads. We used structural equation modeling (SEM) to create a 9-month iron composite and to assess direct and indirect contributions of multiple influences on 9-month iron status. Logistic regression was used to calculate odds ratios (OR) for iron deficiency (ID), iron deficiency anemia (IDA), and anemia.

RESULTS—Approximately 15% (78/523) of infants were born with cord SF<75 μg/l, suggesting fetal-neonatal ID. At 9 months, 34.8% (186/535) and 19.6% (105/535) of infants had ID and IDA, respectively. The following factors were independently associated with poorer 9-month iron status: higher cord zinc protoporphyrin/heme (ZPP/H) (adjusted estimate −0.18, $P< 0.001$) and serum transferrin receptor (sTfR) (−0.11, $P=0.004$), lower cord hemoglobin (Hb) (0.13, $P=0.004$), lower birth weight (0.15, $P< 0.001$), male sex (0.10, $P=0.013$), older age at testing (−0.26, $P<0.001$), higher 9-month weight (−0.12, $P=0.006$) and breastfeeding (0.38, $P<0.001$). Breastfeeding at 9

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AUTHOR CONTRIBUTIONS

JS was a co-investigator in the Brain and Behavior in Early Iron Deficiency study and principal investigator (PI) in the grant from NSFC; BL was the overall PI of the study; BL and JS were responsible for designing and conducting the research, writing and interpreting results; NK conducted methodology and formal analysis; BR conducted data extraction and analysis; BZ was responsible for investigation and data collection. KMC contributed to writing, review and editing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest for the authors or acknowledged individuals.

months showed the strongest association, adjusting for all other factors. Compared to formula-fed infants, the odds of IDA were 19.1 (95%CI: 6.92, 52.49, $P < 0.001$) and 3.6 (95%CI: 1.04, 12.50, $P=0.043$) times higher in breastfed and mixed-fed infants, respectively.

CONCLUSIONS—Indicators of iron status at birth, postnatal iron needs, and iron sources independently related to iron status at 9 months. Sex was an additional factor. Public health policies to identify and protect infants at increased risk of ID should be prioritized.

INTRODUCTION

Adverse effects on neurodevelopment are worrisome concerns about iron deficiency (ID) and iron deficiency anemia (IDA) in infancy.[1] Although routine iron supplementation is often recommended,[2] 43% of the world's children < 5 years were anemic in 2011, about half due to ID.[3] In rural regions of China where our study was conducted, 49.5% of 6- to 23-month-olds were anemic in 2010.[4] ID also remains a concern in higher income countries. For example, the National Health and Nutritional Examination Survey 2003–2010 found ID in 15.1% of all US 12- to 23-month toddlers;[5] prevalence was higher among poor and/or minority toddlers.[6] Screening for anemia is often recommended late in the 1st year,[7] but brain development may already have been affected. However, there is recent debate about the value of screening,[8] making it even more important to identify early-life factors that put infants at risk for ID.

A physiologic framework indicates that infant iron status will be determined by iron status at birth and postnatal iron needs, availability, and losses.[9] This framework is implicit or explicit in previous studies of predictors of iron status in infancy. In our previous study in Chile,[9] risk factors varied depending on iron supplementation, but lower hemoglobin (Hb) at 6 months was the strongest predictor of ID/IDA at 12 months. We postulated that 6-month Hb largely reflected iron status at birth, but, like most other studies, did not directly assess it.[9]

Few studies have explored multiple potential determinants in a single cohort. Some of those studies were limited by small samples or single indicators.[10–13] Two studies considered cord transferrin receptor (sTfR, a measure of erythropoiesis) and serum ferritin (SF, a measure of iron stores) in larger samples[14,15] but did not include multiple factors related to iron status in infancy. For instance, they did not consider feeding, although associations between feeding type and iron status of infants in the first year of life have been reported in cross-sectional studies.[16,17] Therefore, the aim of the present study was to consider multiple measures of iron status at birth and other factors that affect iron status at 9 months in an extensively characterized sample of healthy term infants from a prospective, longitudinal maternal-infant birth cohort. We expected that lower cord SF would predict poorer iron status later in infancy.

MATERIALS AND METHODS

Study design and subjects

Infants were part of the Brain and Behavior in Early Iron Deficiency study to evaluate the impact of the timing of early ID on neurodevelopment.[16] Pregnant women receiving prenatal care at Fuyang Maternal and Children's Health Care Hospital in southeastern China were

randomly recruited at routine visits at 36–37 weeks gestation between December 2008 and November 2011. Those with uncomplicated singleton pregnancies were invited to participate. Using chart review and maternal interview after delivery, entrance criteria were confirmed as previously reported:[18] singleton birth with gestational age ≥37 weeks, birth weight ≥2500g, 5-minute Apgar score ≥7. Exclusion criteria were *in vitro* fertilization, chronic disease, pregnancy complications (maternal severe hypertention, diabetes mellitus), antibiotics use (an indirect measure of infection), placental abruption/uterine rupture, birth injury or congenital deficits, and hemolytic or metabolic diseases. A total of 1196 newborns had cord blood collected with parental signed consent. Based on cord-blood iron measures (see Figure 1), 436 infants were invited to participate in the developmental study, with a venous blood sample at 9 months to assess iron status in infancy. The remaining 760 infants received usual health care at community clinics (routine visits at 6 weeks and 3, 6, and 9 months) with anemia screening by capillary blood test at 9 months. Those found to be anemic were invited to have a venipuncture for iron measures. The study was approved by the Institutional Review Boards of the University of Michigan and the Children's Hospital Zhejiang University.

