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Breastfeeding, Mixed, or Formula Feeding at 9 Months of Age and the Prevalence of Iron Deficiency and Iron Deficiency Anemia in Two Cohorts of Infants in China

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Objective To assess associations between breastfeeding and iron status at 9 months of age in 2 samples of Chinese infants.

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

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

Conclusions In both cohorts, the odds of iron deficiency/IDA at 9 months of age were increased in breastfed and mixed-fed infants, and iron deficiency/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 iron deficiency/IDA in many settings. Protocols for detecting and preventing iron deficiency/IDA in breastfed infants are needed. *(J Pediatr 2017;181:56-61).*

Trial registration ClinicalTrials.gov: NCT00642863 and NCT00613717.

Iron deficiency is among the most common single nutrient deficiencies in the world, affecting women and young children disproportionately.[1] Iron deficiency is the most common cause of anemia. Both iron deficiency 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 iron deficiency 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 one-half year of life in otherwise healthy infants may be another risk factor. In the US, infants breastfed for 6 months or more were reported to be at higher risk of iron deficiency than infants who were not.[4] Other studies worldwide[5-16] reported associations between poorer iron status and breastfeeding into the second one-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 of age. Studies restricted to breastfed infants alone do not address the question, but show the wide range in prevalence of iron deficiency or IDA in breastfed infants in different settings.[18-21]

It is important to ascertain associations between iron deficiency and breastfeeding over 6 months in different contexts. The World Health Organization (WHO)

Hb	Hemoglobin
IDA	Iron deficiency anemia
sTfR	Serum transferrin receptor
WHO	World Health Organization
ZPP/H	Zinc protoporphyrin/heme

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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 iron deficiency, because iron requirements during the second one-half year of life are greater 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 of age and iron status in 2 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 of age, obtained in the course of 2 studies on neurodevelopmental effects of iron deficiency 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 of age 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 protein succynilate from 6 weeks to 9 months). Infants were assigned randomly to receive the same supplement or placebo from 6 weeks to 9 months of age 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 protein succynilate from 6 weeks to 9 months of age) and not randomized. They were included in the current analysis if they had data on feeding. Other infants were assigned randomly to the 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 of age. The breastfed group consisted of infants receiving breast milk as the sole source of milk. The mixed-fed group included those receiving some human milk and some formula. The formula-fed group consisted of those receiving formula as the sole source of feeding. 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.[33] However, complementary feeding of nutrient-containing solids and liquids besides breast milk typically starts at or around 4-6 months of age in China.[34]

Iron status at 9 months of age 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] Body iron was calculated using serum ferritin and sTfR according to the formula in Cook et al[35]: Body iron (mg/kg) = -[log10(sTfR × 1000/ferritin) – 2.8229]/0.1207. This formula used a sTfR assay described in Flowers et al.[36] 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,[37] the Ramco assay was similar to Flowers et al. Ramco and Beckman Coulter assays (Beckman Coulter, Inc, Brea, California) were part of a WHO study that used a standard reference reagent for sTfR.[38] The Ramco assay (Ramco Laboratories, Inc, Stafford, Texas) 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. The assay used in Zhejiang did not require conversion.

Body iron ≥ 0 mg/kg indicates iron surplus in stores; body iron < 0 mg/kg indicates iron deficit in tissues.[39] Iron deficiency at 9 months of age was defined as body iron < 0 mg/kg.[39] IDA was defined as iron deficiency and anemia (Hb < 110 g/L per WHO guidelines[1]) and iron sufficiency as body iron ≥ 0 mg/kg and no anemia. As previously reported,[30,31] we considered fetal-neonatal iron deficiency 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 (Released 2013; IBM Corp, Armonk, New York). We used *t* tests for continuous measures and χ^2 for categori-