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Iron metabolism in infants: influence of bovine lactoferrin from iron-fortified formula

Chen Ke Ph.D. [a], Zhang Lan B.D. [a], Li Hua B.D. [b], Zhang Ying B.D. [c], Xie Humina B.D. [d], Shang Jia B.D. [e], Tian Weizheng B.D. [f], Yang Ping B.D. [g], Chai Lingying Ph.D. [h], Mao Meng Ph.D. [i,*]

[a] Department of Child Health Care, Chengdu Women's and Children's Central Hospital, Chengdu, Sichuan, China
[b] Department of Preventive Health Care, Women and Children's Health Care Hospital of Qing Baijing, Qing Baijing District, Chengdu, Sichuan, China
[c] Department of Child Health Care, Women and Children's Health Care Hospital of Jinniu, Jinniu District, Chengdu, Sichuan, China
[d] Department of Child Health Care, Women and Children's Health Care Hospital of Dayi, Dayi County, Chengdu, Sichuan, China
[e] Department of Child Health Care, Women and Children's Health Care Hospital of Chenghua, Chenghua District, Chengdu, Sichuan, China
[f] Department of Pediatrics, Women and Children's Health Care Hospital of Dongpo, Dongpo District, Meishan, Sichuan, China
[g] Department of Child Health Care, Women and Children's Health Care Hospital of Xindu, Xindu District, Chengdu, Sichuan, China
[h] Beingmate Baby & Child Food Co., Ltd, Hangzhou, Zhejiang, China
[i] Chengdu Women's and Children's Central Hospital, Chengdu, Sichuan, China

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ABSTRACT

Objective: The aim of this study was to evaluate whether an iron-fortified formula with a concentration of lactoferrin would significantly improve the hematologic indexes and iron status in term infants compared with those same values in infants fed an iron-fortified formula without lactoferrin.

Method: In this prospective, multicenter, controlled intervention study, 260 infants ages 4 to 6 mo were selected from six maternal and children's health care hospitals in the area. All infants were divided into two groups with the sequence of outpatient: lactoferrin-fortified formula milk group (fortified group, FG, containing lactoferrin 38 mg/100 g milk and iron element 4 mg/100 g milk) and no lactoferrin fortified milk (control group, CG, containing lactoferrin 0 mg/100 g milk and iron element 4 mg/100 g milk) for 3 mo. The levels of weight, height, and head circumference and the concentration of hemoglobin (Hb), serum ferritin (SF), and serum transferring receptor (sTfR) were measured and sTfR-SF index (TFR-F index), total body iron content (TBIC) and low height for age (HAZ), low weight for age (WAZ), and low weight for height (WHZ) were computed before and after the intervention, respectively.

Results: In all, 213 (115 in FG and 98 in CG) infants completed the intervention trial and all measurements of biochemical indicators. There were no significant differences in the average amount of daily intake of formula milk (94.3 ± 9.8 g versus 88.2 ± 8.7 g for FG and CG; $P > 0.05$) and iron element (3.8 ± 0.4 mg versus 3.7 ± 0.6 mg for FG and CG; $P > 0.05$). The average amount of daily intake of lactoferrin for infants in FG group was 35.8 ± 3.7 mg. The levels of weight, WAZ, WHZ, Hb, SF, TFR-F index, and TBIC after intervention of infants in FG were all significantly higher than those of infants in CG weight, 8723 ± 245 g versus 8558 ± 214g; WAZ, 1.02 ± 0.31 versus 0.44 ± 0.18; WHZ, 0.98 ± 0.31 versus 0.41 ± 0.12; Hb, 125.5 ± 15.4 g/L versus 116.9 ± 13.1 g/L; SF, 44.7 ± 17.2 μg/L versus 31.6 ± 18.4 μg/L; TFR-F index, 1.88 ± 0.41 versus 1.26 ± 0.39; TBIC, 6.12 ± 0.78 mg/kg versus 5.26 ± 0.55 mg/kg for FG and CG; $P < 0.05$), but significantly lower ($P < 0.05$) for the prevalence of anemia (4.1% versus 7.5%), iron deficiency (13.9% versus 24.4%), and iron-deficient anemia (1.7% versus 6.1%).

Conclusion: When infants who were exclusively breastfed were supplemented with lactoferrin-fortified milk, significant increases in TBIC and iron absorption in the intestine were seen.

* Corresponding author. Tel.: +86 288 663 0477; fax: +86 288 626 0292.
E-mail address: mmcdfy@163.com (M. Meng).

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Introduction

Anemia is a worldwide public health problem. The global prevalence of anemia in preschool-aged children, pregnant women, and nonpregnant women is 47.4%, 41.8%, and 30.2%, respectively [1]. In May 2002, the General Assembly of the United Nations [2] reemphasized that control of nutritional anemia should be one of the global development goals to be achieved in the early years of this new millennium. Despite this considerable programmatic experience and a vast amount of compiled scientific data on iron metabolism, the global prevalence of anemia has hardly declined in the past decade. Reasons for this lack of improvement include the multifactorial etiology of anemia, underfunding, and poor program implementation, which often assumes that the sole cause of anemia is iron deficiency (ID).

In response to this situation, the World Health Organization (WHO) created a set of measures to address ID and anemia. This package is being used in countries characterized by high levels of ID and anemia, malaria, helminthes infections, and schistosomiasis [3]. These measures include increasing iron intake, controlling infection, and improving nutritional status. The priority was the enhancement of iron absorption, especially for areas with a high incidence of anemia, poor economy, and underserved food-additive technology.

