Iron Concentrations in Breast Milk and Selected Maternal Factors of Human Milk Bank Donors

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Abstract

The aim of this study was to evaluate the relationship between iron concentration in mature breast milk and characteristics of 136 donors of a Brazilian milk bank. Iron, vitamin A, zinc, and copper concentrations were assessed in human milk and maternal blood. Data were collected on maternal anthropometrics, obstetric, socioeconomic, demographic, and lifestyle factors. Iron, zinc, and copper in milk and zinc and copper in blood were detected by spectrophotometry. Vitamin A in milk and blood was determined by high-performance liquid chromatography. Hemoglobin was measured by electronic counting and serum iron and ferritin by colorimetry and chemoluminescence, respectively. Transferrin and ceruloplasmin were determined by nephelometry. According to multivariate linear regression analysis, iron in milk was positively associated with vitamin A in milk and with smoking but negatively associated with timing of breast milk donation ($P < .001$). These results indicate that iron concentration in milk of Brazilian donors may be influenced by nutritional factors and smoking. *J Hum Lact*. 26(2):175-179

Keywords: human milk, breastfeeding, human milk banking, iron, retinol, smoking, zinc, copper

Iron deficiency anemia is the most prevalent nutritional deficiency in the world, mainly affecting pregnant and lactating women, infants, and preschool children.[1] Anemia interferes in children's growth and development, impairing their mental, motor, and language development and determining behavioral and psychological disorders, such as lack of attention, fatigue, and reduction of physical activity.[2,3]

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It is assumed that the normal full-term infant has a considerable iron store that is mobilized during the first 4 to 6 months of life to meet physiological requirements. Consequently, the amount of iron provided from stores at birth plus intake from breast milk can provide sufficient iron for 6 months. However, the stores can be influenced by maternal prenatal iron status, birth weight, and timing of umbilical cord clamp.[4] Studies using stable isotopes have shown that Swedish infants at 6 months of age absorbed much less iron from human milk (15.7%)[5] than Peruvian infants (42.6%) at 5 to 6 months,[6] who are probably more iron deficient.

Iron concentrations in human milk do not seem to be influenced by iron status of lactating women,[7-10] maternal anthropometry,[11] age,[12] iron intake,[7,13] use of oral contraceptive,[7] and smoking,[7] but they may be affected by the timing of breast milk donation[7,9,10,14] and maternal parity.[12] Interactions with other micronutrients, such as vitamin A, zinc, and copper, can also modulate iron content in mammalian milk.[15,16]

In view of the importance of the human milk banks in Brazil[17] and the extensive number of milk donors, it is important to investigate iron concentrations in the milk of those apparently healthy mothers and the factors associated with the concentration of this micronutrient in milk.

The aims of the present study were to assess the relationship between iron concentrations in human milk and (1) retinol, zinc, and copper concentrations in maternal milk; (2) iron, retinol, zinc, and copper concentrations in maternal blood; and (3) maternal anthropometric, obstetric, and socioeconomic-demographic factors and lifestyle.

Materials and Methods

Population

All of the 161 lactating women who were donors of a human milk bank in Marilia, Brazil, were included in this study. Screening of donors includes collection of information, such as number of prenatal appointments, weight at the beginning and end of pregnancy, hematocrit values, serological tests (including Venereal Disease Research Laboratory [VDRL]), and history of morbidity during the prenatal period. Donors must have been healthy, breastfeeding, or extracting breast milk for their own children and must have had pre- and postnatal tests compatible with the donation. Moreover, donors could not smoke more than 10 cigarettes/day, take any medicine incompatible with breastfeeding, and use alcohol or illicit drugs. The milk recipients were usually preterm, low birth weight, or sick newborns.[17]

Samples and Data Collection

Samples of mature milk (20-60 days postpartum) were obtained from the 161 milk donors between February 2003 and May 2004. The donors were advised by the human milk banks to withdraw small samples of milk manually from both breasts throughout the nursing period (usually before and after the child breastfed and in a middle period) over 24 hours and to store the milk in a refrigerator at 6°C in a dark plastic container washed with 0.5% nitric acid solution to remove trace elements. On the next day, the milk was collected, divided into aliquots, and stored at –70°C until the time for analysis. On the day of collection of the milk samples, anthropometric measurements were obtained from the donors, and a questionnaire was applied to obtain sociodemographic and obstetric data and information regarding lifestyle. In addition, a blood sample was collected, by venipuncture, for micronutrient determination.

Analytical Methods

Iron, zinc, and copper were determined simultaneously in breast milk, in triplicate, by the method of Morgano et al,[18] using inductively coupled plasma-optic emission spectrometry with axial view (ICP-OES; Vista MPX, Varian, Mulgrave, Australia). The micronutrients were determined at the following wavelengths (nm): iron, 259.94; zinc, 206.20; and copper, 324.754. The National Research Council (NRC) cutoff points[19] for low concentrations of iron (< 0.27 mg/L), zinc (< 0.2 mg/L), and copper (< 2 mg/L) in breast milk were adopted.

