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Breastfeeding, mixed or formula feeding at 9 months and the prevalence of iron deficiency and iron deficiency anemia in two cohorts of infants in China

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Abstract

Objective—To assess associations between breastfeeding and iron status at 9 months in two samples of Chinese infants.

Study design—Associations between feeding at 9 months (breastfed [BF] as sole milk source, mixed-fed [MF], or formula-fed [FF]) and iron deficiency anemia (IDA), iron deficiency (ID), and iron sufficiency were determined in infants from Zhejiang and Hebei provinces (*n*s = 142 and 813). ID was defined as body iron < 0 mg/kg, IDA as ID + hemoglobin < 110 g/L. Multiple logistic regression assessed associations between feeding pattern and iron status.

Results—Breastfeeding was associated with iron status (*P*-values < .001). In Zhejiang, 27.5% of BF infants had IDA compared with 0% of FF infants. The odds of ID/IDA were increased in BF and MF infants compared with FF: BF vs. FF odds ratio (OR): 28.8, 95% CI: 3.7–226.4; MF vs. FF OR: 11.0, 95% CI: 1.2–103.2. In Hebei, 44.0% of BF infants had IDA compared with 2.8% of FF infants. With covariable adjustment, odds of IDA were increased in BF and MF groups: BF vs. FF OR: 78.8, 95% CI: 27.2–228.1; MF vs. FF OR: 21.0, 95% CI: 7.3–60.9.

Conclusions—In both cohorts, the odds of ID/IDA at 9 months were increased in BF and MF infants, and ID/IDA was common. Although the benefits of breastfeeding are indisputable, these findings add to the evidence that breastfeeding in later infancy identifies infants at risk for ID/IDA in many settings. Protocols for detecting and preventing ID/IDA in BF infants are needed.

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Keywords

feeding; human; breastfeeding; iron deficiency

Iron deficiency is among the most common single nutrient deficiencies in the world, affecting women and young children disproportionately.[1] ID is the most common cause of anemia. Both ID and iron deficiency anemia (IDA) in early life are associated with poorer cognitive, motor, and social- emotional development as well as neurophysiologic alterations.[2]

The risk for developing ID in infancy increases with factors such as male sex, rapid growth, economic stress and other family disadvantages.[3] Evidence is accumulating that breastfeeding into the second half-year of life in otherwise healthy infants may be another risk factor. In the U.S., infants breastfed for 6 months or more were reported to be at higher risk of ID than infants who were not.[4] Other studies worldwide[5–16] reported associations between poorer iron status and breastfeeding into the second half-year of postnatal life and beyond. A study in Bangladesh,[17] however, did not find an association between breastfeeding and anemia at 6 months. Studies restricted to breastfed infants alone do not address the question but show the wide range in prevalence of ID or IDA in breastfed infants in different settings.[18–21]

It is important to ascertain associations between ID and breastfeeding over 6 months in different contexts. The World Health Organization (WHO) recommends continuation of breastfeeding for up to 2 years of age or beyond, in addition to nutritionally adequate and safe complementary foods. The 6- to 24-month age period coincides with the maximal risk of ID, because iron requirements during the second half-year of life are higher than at any other time of life.[22] However, there is debate about the iron needs of breastfed infants[4,23,24] and the risks of giving iron to iron-sufficient infants.[23,25] The purpose of this secondary analysis was to determine associations between breastfeeding at 9 months and iron status in two cohorts of healthy Chinese infants.

Methods

This secondary analysis of an observational study involved Chinese infants with data on iron status and feeding at 9 months, obtained in the course of two studies on neurodevelopmental effects of ID in early life (ClinicalTrials.gov: NCT00642863 and NCT00613717). For the cohort in Zhejiang province, in southeastern China, 142 of 204 infants with feeding data at 9 months had classifiable iron status; they constitute about 12% of 1196 infants screened for study participation at birth. For the cohort in Hebei province, in northeastern China, 813 of 927 infants with feeding data at 9 months had classifiable iron status; they constitute about 54% of 1512 infants considered at birth. Signed informed consent was obtained from parents. Studies were approved by the Institutional Review Boards of the University of Michigan and Zhejiang University School of Medicine (Zhejiang cohort) or Peking University First Hospital (Hebei cohort). A program of multimicronutrient fortification that was being rolled out in some regions of China at the time[26] did not involve the areas where our participants lived.