Data collection

Information regarding basic family background, maternal medical history, cigarette and alcohol use, maternal anthropometric (height and weight) and clinical measurements (blood pressure, Hb), mode of delivery, infant birth weight, sex and gestational age were collected after delivery. The time of cord clamping was not individually recorded but, per local clinical practice, occurred "immediately" after vaginal birth and within about 60 seconds of birth by caesarian section. Information on family socioeconomic status, occupation, education, infant anthropometric data to index iron needs for growth, and iron sources such as breast/bottle feeding and nutritional supplement use were recorded by trained project personnel at 6-weekand/or 9-month developmental assessments. This information was not available for most infants receiving community care, except for anthropometric data.

Iron status measures and iron supplementation

Measures of maternal iron status consisted of Hb in mid- and late pregnancy and at delivery, obtained by chart review. Samples of 15 ml umbilical cord blood at birth and 2 ml venous blood at 9 months were collected for study infants. In Fuyang, whole cord blood for zinc protoporphyrin /heme (ZPP/H) was stored at 4°C and protected from light; serum for other iron measures was stored at-20°C. Samples were transferred twice a week to the central laboratory of the Children's Hospital, Zhejiang University, where iron status included Hb, SF, sTfR, ZPP/H and serum C-reactive protein were measured. At 9 months, mean corpuscular volume (MCV) and red cell distribution width (RDW) were included. Laboratory methods were previously specified:[18] SF by electro-chemiluminescent immunoassay (Cobas 6000–601, Roche Diagnostics Corp., Basel, Switzerland), sTfR by Chemiluminescent immunoassay (IMMULITE, Diagnostic Products Corporation, DPC, Los Angeles, California), ZPP/H by ZP Hematofluorometer (model 206D; AVIV Associates, Lakewood, New Jersey), and Hb, MCV and RDW by autoanalyzer (Sysmex SE-9000 Auto Hematology Analyzer, Kobe, Japan).The laboratory maintained standard quality control procedures.

Drawing on review data for term infants,[19] the study considered cord Hb< 130 g/L as low (corresponding to about < 2 SD), 130 – 140 g/L as marginally low, and 150–175 g/L as normal (0.75 SD to 1.25 SD). For cord SF, we initially considered < 60 μg/L as low and 150–250 μg/L as normal. This cutoff for low SF corresponded to <5th percentile and the normal range to about the mean ± 0.5 SD in our previous study in the same province.[20] Since most iron in a neonate's body is in red cells, we reasoned that low Hb at birth might increase risk of ID in later infancy. Consequently, infants with low cord blood Hb were considered to have low birth iron status and provided with iron supplements (~1 mg/kg per day iron as oral iron proteinsuccynilate [Lee's Pharmaceutical Holdings Limited, Hong Kong]), beginning at 6 weeks. Infants with cord blood Hb 130–140 g/L or cord SF< 60 μg/L were considered to have marginal iron status and were randomly assigned in a 1:2 ratio to the same supplement or placebo (Figure 1). Our rationale for a small randomized controlled trial (RCT) (www.Clinicaltrials.gov.NCT.00642863) was uncertainty about whether such infants were at increased ID risk or would benefit from early iron supplementation. Infants with normal cord blood Hb and SF received placebo to avoid giving iron to iron-sufficient infants. Iron supplements and placebo were prepared in identical bottles with identical packaging except for a unique supplement number for each child. Parents and project staff were unaware of which supplement infants received. An automated program utilizing cord blood Hb and SF, created by the project's statistician in the US, assigned the supplement numbers.

For data analysis, we defined fetal-neonatal ID as in previous developmental studies,[20–24] specifically cord SF < 75 μg/l. At 9 months, anemia was defined per WHO guidelines as Hb< 110 g/l.[25] ID was defined as 2 or more abnormal measures using the following cutoffs for 4 measures: MCV < 74 fl,[26] RDW > 14.5%,[27] SF< 12.0 μg/l,[28] and ZPP/H > 69 μmol/molheme.[29] IDA was defined as ID plus anemia. We did not use sTfR to classify iron status, since methods differ and norms were not standardized for infants. However, we analyzed sTfR and body iron (BI) as continuous variables. BI was calculated as our previous study[16] (body iron (mg/kg) = 2[log10(sTfR*1000/ferritin) 2.8229]/0.1207).[30–32] Missing ZPP/H data were imputed using IVEware (version 0.2), as equipment for ZPP/H was not available at the start of the study. Infants with IDA at 9 months were treated with oral iron therapy per local clinical practice.