Retinol was extracted from serum by the method of Arnaud et al[20] and from breast milk by the simplified method of Cassettari et al[21] using a Shimadzu high-performance liquid chromatograph (HPLC). The methanol/water (95:5, v/v) elution used in the original method of Cassettari et al was replaced with a mobile phase consisting of acetonitrile/dioxane/methanol (82%/15%/3%). For construction of the calibration curve and calibration of the HPLC, a stock solution of the retinol standard of known concentrations was eluted after each 10 serum or milk samples. To monitor the reproducibility of the method, retinol was determined in duplicate. The following cutoff points—0.7 mmol/L and 1.05 mmol/L[22]—described deficient concentrations of vitamin A in blood and low concentrations of vitamin A in milk, respectively. Internal controls were from Sigma (St. Louis, Missouri) and external controls from the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia).

Serum iron and ferritin concentrations were determined, respectively, by a colorimetric method (using ferrozine/ascorbic acid) and by chemoluminescence; reference values ranged from 49.0 to 151.0 μg/dL for serum iron and from 10 to 291 ng/mL for ferritin. Hemoglobin was measured by an automated CELL-DYN 1400 counter (Abbott, Abbott Park, Illinois). Serum transferrin and ceruloplasmin concentrations were determined by nephelometry; reference values ranged from 212.0 to 360 mg/dL and from 25.0 to 63.0 mg/dL, respectively. Serum zinc and copper concentrations were analyzed by atomic absorption spectrophotometry; reference values ranged from 70.0 to 120.0 μg/dL for zinc and from 85 to 155 μg/dL for copper. Iron, zinc, and copper in milk and vitamin A in milk and blood were determined respectively at the Institute of Food Technology and at the Laboratory of Micronutrients, University of São Paulo. Serum iron, ferritin, hemoglobin, transferrin, ceruloplasmin, serum zinc, and serum copper were analyzed at Laboratory Labclin, which has the Quality Certificate of the Brazilian Society of Clinical Pathology.

Anthropometric Measurements

Weight and height of the donors were measured respectively with a Soehnle balance (precision of 100 g) and with a CMS Weighing Equipment Ltd anthropometer (precision of 1 mm) by the method of Frisancho.[23] The nutritional status of the women was assessed by their body mass index (BMI).[24]

Statistical Analysis

From the 161 women initially selected for the study, 136 completed all the hematological and biochemical tests and were included in the statistical analysis. Because the variables were not normally distributed, the tests used were for nonparametric analysis. The differences in iron concentrations in human milk, according to age, education, BMI, and postpartum timeframe categories, were assessed by the Kruskal-Wallis test. The differences in iron concentrations in human milk regarding maternal marital status, occupation, family income, parity, smoking, and oral contraceptive were assessed by the Mann-Whitney test. The associations between iron concentrations in milk and the variables investigated (maternal age; marital status; occupation; education; family income; gestational age; smoking; BMI; timing of breast milk donation; serum concentrations of iron, vitamin A, zinc, and copper; breast milk concentrations of vitamin A, zinc, and copper; hemoglobin; ferritin; transferrin; and ceruloplasmin) were evaluated by multivariate linear regression analysis. The results were considered statistically significant for a descriptive value of $P < .05$. Data were analyzed with Stata Version 9.0.[25]

Ethical Issues

The study was approved by the Ethics Committee of the School of Public Health, University of São Paulo, based on Resolution 196/96 of the National Health Council/Brazilian Ministry of Health.

Results

The mean concentration of iron in the milk donated was 0.35 mg/L (0.06 µmol/L; not shown). Mean iron concentration was higher in the milk of smokers compared to nonsmokers ($P = .012$) and inversely associated with timing of breast milk donation ($P < .001$; Table 1). Mean concentrations of iron, vitamin A, zinc, and copper in blood and breast milk from donors of the Marilia milk bank are given elsewhere.[26]

The mean age of the women included in the study (n = 136) was similar to the mean age of the excluded ones (n = 25; mean ± SD, 26.82 ± 6.0 vs 25.20 ± 5.8; $P = .215$). Regarding per capita income, no significant difference was observed between the women included (1.76 ± 1.6) and excluded (1.80 ± 1.7) from the study ($P = .928$).

According to the multivariate linear regression analysis, iron concentration in milk was positively associated with vitamin A in milk ($P < .001$) and smoking ($P = .001$) and negatively associated with timing of breast milk donation ($P < .001$; Table 2).