It is known that the concentration of iron in human milk is low and decreases during the course of lactation [4,5], but its bioavailability has been assumed to be high because exclusively breastfed infants do not show signs of ID during the first 6 mo of life. It has been suggested that lactoferrin (LF) is a possible promoter for iron absorption from breast milk [6]. This protein has been proposed as a delivery system by which iron can be absorbed from human milk because it is the major iron-binding protein present [7]. Bovine and human LF are structurally and biochemically similar and have comparable bioactivity, according to in vitro and animal model assessments [8]. Bovine lactoferrin (bLF) has been proven safe in multiple studies of iron metabolism in human infants [9,10]. Although efficacy has not been adequately studied in humans, bLF is readily available and is being used for its putative health benefits.

Studies have been performed to explore the effect of bLF on iron absorption. One study [11] showed that oral bLF for pregnant women increased levels of hemoglobin (Hb), total serum iron, and ferritin and decreased the prevalence of ID and iron-deficient anemia (IDA). Moreover, although ferrous sulfate and LF supplement can significantly improve the body's iron stores to the same extent, gastrointestinal side effects of LF was significantly lower than that of ferrous sulfate [12].

Although the iron absorption role of LF has been debated for some time in studies on adults, no conclusive data has been reported to clarify the importance of this protein in iron absorption from bLF-fortified formulas in infants. Three studies [9,13,14] found that formula fortified with bLF could significantly increase hematocrit, serum ferritin, and reticulocyte levels; however, a fourth [10] reported that no significant improvement of serum iron levels was observed from LF-fortified formula.

In the present study, we explored the effect of iron-fortified infant formula that contained potential enhancers of iron absorption on 4- to 6-m-old infants who were exclusively breastfed before the study. The primary goal was to evaluate whether the iron-fortified formula with a concentration of bLF would significantly improve the hematologic indexes and iron status in term infants compared with those values in infants fed an iron-fortified formula without bLF.

Materials and methods

Participants and ethical approval

This randomized, controlled, and blinded intervention study was performed in the Qing Baijing, Jinniu, Dayi, Chenghua, Meishan, and Xindu districts of Chengdu city, Sichuan province, western China from March 2012 to March 2013. Approximately 260 infants who previously were exclusively breast-fed, but required formula feeding at 4 to 6 mo, were randomly recruited from six Women and Children's Health Care Hospital in the six described regions. The eligibility criteria for participation were as follows:

1. Apparent good health without common obstetric risk factors;
2. Hb concentration >60 g/L;
3. Serum C-reaction protein (CRP) < 10 mg/L;
4. Parent's or guardian's approval for participating in all aspects of the study;
5. Parent's or guardian's agreement to avoid additional use of infant formulas and foods and nutrients fortified by iron during the investigation.

Exclusion criteria included a history of severe, persistent, or chronic diarrhea; severe malnutrition; serious infections requiring hospitalization in the month prior; serious chronic illness; a personal or family history of allergy to cow's milk or infant formula; eczema; allergic rhinitis; or asthma.

The primary objective for the present study was to measure the change in serum Hb before and after intervention. A sample size of about 60 infants per group was sufficient to allow the detection of a 10 g/L Hb SD of difference after fortification with 95% power and $\alpha = 0.05$ for a two-sided t test. The initial recruitment size was about 130 children per group; however, this declined throughout the testing as a number of children were withdrew from the study.

We conducted a census in each regional hospital to determine which households included a weaned, 4- to 6-mo-old infant. Then field health workers conducted a food-intake survey to determine which infants were weaned. A fieldworker who visited the eligible families explained the protocol, answered questions, and obtained written informed consent from parents or guardians. The enrollment and research plan were reviewed and approved by the institutional ethics committee of the Chengdu Women's & Children's Central Hospital of Chongqing Medical University in Sichuan province, China. The present study complied with the code of ethics of the World Medical Association (Declaration of Helsinki).

Intervention

Two different fortification styles were performed in the present study and eligible infants were randomly assigned to one of the two groups. Infants in the fortified group (FG) took a commercially available bLF-fortified formula (Beingmate Baby & Child Food Co., Ltd, Hangzhou, China), which had a bLF concentration of 38 mg/100 g (Production No.: 20121008 B89 M). Infants in the control group (CG) took a formula free of LF (Beingmate Baby & Child Food Co., Ltd, Hangzhou, China. Production No.: 20100908 D05 Z). Both formulas contained the same composition of nutrients except for LF and the iron content of the two formulas were both 4 mg/100 g. Immediately after recruitment, infants were assigned a study number that had been previously randomly assigned to the fortified or control group with fixed, equal allocation to each group prepared by a third party. The physicians, nurses, field health workers, parents, and laboratory personnel were blinded to the treatment assignment of each infant throughout the study period. The data manager, statistician, and all investigators remained blinded to group assignments until the end of data analysis. The total duration of fortification was 3 mo.

Questionnaire interview

A trained field health worker conducted a 30-min questionnaire after recruitment. The questionnaire included questions on demographic information (infant's age, sex), family socioeconomic information related to educational levels of main caregivers (who were responsible for at least half of the infant's care time), family monthly income, and use of vitamin/mineral supplement before trial.

Anthropometric measurements

The same trained anthropometric nurses (N = 6) from each hospital conducted the anthropometric examinations at their specific hospitals at baseline and 3-mo follow-up using standardized techniques to eliminate intra-examiner error. Duplicate measurements were performed for all infants. The inter-examiner coefficient of variation of weight, length, and head circumference (HC) for each examiner in FG and CG was <5%. Weight was recorded using a weighing scale (100 Med, Beijing, China) to the nearest 100 g with infants in minimal clothing and bare feet. Similarly, length was measured in the standard supine position by a supine scale (Haode, Guangzhou, China) to the nearest 0.1 cm. By using reference data from the WHO (2005), the Z scores were