Effect of iron supplementation during lactation on human milk composition

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There are no reports of studies specifically designed to test the effect of maternal iron supplementation on human milk composition. The purpose of this study was to evaluate the effect of moderate (40 mg Fe/day as $FeSO_4$) iron supplementation of nursing women for 3 months after delivery (non-supplemented, n = 14; supplemented, n = 14) on levels of iron, lactoferrin, total iron-ligands, and zinc in milk. Iron supplementation did not alter significantly iron and zinc levels in milk and the low iron to lactoferrin ratio was maintained, thus preserving the important functions of lactoferrin for the infant organism. However, iron supplementation increased total iron ligands in milk as measured by the total iron-binding capacity and increased the proportion of lactoferrin in total protein secreted. Also, lactoferrin levels tended (P = 0.059) to be higher in milk of the supplemented women. Our results suggest that the increase in total iron ligands and in the proportion of lactoferrin in total protein in milk after iron supplementation may be a response of the mammary gland to a higher plasma transferrin saturation. (J. Nutr. Biochem. 5:331–337, 1994.)

Keywords: iron supplementation; human milk; iron status

Introduction

Iron supplementation of nursing women may be beneficial for the maternal organism because pregnancy imposes a large demand on the maternal iron stores. The effect of use of iron supplements by nursing mothers on milk composition has been evaluated mainly by a limited number of observational studies[1,2] concerned particularly with the total iron concentration of milk. These studies concluded that iron supplementation has no effect on human milk iron content. Indirect support to this conclusion has also been obtained through the observation of no correlation between maternal iron intake and human milk iron levels[1–3] and between maternal iron status and milk iron.[4–7] However, the use of iron supplements has not been related to lactoferrin, lactoferrin-bound iron, and other iron ligands in milk. Alterations of these iron-related components that could occur with maternal iron supplementation may have implications for the infant organism in terms of iron bioavailability from milk and in terms of intestinal protection against bacterial overgrowth.[8]

Comparisons have been made between lactoferrin levels in milk of lactating women of different geographical regions and dietary habits, but results are not easily interpreted. Higher lactoferrin levels were observed in milk of Ethiopian women with very high dietary iron intake[9] and in women from India, including both severely anemic and non-anemic,[10] when compared with Swedish women. In the latter study, milk iron levels were much higher in the severely anemic Indian women, while Ethiopian women appear to have milk iron levels similar to Swedish women.[3]

Comparisons of milk composition between women of different geographical regions and socioeconomic status may be confounding because of cultural and environmental differences that may affect composition. Regional differences in trace element content in human milk may be partially due to differences in soil composition.[11,12] In the case of lactoferrin, the high levels observed in milk of women of underdeveloped countries may be a response to poor sanitary conditions and/or to latent infection state rather than to iron status or intake, as suggested by Lönnerdal et al.,[9] although this has not been confirmed.

There are no studies reported in the literature specifically designed to relate maternal iron supplementation and milk composition in nursing women of the same community with levels and period of use of supplementation adequately controlled. Also, if the effect to be pursued is specifically on

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milk composition, such a study should exclude iron-deficient mothers. Maternal iron deficiency or marginal status may be a complicating factor when evaluating the relationship of iron supplements with milk composition. Moreover, the use of iron supplements by nursing mothers may be of value even in those who are non-anemic as a means to restore iron reserves usually diminished during pregnancy despite the use of iron supplements.

The purpose of this study was to evaluate longitudinally the effect of moderate maternal iron supplementation during the first 3 months of lactation on milk iron levels and iron-related milk components of iron-sufficient nursing women of the same community. Milk zinc levels were also measured in relation to maternal iron supplementation due to the postulated competitive interaction between zinc and iron.[13]

Methods and materials

Subjects and study design

Twenty-eight volunteer nursing women of low socio-economic status participated in the study after informed consent. The women were recruited at the maternity ward of a public clinic in Rio de Janeiro, Brazil (Maternidade Escola/UFRJ) immediately after giving birth. Their ages ranged from 19 to 35 years (average of 27); they had prenatal care, received iron supplements during the second half of gestation (30 to 60 mg Fe per day), had uncomplicated pregnancies and deliveries as determined from medical records, delivered full-term healthy infants, were non-smokers, and presented biochemical indication of iron sufficiency at the beginning of the study *(Table 1)*.