Statistical analysis

Analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). *T*-tests were used to determine if infants with or without 9-month iron status data differed with respect to cord blood iron measures and sample characteristics and to assess sex differences.

Since several factors influence iron status in infancy, our primary statistical approach was multivariate. However, in an initial step focusing solely on iron status at birth, we examined Pearson correlations between cord-blood and 9-month iron measures to identify significant relations that warranted further multivariate analysis. To assess how different potential influences on iron status in infancy affected each other and 9-month iron status, we applied structural equation modeling (SEM). SEM is a powerful statistical technique that combines measurement of latent variables via factor analysis and path analysis. This statistical

technique is designed to examine complex relations among variables and produce visual representations of those relations. The path modeling aspect of SEM assesses main effects, confounders, and mediators simultaneously. Resulting path modeling can display direct and indirect effects that are simultaneously adjusted for multiple confounders. Thus, the independent effects of each variable can be determined, as well as the path by which that variable relates to the outcome. In this study, 9-month iron status was the outcome. We used SEM to derive a 9-month iron status composite via factor analysis of all 9-month iron measures (Hb, MCV, RDW, ZPP/H, SF, and sTfR) with their respective factor loading coefficients. We also performed similar SEM with 9-month BI as the outcome.

In our SEM application, variables considered potentially related to iron status at birth were maternal Hb (mid- and late pregnancy, delivery); delivery type (vaginal vs. C-section); cord Hb, SF, sTfR, and ZPP/H; gestational age and birth weight. Weight at 9 months and age at testing were used to capture iron needs. The iron available from external sources was captured by type of milk feeding at 9 months (breastfeeding only [BF], mixed breast and formula [MF], or formula only [FF]),[16] age at introduction of other foods, and iron supplementation between 6 weeks and 9 months (assigned by design based on cord-blood Hb and SF). We considered other potential factors (e.g., infant sex, socioeconomic status, and maternal age). To relate to clinical practice, we used logistic regression (Mplus [www.StatModel.com]) to calculate adjusted odds of IDA, ID without anemia, ID total, and anemia at 9 months based on significant paths in SEM models.

RESULTS

Sample characteristics

Of 2315 women screened, 2144 (92.6%) were eligible. Almost all considered participation (2128/2144, 99.3%). Cord blood measures were available for 1196 neonates, 436 of whom were recruited to the initial developmental study and 760 received usual community care. Missing cord blood data was generally due to birth in another health care facility or the obstetrical unit being too busy to collect cord blood. Venous samples for measures of 9-month iron status were obtained for 283 of the infants initially recruited to the developmental study and 262 of those who received anemia screening at the community level. Thus, a total of 545 infants had iron status data at both birth and 9 months (Figure 1). Infants with or without 9-month iron status data had similar characteristics (data not shown), with the exception of iron status at birth. As expected by design, infants with 9-month hematology data had slightly worse iron status at birth than those without (cord Hb: 150.0 ± 18.7 g/l vs. 153.2 ± 16.7 g/l, $P = 0.002$; cord SF: 171.6 ± 87.0 g/l vs. 188.7 ± 89.8 g/l, $P = 0.001$; cord BI: 11.1 ± 3.1 mg/kg vs.11.7 ± 2.8 mg/kg, $P < 0.001$).

Characteristics of participants with data at 9 months are shown in Table 1. Mothers were in their mid- to late-twenties, most of whom (n=176/308) had completed middle school. Households almost uniformly included the father and at least one grandparent. Infants were born at term, weighing 3.4 kg on average. Males and females were represented roughly equally. Infants averaged 9.3 months at 9-month testing, range 8.0 to 10.6 months. At 9 months, mean weight-for-age and height-for-age z-scores were 0.63 and 0.53, respectively.[33]

On average, males weighed 100 (SD399) g more than females at birth (P= 0.008) and gained 580 (SD 987) g more between birth and 9 months (P<0.001).

Hematology profiles

Approximately15% (78/523) of infants were born with cord SF<75 μg/l, indicating fetal-neonatal ID. At 9 months, 34.8% (186/535) of infants had ID: 19.6% (105/535) had IDA, and 15.1% (81/535) had ID without anemia. An additional 13.8% (74/535) were anemic but not ID. Iron status for 10 infants could not be classified. Table 2 describes iron measures depending on iron status at 9 months.[30–32]

Correlations between iron measures at birth and 9 months

Table 3 shows correlations between individual cord-blood and 9-month iron measures. Cord-blood measures commonly used in previous studies, such as Hb and SF, showed no correlation with most indicators of iron status at 9 months. Cord sTfR and BI showed statistically significant low-order correlations with several indicators. In contrast, all correlations between cord ZPP/H and 9-month iron measures were statistically significant and generally higher, although still modest.

Factors contributing to poorer 9-month iron status in structural equation models (SEM)

All statistical indices showed a good fit for both iron composite and BI models: Bentler's Comparative Index = 0.99/0.98, Chi-Square = 0.23/0.21, and standardized root mean-square residual= 0.039/0.038. The latent variable of 9-month iron status was based on factor analysis with the following loading coefficients for the individual iron measures: Hb 0.63, MCV 0.77, RDW −0.69, ZPP/H −0.57, SF 0.48, and sTfR 0.40.