In Zhejiang, mother-neonate pairs in Fuyang County were enrolled between December 2008 and November 2011. Pregnant women with normal, uncomplicated pregnancies were invited to participate after random screening at routine prenatal visits at 36–37 weeks gestation. After birth, inclusion criteria were confirmed as previously reported.[27] Infants with cord hemoglobin [Hb] 2 SD below the mean for term infants (< 130 g/L)[28] were provided iron supplements on ethical grounds (~1 mg/kg per day iron as liquid iron proteinsuccynilate from 6 weeks to 9 months). Infants were randomly assigned to receive the same supplement or placebo from 6 weeks to 9 months if they had marginally low cord Hb (130 – 140 g/L) or cord serum ferritin < 60 μg/L (<5th percentile in our previous study in the same province[29]). Other infants did not receive iron.

For the Hebei cohort, infants in Sanhe County were invited to participate in a randomized controlled trial of iron supplementation[30] if their mothers had participated in a Peking University First Hospital randomized controlled trial of iron supplementation in pregnancy.[31] Enrollment in the infancy study occurred between December 2009 and June 2012. Inclusion/exclusion criteria for mothers have been previously published.[30,31] Infants with very low cord serum ferritin (< 35 μg/L) were provided iron supplements (~1 mg/kg per day iron as oral iron proteinsuccynilate from 6 weeks to 9 months) and not randomized. They were included in the current analysis if they had data on feeding. Other infants were randomly assigned to same iron supplement or placebo.[30] Background characteristics of the Zhejiang and Hebei cohorts are shown in Table I.

In each cohort, project personnel obtained information on feeding from mothers at a 9-month developmental assessment. We grouped infants based on feeding at 9 months. The breastfed (BF) group consisted of infants receiving breast milk as the sole source of milk. The mixed-fed group (MF) included those receiving some human milk and some formula. The formula-fed (FF) group consisted of those receiving formula as the sole source of milk. Formula in both settings was typically commercially prepared and iron-fortified. Because data on consumption of juice and solids were not complete, we could not determine exclusive breastfeeding as defined by WHO.[37] However, complementary feeding of nutrient-containing solids and liquids besides breast milk typically starts at or around 4–6 months of age in China.[38]

Iron status at 9 months was determined from venous blood samples in Zhejiang and capillary samples in Hebei. The measures of iron status included Hb, serum ferritin, serum transferrin receptor (sTfR), and zinc protoporphyrin/heme (ZPP/H). Serum C-reactive protein was also analyzed in Hebei. Laboratories maintained standard quality control procedures; methods have been previously published for both cohorts.[27,30] BI was calculated using serum ferritin and sTfR according to the formula in Cook et al[33]: Body iron (mg/kg) = −[log10(sTfR*1000/ferritin) − 2.8229]/0.1207. This formula used a sTfR assay described in Flowers et al[34] The Beckman Coulter sTfR concentrations in the Hebei project required conversion for use in the formula. To do so, we built on published data for Flowers, Ramco, and Beckman Coulter sTfRs. As reported in Pfeiffer et al,[35] the Ramco assay was similar to

Flowers et al. Ramco and Beckman Coulter assays were part of a World Health Organization study that used a standard reference reagent for sTfR.[36] 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 x Beckman Coulter sTfR. The assay used in Zhejiang did not require conversion.

BI ≥ 0 mg/kg indicates iron surplus in stores; BI < 0 mg/kg indicates iron deficit in tissues.[39] ID at 9 months was defined as BI < 0 mg/kg.[39] IDA was defined as ID and anemia (Hb < 110 g/L per WHO guidelines[1]) and iron sufficiency (IS) as BI ≥ 0 mg/kg and no anemia. As previously reported,[30,31] we considered fetal-neonatal ID as cord serum ferritin < 75 μg/L or ZPP/H > 118 μmol/mol. The serum ferritin cutoff has been used in studies of neurodevelopmental effects;[40–42] the ZPP/H cutoff is the US 90th percentile.[43]

Statistical Analyses

Statistical analyses were conducted using IBM SPSS Statistics Version 22 (IBM Corp. Released 2013). We used t-tests for continuous measures and χ^2 for categorical data to describe and compare cohorts. Associations between feeding pattern and iron status were analyzed separately for each cohort using χ^2 and logistic regression. A single unified analysis was not appropriate due to differences in study design, prevalence of ID, and background characteristics. Separate analyses could also make results more generalizable if findings were similar in the different contexts.