Table 1 Hematological indices of the participating women at the beginning and end of the study

	Days after delivery	
	1–2	90–100
Hematocrit (vol. fraction)		
Group NS***	0.35 ± 0.03	0.40 ± 0.02
Group S***	0.36 ± 0.04	0.40 ± 0.03
Plasma iron (μmol/L)		
Group NS	15.8 ± 6.1	19.9 ± 7.9
Group S**	16.3 ± 3.8	21.9 ± 5.9
Unsaturated iron-binding capacity (μmol/L)		
Group NS	43.3 ± 8.6	43.0 ± 11.5[c]
Group S**	45.8 ± 8.6	29.2 ± 8.4[c]
Transferrin saturation (%)		
Group NS	27 ± 10	32 ± 13[a]
Group S***	27 ± 7	43 ± 11[a]
Plasma ferritin (μg/L)		
Group NS	49 ± 30	36 ± 21[c]
Group S***	47 ± 18	80 ± 26[c]

Group NS: nonsupplemented (n = 14).
Group S: supplemented (n = 14).
Values are mean ± SD.
Significant differences between groups at the same time period.
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
Significant differences during the period of the study in the same group.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

The women were recruited 1 to 2 days post-partum and randomly assigned to the supplemented or unsupplemented groups. Iron supplements were given in the form of iron sulfate ($FeSO_{4.7}H_2O$) tablets containing 40 mg of Fe each. Women in the supplemented group were instructed to take one pill daily of the supplement after a main meal for 90 days, starting 1 to 2 days after delivery. Compliance was evaluated by counting the remaining pills in the middle and end of the study. All women were asked not to take any other vitamin or mineral supplement during the period of the study. Blood samples were taken at the beginning (1 to 2 days after delivery) and at the end of the study (90 to 100 days post-partum), and milk samples were taken at 1 to 2, 30 to 40, and 90 to 100 days post-partum. This protocol was approved by the Human Research Committee of the Maternidade Escola/UFRJ.

All participating women maintained exclusive breast feeding during the period of the study and had no problems of mastitis or other infections in that period. Compliance of use of the iron supplement was 100%.

Body mass index and habitual dietary intake of iron and zinc were similar at the beginning and during the study in both groups of nursing women. Body mass index was on average 22.3 kg/m^2 (17.2 to 29.3). Daily dietary intakes averaged 14 (6 to 23) mg for iron and 11 (5 to 18) mg for zinc, with 38% and 43% from animal foods, respectively. Weight of infants were also similar in both groups, at birth and during the study, and within the values expected for a healthy population.[14]

Sample collection

Blood samples of the volunteers (ca 10 mL) were collected by peripheral venipuncture in vacuntainer tubes with EDTA after an overnight fast. Aliquots were taken for determination of hematocrit, and plasma was separated by centrifugation, divided in aliquots, and finally stored at −20° C until analyzed.

Milk samples (ca 10 to 15 mL) were collected between 8:00 and 10:00 a.m. by manual expression of both breasts into metal-free plastic tubes. The samples were immediately divided in aliquots that were stored at −20° C until analyzed.

At the time of each milk collection, the maternal dietary intake was evaluated by a food frequency questionnaire.

Other information regarding mother and infant, such as history of previous pregnancies, weight and length of infants at birth, weight gain of the infants during the study, and age and weight for height of the mothers, were obtained from medical records.

Laboratory assays

Hematological indices. Hematocrit was determined by the capillary technique using a Hemo-Spin centrifuge (Incibras, Brazil). Plasma iron was measured with a commercial kit (Merck, Rio de Janeiro, Brazil) after precipitation of proteins with HCl/TCA (final concentrations 0.3 N and 5%, respectively). Unsaturated iron binding capacity was also measured with a kit (Merck) after coagulation of samples with $CaCl_2$ (final concentration 0.01 M). Saturation of transferrin was calculated based on measurements of plasma iron and unsaturated iron-binding capacity. Plasma ferritin levels were measured by radioimmunoassay using a commercial kit (Diagnostic Products, Los Angeles, CA, USA). All determinations were run in duplicate.

Milk samples. Concentrations of iron and zinc in milk were measured in dry-ashed samples obtained at 525°C for 12 hours and dissolved in 1.2 N HCl (Suprapur, Merck). Iron was analyzed colorimetrically with sulfonated bathophenantroline by adaptation of methods used for serum iron. Briefly, appropriate aliquots of ash solutions were mixed with ascorbic acid, sodium acetate, and bathophenantroline-disulfonic acid (Sigma Chemical Co., St.

Louis, MO USA) prepared in NaH_2PO_4/Na_2PO_4 0.4 M, pH 5.5 (final concentrations 1.8%, 8%, and 0.01%, respectively), and absorbance was measured between 10 and 60 minutes at 540 nm. Zinc was analyzed by flame atomic absorption spectrophotometry using a Varian (San Francisco, CA, USA) model AA 1475. Standards were prepared with $FeCl_3$ (Titrisol, Merck) and $ZnCl_2$ (Titrisol, Merck). The accuracy and precision of these methods were evaluated using a certified non-fat milk powder (National Bureau of Standards, 1549). Values obtained were 1.81 ± 0.08 μg/g dry weight for Fe and 46.8 ± 1.9 μg/g dry weight for Zn. Certified values were 1.78 ± 0.10 μg/g and 46.1 ± 2.2 μg/g for Fe and Zn, respectively.