There were direct paths to iron status at 9 months for indicators of relevant components of our physiologic framework. Figure 2 shows direct and indirect paths for the iron composite model. Results for the BI model were generally similar but not as strong (data not shown). Regarding indicators of iron status at birth (shown in yellow in Figure 2), higher values for cord ZPP/H and sTfR were directly related to poorer 9-month iron composite (P< 0.001 and 0.004, respectively). Lower cord Hb and birth weight also had direct relations with poorer 9-month iron status (P= 0.004 and < 0.001, respectively). ZPP/H was the strongest among the indicators of iron status at birth. These indicators were in turn influenced by other factors. Cord Hb was positively affected by maternal Hb at delivery (P= 0.001) and negatively affected by delivery type (P= 0.023) (lower Hb with caesarian section). Birth weight increased with gestational age (P< 0.001) and maternal BMI in early pregnancy (P< 0.001) and was lower in females (P< 0.001). Cord SF did not show any relation to 9-month iron status, although it was associated with iron supplementation, as expected by design (P< 0.001).

Regarding indicators of iron needs (shown in blue in Figure 2), older infant age, even within the relatively narrow age range of 8.0 to 10.6 months, was directly associated with poorer iron status in both models(P-values<0.001). Higher weight at 9 months was also directly associated with concurrent iron status (P= 0.006). Indirect paths to higher 9-month weight

included higher birth weight (P<0.001), male sex (P< 0.001), later age at starting solids (P= 0.007), and older age at testing (P= 0.002).

Regarding iron sources (shown in green in Figure 2), type of milk feeding at 9 months related to 9-month iron status – more breastfeeding at 9 months was associated with poorer iron status (P-value< 0.001). In fact, type of feeding was the strongest single contributor to 9-month iron status, independent of all other factors. Iron supplementation in the small RCT showed better iron status at 9 months (P= 0.024).

Infant sex was a significant predictor (P= 0.013). Most of the effect was through growth (birth weight and 9-month weight, both P-values <0.001), but males had poorer 9-month iron status than females after taking these growth parameters into account.

To indicate the contribution of cord-blood iron measures relative to other factors, we estimated their proportion of the variance explained. Cord Hb, ZPP/H, and sTfR combined accounted for 20.5% of the explained variance in the iron composite model. Thus, other factors accounted for 79–86% of the explained variance.

Risk factors contributing to anemia and ID in later infancy

Table 4 shows the odds for risk factors related to clinical diagnoses, i.e., anemia, IDA, ID without anemia, and ID overall at 9 months. Breastfeeding was the only factor that increased the odds of all 4 clinical outcomes, with highest odds for IDA. Compared to formula-fed infants, the odds of IDA were 19.1 and 3.6 times higher in breast- and mixed-fed infants, respectively. For age at testing, the odds of IDA and ID overall increased 8.4 and 6.5 times, respectively, with each added month in age (odds for testing age in weeks X 4.33 weeks/month). Regarding sex differences, the odds of ID without anemia or ID overall were about 2 times higher in males than females. Statistically significant but smaller odds of one or more clinical outcome were also observed for lower cord Hb, higher cord sTfR and ZPP/H, and lower birth weight.

DISCUSSION

This study simultaneously considered pre- and postnatal factors that might influence iron status in later infancy in a prospective maternal-infant birth cohort. We found that indicators of iron status at birth, postnatal iron needs for growth, and external iron sources independently predicted iron status at 9 months in this sample of term Chinese infants. Our results also confirmed male-female differences in iron status in infancy,[34] taking growth-related sex differences into account. The strongest individual factors related to poorer iron status were breastfeeding at 9 months, older age at assessment, and higher cord-blood ZPP/H.

Although specific relations between contributing factors and infant iron status are likely to vary depending on context, our main findings have implications for pediatric practice. Our results show that fetal-neonatal iron status contributed to iron status in later infancy but not through cord SF. In SEM, cord SF, which has been used in several previous studies, did not contribute significantly by itself. Rather, cord ZPP/H, sTfR, and Hb were each significant

indicators of the 9-month iron composite. ZPP/H and sTfR are not commonly used in clinical practice, but ZPP/H is promising as a clinical tool. It reflects the bone marrow iron available for erythrocyte production,[35] is the first measurable biochemical change in erythrocytes after a decline in iron status,[36,37] and is more sensitive than SF or Hb in detecting pre-anemic ID in children.[38] It can be done at low cost requiring only a drop of blood or even non-invasively.[39] Our finding that cord ZPP/H showed the strongest association to iron status at 9 months among iron measures at birth points to a possible screening measure to identify neonates at increased risk for poor iron status in later infancy.