Table 1. Mean (SD) Concentrations of Iron in Human Milk (mg/L) According to the Characteristics of the Lactating Women

Variables	*n*	*%*	*Mean (SD)*	*P*
Age				
16-19	18	13.2	0.40 (0.21)	.161
20-29	74	54.4	0.32 (0.20)	
30-41	44	32.4	0.39 (0.54)	
Marital status				
Married/stable union	114	83.8	0.33 (0.20)	.745
Single/divorced	22	16.2	0.47 (0.74)	
Occupation				
Work	61	44.9	0.35 (0.22)	.744
Unemployed or housekeeper	75	55.1	0.36 (0.43)	
Education, y				
0.0-8.0	41	30.1	0.33 (0.19)	.945
8.1-11.0	73	53.7	0.33 (0.21)	
> 11	22	16.2	0.46 (0.74)	
Family income (BMW[a])				
0.0-4.0	51	37.5	0.36 (0.22)	.257
> 4.0	85	62.5	0.35 (0.41)	
Parity				
Primiparous	78	57.4	0.39 (0.43)	.100
Multiparous (2-7)	58	42.6	0.30 (0.17)	
Gestational age				
Preterm (< 37 weeks)	21	15.4	0.38 (0.22)	.293
Term (37 weeks)	115	84.6	0.35 (0.37)	
Smoking				
Yes	20	14.7	0.56 (0.75)	.012
No	116	85.3	0.32 (0.20)	
Oral contraceptive				
Yes	17	12.5	0.32 (0.17)	.966
No	119	87.5	0.36 (0.37)	
BMI, kg/m^2				
17.0-25.0[b]	77	56.6	0.38 (0.43)	.185
25.1-30.0	41	30.2	0.29 (0.19)	
30.1-41.0	18	13.2	0.36 (0.22)	
Timing of breast milk donation, d				
20-30	53	39.0	0.47 (0.50)	< .001
31-40	40	29.4	0.32 (0.18)	
41-65	43	31.6	0.23 (0.12)	
Total	136	100.0	0.35 (0.35)	

[a]One Minimum Brazilian Wage = 82 US dollars.
[b]Five women had body mass index (BMI) between 17 and 18.5 kg/m^2.

Discussion

There was a relationship between iron and vitamin A concentrations in human milk. According to Mbofung et al[27] and Dorea,[7] half of the iron in breast milk is

Table 2. Multivariate Linear Regression Model With Iron Concentrations in Breast Milk as the Dependent Variable and the Other Maternal Characteristics Investigated as Independent Variables

	Coefficient	*SE*	*P*
Vitamin A in milk	0.5303387	0.0803182	< .001
Timing of breast milk donation	–0.6073459	0.1346873	< .001
Smoking	0.4077566	0.1171941	.001

linked to protein fractions and the other half with fat, which can explain the positive association with vitamin A.[28] Moreover, several studies have shown that there is an interaction between iron and vitamin A.[29,30] Kelleher and Lönnerdal[31] investigated whether low vitamin A intake during lactation may result in low iron concentration in maternal milk in rats. The authors observed lower levels of divalent metal transporter 1, ferroportin, transferrin receptor, and serum ferritin in the group given the low vitamin A diet. These findings indicate a reduction of the transcription and translation of iron transport proteins in mammary tissue in rats with low vitamin A intake.

Similar to other results,[32] iron concentration in milk had a positive association with smoking habit and a negative association with timing of breast milk donation.[9,10,15,27] Kwapulinski et al[32] reported higher concentrations of iron in the milk of active and passive smokers in comparison to nonsmokers. It is known that smoking induces an increase in hemoglobin concentration[33] to compensate the low availability of free hemoglobin to be linked to oxygen. It is possible that a similar phenomenon is triggered to guarantee an appropriate concentration of iron in the milk of smokers. Although in the present study, smoking was associated with higher concentrations of iron in milk, there was no difference in the concentration of hemoglobin between smokers and nonsmokers (data not shown). The prevalence of smokers was 14.7%, similar to another study involving Brazilian milk donors.[34] Those women smoked fewer than 10 cigarettes a day, which is within the milk donation criteria adopted by the Brazilian milk banks. Shashiraj et al,[9] Raj et al,[10] Leotsinidis et al,[35] Mastroeni et al,[14] Trugo et al,[36] and Mbofung et al[27] analyzed colostrum and mature milk samples from lactating Indian, Greek, Brazilian, and Nigerian women and described a decrease in mineral content in human milk with an increase in timing of breast milk donation.

In accordance with other studies,[7-10,36,37] iron concentration in human milk was not associated with sociodemographic and obstetric factors or maternal iron status.

Twenty-one (15.4%) of the babies included in this study were preterm. There was no statistically significant difference in the content of iron in mature milk between the mothers who delivered preterm and term babies (Table 1), in agreement with another study carried out in Brazil.[36] The mean concentration of iron in milk was 0.35 mg/L (0.06 µmol/L), compatible with the value described by the Institute of Medicine,[38] based on several studies from developed and developing countries.

A limitation of this study is the relatively broad postpartum time period investigated (20-60 days postpartum), with variability of some of these micronutrients in mature milk,[7,39] especially zinc.[40]

In conclusion, the results of this study indicate that nutritional factors and smoking may have an influence on mature breast milk concentrations of iron in Brazilian donors.

Considering the importance of iron, especially in the prevention of anemia, and the role of human milk banks, we recommend further epidemiological studies in countries where milk banks are integrated into the health system to confirm our results.

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