We conducted univariate logistic regression to determine associations at 9 months between the predictor (feeding group) and the dependent variable (iron status group) in each cohort. Anemic infants (Hb < 110 g/L)[1] who did not meet criteria for ID were excluded, because their iron status could not be categorized: 62 in Zhejiang and 114 in Hebei, leaving final *n*s of 142 and 813, respectively. Multiple logistic regression modeling was used to adjust for clinically relevant, potentially important covariables (sex, age at testing, birth weight, gestational age, parental education, weight gain from birth to 9 months, iron supplementation in infancy, fetal-neonatal ID, and inflammation as measured by C-reactive protein [available for Hebei but not Zhejiang]). Covariates were included and then removed if they were not at least marginally significant ($P < .10$) until the most parsimonious model remained.

Results

Sample characteristics are shown in Table I. In both cohorts, there were slightly more males than females, infants were born at term with mean birth weights > 3 kg, and continued to grow well. The cohorts differed in most other background characteristics (Table I). Infants in the Hebei cohort had lower length-for-age *z*-scores but higher weight-for-length *z*-scores at 9 months, meaning that, on average, they were shorter and heavier than infants in the Zhejiang cohort. A greater percentage of families in the Hebei cohort than the Zhejiang cohort were low income and parents were less educated. A higher proportion of Hebei cohort infants had ID or IDA at 9 months.

Zhejiang Cohort

At 9 months, feeding pattern was strongly associated with iron status (overall *P*-value < .001; Figure). Because no FF infants had IDA, we combined ID and IDA in further analyses. BF and MF groups differed significantly in ID/IDA from the FF group (*P* values=.001 and .04), but BF and MF groups did not (*P* = .12). Nearly one-third of BF infants (31.4%) had ID/IDA. The odds of ID/IDA were increased in both BF and MF groups, compared with the FF group (Table II). No other variables were significant in the model.

Hebei Cohort

Feeding pattern was also strongly associated with iron status at 9 months in Hebei (overall *P*-value <.001; Figure). Forty-four percent of BF infants had IDA and 27.5% had ID. BF, MF, and FF groups differed significantly from one another in iron status (*P* <.001). The odds of IDA were significantly increased in both the BF and MF groups compared with the FF group (Table II). Male sex, fetal-neonatal ID, lower birth weight, higher weight gain from birth to 9 months, and age at testing remained as significant covariables. For ID, the odds were also increased in both the BF and MF groups compared with the FF group (Table II): Male sex and higher C-reactive protein were significant covariables.

Discussion

A high percentage of breastfed infants had IDA at 9 months in two separate Chinese cohorts. The relation between breastfeeding as the sole source of milk and ID/IDA at 9 months was similar in both Hebei and Zhejiang cohorts despite differences in background characteristics. These findings in two distinct regions of China mirror those in other studies,[4–16] indicating that breastfeeding beyond 6 months of age is associated with poor iron status in a wide range of settings.

We identified 13 studies that examined associations between breastfeeding for 6 months or more and infant iron status.[4–16] In all but four,[5,7,11,16] iron status was assessed only by Hb or Hb in combination with serum ferritin. Our panel of iron status measures was more comprehensive, particularly in its inclusion of sTfR, which allowed BI to be calculated. The US Centers for Disease Control included BI < 0 mg/kg as a way to define ID in recent National Health and Nutrition Examination Surveys,[39] as BI captures both iron storage and function in a single index and does not depend on specific cutoffs for individual iron status measures. Nonetheless, BI may be a conservative indicator of ID in infancy, because other iron measures, such as ZPP/H or reticulocyte Hb, may indicate iron insufficiency before serum ferritin or sTfR are altered enough to make the BI calculation < 0 mg/kg. We could not consider these options, however, because ZPP/H data were missing for about a third of the Hebei cohort, and the hospitals were not set up to measure reticulocyte Hb.

The infants in our study were relatively heavy and growing rapidly, as evidenced by birth weight, weight gain, and *z*-scores. Their rapid growth could explain the limited ability of breastfeeding to protect against IDA. Although the benefits of breastfeeding are indisputable, rapid growth of infants in many settings may now exceed the capacity of breast milk to meet iron needs. However, the ability to consider the importance of growth in most previous studies is limited. Four studies did not report birth weight or growth rate[9–11,16] and four others reported birth weight only.[4,7,8,13] Nonetheless, it is likely that birth weights and growth rates are higher now than in most of human history.[44] That infants in Hebei were shorter and heavier at 9 months than infants in Zhejiang raises the question of other micronutrient deficiencies or other factors.