While our results document and quantify the contribution of iron status at birth, the findings emphasize the importance of postnatal factors. Breastfeeding and older infant age were the strongest influences on infant iron status in this sample. We previously summarized 13 studies and reported the relations between breastfeeding> 6 months and infant iron status in this cohort and another larger cohort in northern China.[16] That paper and recent study of Chinese infants[17] show that, across a range of settings and measures of iron status, infants breast fed > 6 months are at increased risk of ID/IDA. The current analysis extends these observations by simultaneously considering other potential influences on iron status in later infancy, including iron status at birth. Our findings should not be interpreted to detract from breast feeding, the advantages of which are well recognized. Rather, the clinical implication is that strategies for preventing ID in breast-fed infants still need improvement. Additional strategies might include screening for ID at birth, more sensitive and/or more frequent screening in infancy, especially in breast-fed infants, iron-rich complementary foods, and iron supplements. Recommended strategies should ideally be tailored to specific contexts, since the prevalence of ID and the duration and intensity of breast feeding varying greatly from country to country and region to region. In China, a public health campaign for iron supplement with a full fat soy powder mixed multiple micronutrient powders (Ying Yang Bao), which was mostly rolled out after our study, has been effective in reducing the prevalence of anemia in children.[4] However, more needs to be done to protect infants from ID, especially those who are breast-fed.

Our finding that older infant age, even within the relatively narrow range of 8.0 to 10.6 months, related to poorer iron status also has clinical implications. It points to how rapidly infant iron needs for growth can outpace external sources of iron later in the first year of life. The implication is that more sensitive and timely ID screening is warranted in populations like those in our study. We found that ID with and without anemia was common by 9–10 months, suggesting that anemia screening at 12 months may be later than optimal in China. In fact, a recent study in Taiwan found that ID and IDA were common in breast-fed infants as young as 4–6 months.[17] Taken together, these findings suggest a need for research specifically designed to inform recommendations on the optimal age and method of ID screening under current conditions in Chinese infants.

This study has several limitations. Although our indicators of fetal-neonatal iron status were more comprehensive than previous studies, maternal Hb was the only measure of iron status in pregnancy, and cord ZPP/H was unavailable for infants born early in the study period. Quantitative data on formula intake or consumption of juice and solid foods were not obtained. The study's limited age range and sample size and its design mean that the study

cannot determine an optimal age for screening for ID. The results may not generalize to contexts where there is less breastfeeding, different iron supplementation practices, or poorer growth. Furthermore, relations between potential predictors and infant iron status may vary depending on such contextual factors as the prevalence of maternal and infant ID and infant feeding practices.

CONCLUSIONS

Concerns about long-term developmental deficits after transient early ID emphasize the importance of identifying children who are at risk for ID in infancy and preventing its occurrence. In this prospective maternal-infant cohort, we found that indicators of iron status at birth, postnatal iron needs, and external iron sources independently contributed to iron status at 9 months, as did sex, with breastfeeding being the single strongest risk factor. Public health policies to identify, monitor, and protect those infants at increased risk for poor iron status in later infancy are potential strategies.

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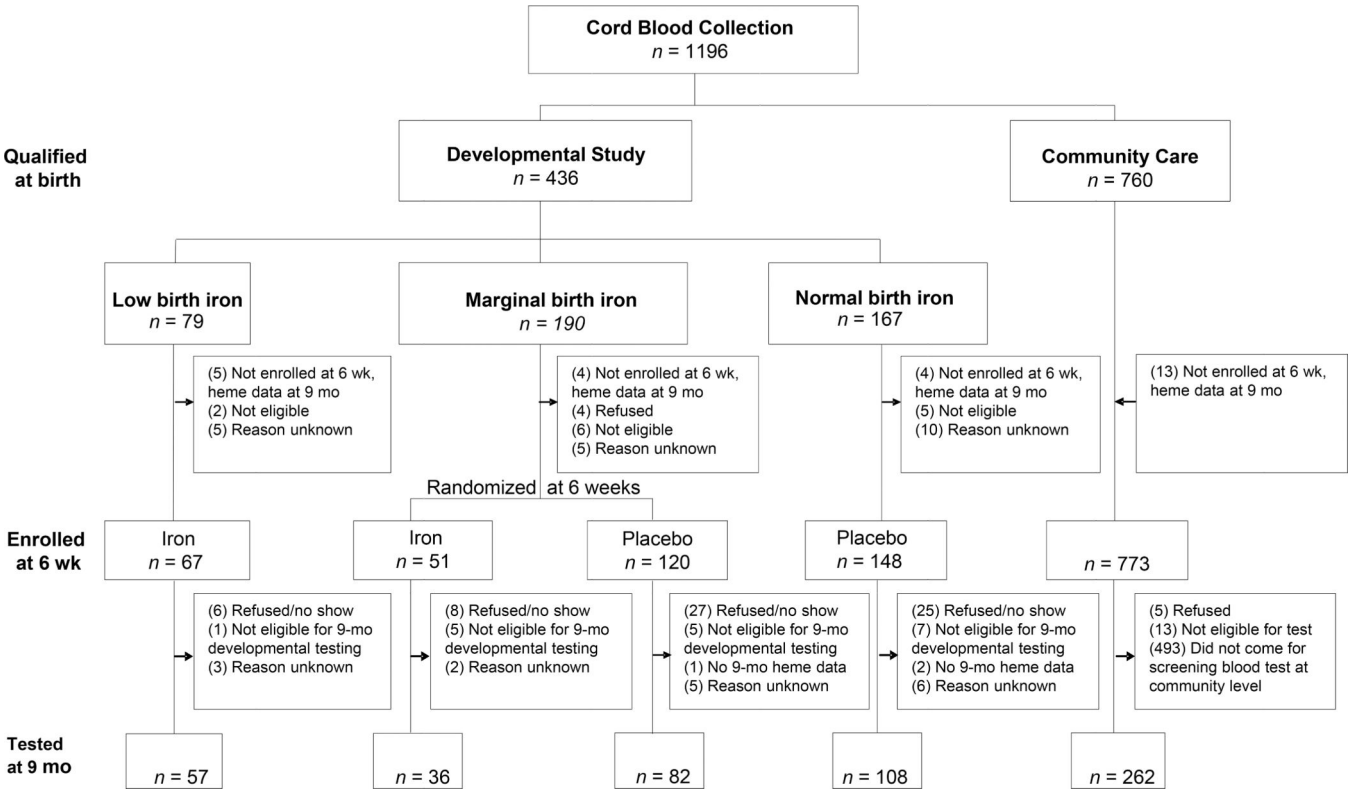

Figure 1.

Flow chart of participants.

The Community Care cohort was not part of the randomized controlled trial (RCT), and individual reasons for drop out were not tracked. 13 infants who qualified for the Developmental Study but were not enrolled at 6 weeks provided hematology data at 9 months. They are shown as joining Community Care. Abbreviations: heme hematology.

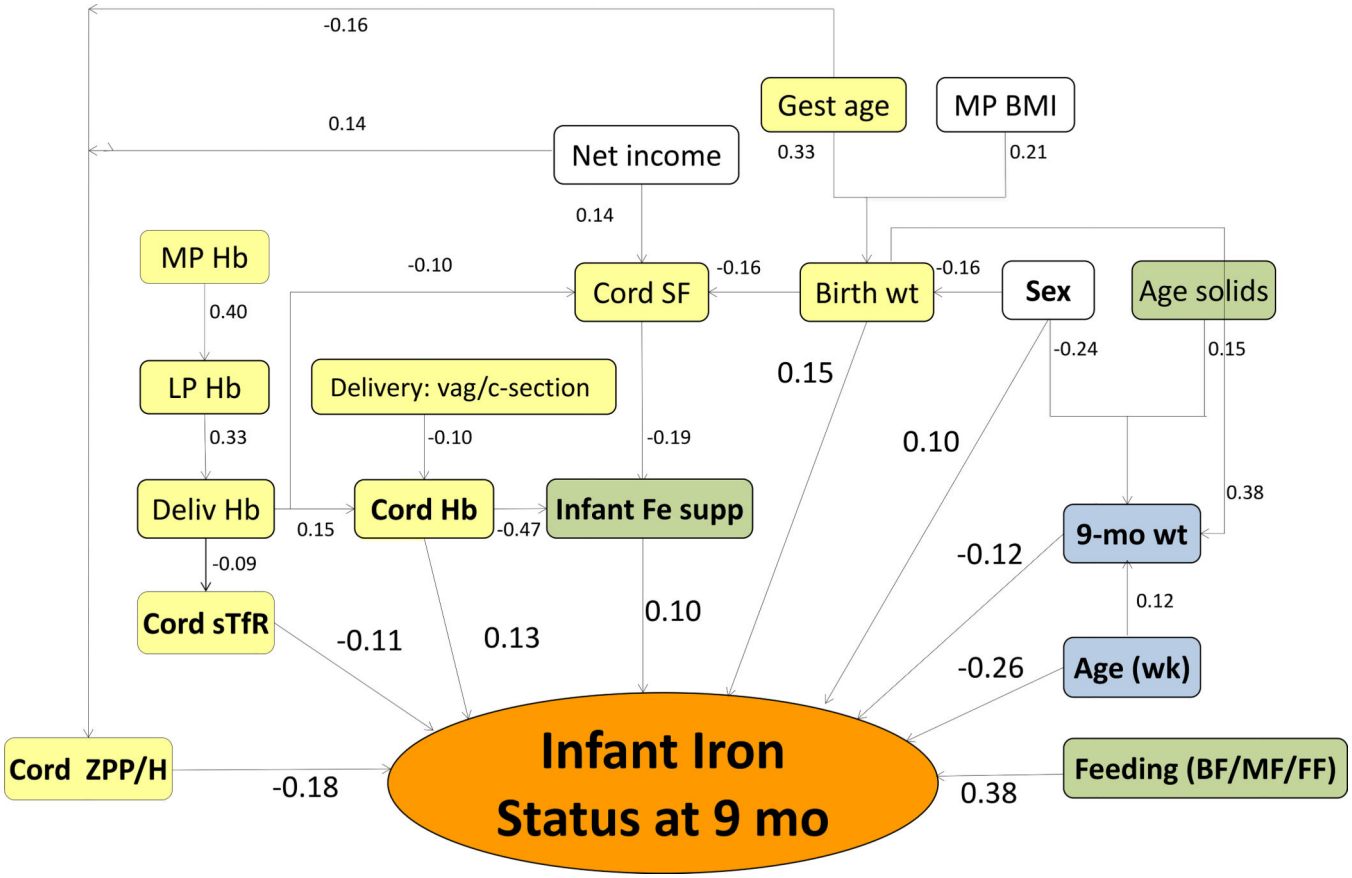

Figure 2.