The observational nature of our study means that it cannot establish a causal relation between breastfeeding and ID/IDA. It is not ethical to randomize women to breastfeeding or for different durations. Therefore, making a causal connection must rely on the robustness of the relation in the face of different contexts, populations, study designs, and definitions of breastfeeding and iron status. Careful statistical control of potential confounding factors is also critical. Our study adds to the literature in these respects. Biological plausibility is another consideration. A new study by Cai et al provides an explanation for the low iron content of breast milk and the limited capacity of breast milk to meet infant iron needs.[45] The study found that human mammary epithelial cells do not have the only known membrane iron transporter and thus apparently have no way to secrete iron into breast milk.

The clinical challenge is to how best to prevent ID/IDA in breastfed infants globally. In our cohorts, some infants received supplemental iron as drops from 6 weeks to 9 months. As reported previously for the Hebei study,[30] there was limited hematologic response to iron supplementation, likely due to insufficient supplement intake and rapid growth. Further studies with carefully supervised early iron supplementation are warranted. Home fortification is another promising approach for breastfed infants. Adding to the growing body of literature, a recent study of China's home fortification program showed its effectiveness in reducing anemia in 6- to 23-month-old children.[26] An unanswered question is whether starting at 6 months is too late to protect the developing brain from ID effects. In an editorial accompanying the Cai et al paper, Friel challenges the rationale for exclusive breastfeeding for 6 months and argues cogently for providing external sources of iron earlier.[46]

Our study has several further limitations. The analysis was cross-sectional. Feeding information was based on maternal report collected when infants were 9 months old. The duration of exclusive breastfeeding, defined by WHO, was not known. Incomplete data on complementary foods means that we cannot determine if groups within each cohort differed in other facets of feeding or the quality of the diet. We also cannot compare diet across cohorts. The use of venous blood in Zhejiang and capillary blood in Hebei may affect iron status differences across cohorts. ZPP/H was missing for part of the Zhejiang cohort, and we were able to adjust for C-reactive protein in the Hebei cohort only.

Despite limitations in the available studies, including ours, the accumulating evidence has global clinical implications. Specifically, breastfed infants in many settings worldwide warrant additional and earlier monitoring to prevent them from developing ID or IDA and to detect and treat ID/IDA before it becomes chronic and severe. Although more research is needed to determine the optimal timing, vehicle, and amount of iron supplementation, clinicians need updated protocols to guide their care of the breastfed infant.

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Abbreviations

Hb	hemoglobin
IDA	iron deficiency anemia
sTfR	serum transferrin receptor
WHO	World Health Organization
ZPP/H	zinc protoporphyrin/heme

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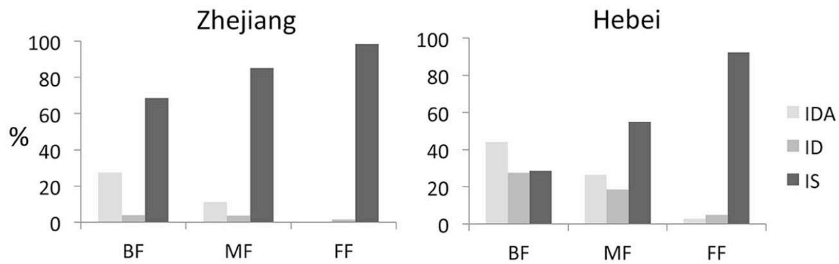

Figure.

Percentage of 9-month-old infants with IDA, ID, and iron sufficiency by feeding pattern in Zhejiang ($n = 142$) and Hebei ($n = 813$). The overall difference in iron status by feeding groups was significant in each cohort: Zhejiang, $\chi^2 = 26.5$, $P < .001$; Hebei, $\chi^2 = 202.5$, $P < .001$.