Structural equation model of iron status at 9 months in healthy term Chinese infants. Variables indexing iron status at birth are shown in yellow. Variables indexing iron needs are shown in blue. Variables indexing external sources of iron are shown in green. Statistically significant ($P < 0.05$) standardized parameter estimates are shown for the 9-month iron composite model; standardized parameter estimates are similar to standardized beta coefficients in multiple regression. Exact P values for all direct and most proximal indirect effects are provided in the text. Abbreviations: BF, breast feeding; BMI, body mass index; c-section, caesarean-section; deliv, delivery; Fe, iron; FF, formula feeding; gest, gestational; Hb, hemoglobin; LP, late pregnancy; MF, mixed feeding; mo, months; MP, mid-pregnancy; SF, serum ferritin; sTfR, serum transferrin receptor; supp, supplementation; vag, vaginal; wt, weight; wk, weeks; ZPP/H, zinc protoporphyrin/heme ratio.

Table 1.

Sample characteristics[1]

Basic family and infant characteristics (*n*= 545 unless indicated)	
Maternal age (years)	27.2 (3.6)
First trimester BMI (kg/m^2)	21.0 (2.86)
Birth by caesarean-section, % (*n/N*)	71.9 (392/545)
Firstborn, % (*n/N*)	74.6 (407/545)
Male, % (*n/N*)	48.4 (264/545)
Gestational age (weeks)	39.5 (1.0)
Birth weight (grams)	3398.9 (400.8)
Age at testing (months)	9.3 (0.4)
Weight gain to 9 months (grams)	5914.5 (1028.4)
Weight-for–age at 9 months (*z* score)[2]	0.63 (0.95)
Length-for–age at 9 months (*z* score)[2]	0.53 (0.95)
Detailed family characteristics (*n* = 356 unless indicated)	
Number people/household	5.2 (1.3)
Parental education ≤ middle school, % (*n/N*)	49.2 (175/356)
Net family income ≤ 50,000 yuan/year, %[3] (*n/N*)	46.7 (163/349)
Feeding at 9months (*n* = 222)	
Breastfed, % (*n/N*)	42.8 (95/222)
Mixed-fed, % (*n/N*)	18.9 (42/222)
Formula-fed, % (*n/N*)	38.3 (85/222)
Received iron outside study from 6 weeks to 9 months, % (*n/N*)	13.5 (31/229)

[1]Values are means (SD) for continuous variables, % (*n/N*) for categorical variables.

[2]*z*-scores based on WHO growth curves.[33]

[3]The income cut off of 50,000 yuan per year was based on the Fuyang County Housing Assistance Policy for Low Income Families, 2012.

Table 2.

Hematology profiles by 9-month iron status classification[1]

	IDA	ID without anemia	IS
N = 461	**105**	**81**	**275**
Newborn			
Hb (g/L)	148.6 (21.6)	153.2 (15.4)	150.7 (18.6)
ZPP/H (μmol/mol)[2]	114.4 (35.7)	102.9 (24.9)	103.2 (28.6)
SF[3] (μg/L)	160.6 (83.2)	179.8 (91.1)	174.1 (86.9)
sTfR (mg/L)	5.3 (2.8)	5.6 (2.5)	4.8 (2.4)
BI[3] (mg/kg)	10.6 (3.0)	10.8 (2.7)	11.3 (3.2)
Infant at 9 months			
Hb (g/L)	101.6 (6.5)	116.5 (5.8)	118.4 (5.8)
ZPP/H (μmol/mol)[2]	160.0 (96.6)	115.7 (38.7)	95.8 (32.2)
SF (μg/L)	21.0 (24.7)	34.0 (28.0)	55.7 (42.0)
sTfR (mg/L)	6.3 (3.3)	4.6 (2.2)	4.0 (1.8)
BI (mg/kg)	1.69 (3.96)	4.9 (3.8)	7.6 (2.9)
MCV (fl)	71.3 (6.0)	75.5 (4.1)	79.8 (3.1)
RDW (%)	15.3 (1.73)	14.8 (1.3)	13.0 (0.9)

[1]Values are means (SD). 84 infants were not included in the table, as they did not fit any iron status category at 9 months: 74 were anemic but not ID, and 10 could not be classified due to one or more missing iron measures. Abbreviations: BI, body iron; Hb, hemoglobin; IDA, iron deficiency anemia; ID, iron deficiency; IS, iron sufficiency; MCV, mean cell volume; RDW, red blood cell distribution width; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP/H, zinc protoporphyrin/heme.

[2]Actual or imputed ZPP/H. ZPP/H was directly measured for 335 infants at birth and 291 at 9 months.

[3]Seven newborns with cord blood SF > 370μg/L (indicating possible infection) were not included in the cord blood SF or BI analyses.

Table 3.

Pearson correlations between iron measures at birth and 9 months

	9 months						
	Hb	**MCV**	**RDW**	**log ZPP/H**	**log SF**	**log sTfR**	**BI**
Cord blood							
Hb[1]	0.11 (544)	0.05 (545)	0.06 (545)	−0.03 (536)	−0.03 (534)	−0.04 (531)	−0.01 (523)
P-value	*0.012*	*0.250*	*0.178*	*0.545*	*0.497*	*0.376*	*0.846*
log ZPP/H[1]	−0.14 (335)	−0.12 (335)	0.16 (335)	0.22 (328)	−0.13 (325)	0.15 (324)	−0.17 (317)
P-value	*0.009*	*0.035*	*0.003*	*<0.001*	*0.018*	*0.008*	*0.002*
SF[1,2]	0.08 (523)	0.06 (523)	0.03 (523)	−0.02 (515)	0.03 (513)	−0.14(509)	0.09 (502)
P-value	*0.054*	*0.199*	*0.540*	*0.629*	*0.485*	*0.002*	*0.052*
log sTfR[1]	−0.03 (543)	−0.09(544)	0.13(544)	0.13 (535)	− 0.08 (533)	0.04 (530)	−0.10 (522)
P-value	*0.512*	*0.032*	*0.002*	*0.003*	*0.060*	*0.349*	*0.026*
BI[1]	0.09 (522)	0.08 (522)	−0.06 (522)	−0.09 (514)	0.07 (512)	−0.12 (508)	0.12 (501)
P-value	*0.044*	*0.066*	*0.147*	*0.046*	*0.117*	*0.008*	*0.008*

[1]Values are the Pearson correlation coefficient (*r*) between iron measures at birth (cord blood) and 9 months; *n* for each correlation is shown in parentheses next to *r*. Abbreviations: BI, body iron; Hb, hemoglobin; MCV, mean cell volume; RDW, red blood cell distribution width; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP/H, zinc protoporphyrin/heme.

[2]Cord SF was normally distributed and therefore not log transformed

Table 4.

Risk factors for poor iron status in Chinese infants at 9 months

	Odds Ratios (95% confidence interval)[1]							
	Anemia[2]	***P***	**IDA[2]**	***P***	**ID without anemia[2]**	***P***	**ID total[2]**	***P***
Risk factor (unit) [3]								
Males vs. females					2.29 (1.31, 4.02)	0.004	2.08 (1.31, 3.31)	0.002
Age at 9-month testing (1 week increase)	1.47 (1.27,1.69)	<0.001	1.93 (1.55, 2.42)	<0.001			1.51 (1.28, 1.78)	<0.001
Birth weight (100 g decrease)					1.09 (1.01, 1.18)	0.027	1.09 (1.03,1.17)	0.007
Cord Hb (10 g/L decrease)	1.14 (1.01,1.28)	0.024						
Cord sTfR (10% increase)			1.07 (1.00,1.14)	0.054	1.11 (1.04, 1.18)	0.001	1.09 (1.03, 1.14)	0.001
Cord ZPP/H (10% increase)			1.19 (1.05, 1.35)	0.007				
Feeding (breast vs. formula)	5.75 (2.87,11.51)	<0.001	19.06 (6.92, 52.49)	<0.001	4.73 (1.65, 13.95)	0.004	10.14 (4.56, 22.52)	<0.001
Feeding (mixed vs. formula)	2.22 (0.94, 5.26)	0.069	3.61 (1.04, 12.50)	0.043	3.04 (0.95, 9.73)	0.061	3.45 (1.35, 8.85)	0.010
Overall ***R-square* (*P-value*)**	**0.24 (<0.001)**		**0.50 (<0.001)**		**0.22 (0.006)**		**0.35 (<0.001)**	
Number abnormal/total *n*[4]	180/544		105/380		81/356		186/461	

[1]The odds ratios for logistic regression coefficients were calculated to reflect the unit change shown in parenthesis after each risk. Abbreviations: BI, body iron; Hb, hemoglobin; sTfR, serum transferrin receptor; ZPP/H, zinc protoporphyrin/heme.

[2]Comparisons: Anemia: anemia vs. no anemia. IDA: iron deficiency anemia vs. iron sufficient. ID without anemia: non-anemic iron deficient vs. iron sufficient. ID total: iron deficient vs. iron sufficient.

[3]Non-significant predictors were excluded from the models. Marginally significant ones (P<0.10) were included to display trends.

[4]Denominator *n*s vary depending on the comparison. For the Anemia comparison, Hb was available for all but one infant, hence, denominator = 544. For the IDA comparison, denominator = 380 (excluding 81 ID without anemia, 74 anemic not ID, and 10 unclassified). For the ID without anemia comparison, denominator = 356 (excluding 105 IDA, 74 anemic not ID, and 10 unclassified). For total ID, denominator = 461 (excluding 74 anemic not ID and 10 not classified).