Table 1

Sample characteristics and comparison of cohorts[a]

	Zhejiang	Hebei	*P* value[b]
N	***142***	***813***	
Sex, % male (*n*)	52.8 (75)	52.5 (427)	.95
Age at testing, days	280.3 (278.6 – 282.0)	282.4 (281.4 – 283.3)	.08
Growth			
Birth weight, g	3388.4 (3321.4 – 3455.4)	3382.7 (3357.4 – 3408.0)	.87
Gestational age, weeks	39.5 (39.4 – 39.7)	39.7 (39.7 – 39.8)	.02
Weight gain to 9 months, g	6103.5 (5922.5 – 6284.5)	6296.2 (6220.3 – 6372.1)	.06
Weight-for-age *z*-score[c]	0.77 (0.60 – 0.94)	0.92 (0.85 – 0.99)	.10
Length-for-age *z*-score[c]	0.66 (0.49 – 0.82)	0.33 (0.26 – 0.40)	.001
Weight-for-length *z*-score[c]	0.65 (0.48 – 0.83)	1.05 (0.98 – 1.13)	.000
Maternal anemia at late gestation/delivery, % (*n*/total *n*)[d]	30.0 (42/140)	24.1 (97/403)	.17
Fetal-neonatal ID, % (*n*/total *n*)[e]	32.6 (46/141)	44.5 (359/807)	.01
Iron supplemented in infancy, % (*n*)	28.2 (40)	53.9 (438)	.000
Iron status at 9 months[f]			
IDA, % (*n*)	12.0 (17)	31.2 (254)	.000
ID, % (*n*)	2.8 (4)	20.7 (168)	.000
Iron Sufficient, % (*n*)	85.2 (121)	48.1 (391)	.000
C-reactive protein at 9 months, mg/L	NA	0.94 (0.61 – 1.27)	NA
Feeding at 9 months			
Breastfed, % (*n*)	35.9 (51)	50.6 (411)	.001
Mixed-fed, % (*n*)	19.0 (27)	32.0 (260)	.002
Formula-fed, % (*n*)	45.1 (64)	17.5 (142)	.000
Parental education, ≥ high school, % (*n*/total *n*)	58.2 (82/141)	27.7 (221/797)	.000
Net family income < 50,000 yuan/year, % (*n*/total *n*)[g]	41.0 (57/139)	83.7 (667/797)	.000

[a]Values are mean (95% CI) for continuous variables, % (*n*) for categorical variables.

[b]*P* values for comparisons between Zhejiang and Hebei samples based on χ^2 and t-test.

[c] *z*-scores based on WHO growth curves.[32]

[d] Prevalence of anemia in the Hebei sample for women who were not randomly assigned to receive prenatal iron supplementation. Including women who received iron supplementation in pregnancy, the prevalence of maternal anemia in Hebei was 17.9% (143/801).

[e] Fetal-neonatal ID was defined as cord blood zinc protoporphyrin/heme > 118 μmol/mol heme or serum ferritin < 75 ng/ml.[30]

[f] Anemic infants who did not meet criteria for ID were excluded, because their iron status could not be categorized (see Methods for details and definitions).

[g] The definition of low income (<50,000 yuan per year) was based on the Fuyang County Housing Assistance Policy for Low Income Families, 2012 (Zhejiang) and the Sanhe Public Housing Benefits Guidelines, Sanhe People's Government, 2013 (Hebei).

Table 2

Logistic regression model for predicting odds of ID/IDA (Zhejiang) and IDA or ID (Hebei)

	Unadjusted		Adjusted	
	Odds Ratio (95% CI)	***P* value**	**Odds Ratio (95% CI)**	***P* value**
ID/IDA vs. IS (Zhejiang)				
BF	28.8 (3.7 – 226.4)	< .001	-	-
MF	11.0 (1.2 – 103.2)	.04	-	-
FF	Reference		-	-
IDA vs. IS (Hebei)				
BF	50.7 (18.2 – 140.7)	< .001	78.8 (27.2 – 228.1)	< .001
MF	15.8 (5.6 – 44.5)	< .001	21.0 (7.3 – 60.9)	< .001
FF	Reference	-	Reference	-
Male sex	-	-	1.7 (1.1 – 2.5)	.01
Fetal-neonatal ID vs. IS	-	-	2.4 (1.6 – 3.5)	< .001
Birth weight[1]	-	-	0.91 (0.86 – 0.96)	.001
Weight gain to 9 months[1]	-	-	1.05 (1.03 – 1.07)	< .001
Age at testing, months	-	-	0.6 (0.3 – 0.9)	.01
ID vs. IS (Hebei)				
BF	18.1 (8.1 – 40.3)	< .001	21.9 (9.7 – 49.7)	< .001
MF	6.3 (2.7 – 14.4)	< .001	6.7 (2.9 – 15.4)	< .001
FF	Reference	-	Reference	-
Male sex	-	-	1.8 (1.2 – 2.7)	.005
Log C-reactive protein at 9 months, mg/L	-	-	0.8 (0.7 – 0.9)	.001

[1]Per 100 g.