Iron concentration was measured in all whole milk samples and in milk whey and whey ultra-filtered in membrane cones (Amicon, Lexington, MA USA; CF25) of samples collected 90 to 100 days after delivery. Whey was obtained by sequential centrifugations to remove fat and casein as described by Fransson and Lonnerdal.[15] Iron concentration in the whey fraction containing high molecular weight components (>25,000 daltons) was calculated as the difference between iron concentrations in whey and ultra-filtered whey. The ratio of the iron concentration in the high molecular weight whey fraction to the lactoferrin concentration (see below) was also calculated (Fe:LF ratio). Total iron-binding capacity of milk was measured by saturation of ligands with excess $FeCl_3$ and precipitation of the unbound iron with magnesium-hydroxi-carbonate as described for serum iron.[16] Lactoferrin was measured by enzyme-linked immunoassay (ELISA) as previously described,[7] and protein by modification of the Lowry procedure.[17] All milk analyses were run in triplicate.

All glassware and plastic-ware used for sample collection and analytical work were previously acid-washed and carefully rinsed with deionized water to prevent spurious metal contamination.

Statistical analysis

Comparisons of hematological indices and milk components between the supplemented and non-supplemented women at the beginning and end of the study were done by Student's *t* test. Comparisons of changes in milk components between the supplemented and non-supplemented women during the period of the study were done by multivariate analysis of variance. Lactoferrin data were log transformed for statistical analysis. Correlations between milk components in each group of women at each collection period were also examined by Pearson's correlation analysis.

Correlations between hematological indices and milk components were tested at the beginning and end of the study using all volunteers irrespective of use of iron supplement. Levels of iron-related milk components at the end of the study were also compared in relation to stratification of the hematological indices at that period using analysis of variance. The criteria for stratification were based in the quartile values for each hematological index observed in all the women at the end of the study.

Results

Hematological values at the beginning of the study were similar in both groups of women *(Table 1)* and compatible with an adequate iron status.[18] After the experimental period, unsaturated iron-binding capacity in plasma was lower ($P < 0.001$), while transferrin saturation and plasma ferritin levels were higher ($P < 0.001$) in the supplemented group. Plasma iron increased ($P < 0.05$) at the end of the study only in this group *(Table 1)*.

Milk composition was similar in both groups at the stage of colostrum *(Table 2, Figure 1)* assuring the homogeneity of the experimental groups. Concentrations of the different components studied were higher in colostrum as expected.

Table 2 Milk composition of the supplemented and non-supplemented nursing women during the study

	Days after delivery		
	1–2	30–40	90–100
Iron (μmol/L)			
Group NS***	18.3 ± 5.0	13.4 ± 6.1	10.0 ± 3.4
Group S**	17.7 ± 5.6	12.5 ± 5.2	12.9 ± 7.5
Total iron-binding capacity (μmol/L)			
Group NS***	81.7 ± 35.1	42.4 ± 10.9	36.9 ± 12.4[a]
Group S*	83.6 ± 39.4	46.2 ± 16.8	58.0 ± 32.6[a]
lactoferrin (g/L)			
Group NS***	7.56 ± 3.95	1.92 ± 0.74	1.77 ± 0.74
Group S***	7.48 ± 4.59	2.17 ± 1.31	4.28 ± 4.70
Protein (g/L)			
Group NS***	47.0 ± 32.0	14.6 ± 3.31	13.5 ± 1.80
Group S***	45.4 ± 26.6	12.3 ± 3.82	13.9 ± 6.00
Lactoferrin: protein (%)			
Group NS*	19.4 ± 7.5	13.2 ± 4.3	13.1 ± 4.9[a]
Group S	20.1 ± 6.2	17.4 ± 8.4	20.3 ± 10.4[a]
Iron#: lactoferrin (μmol/g)			
Group NS	—	—	1.49 ± 0.54
Group S	—	—	1.20 ± 0.75
Zinc (μmol/L)			
Group NS***	107 ± 55.7	31.1 ± 13.2	13.9 ± 8.3
Group S***	116 ± 55.1	25.6 ± 6.4	15.0 ± 9.9

Group NS and Group S: as described in Table 1.
Values are mean ± SD.
#Iron in the whey fraction of high molecular weight components (> 25000 daltons).
Significant differences during the period of the study in the same group.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[a]Significant difference between groups at the same time period ($P < 0.05$).

The decline in concentrations during lactation was not significantly different in the supplemented and non-supplemented women for iron, total iron-binding capacity, and zinc. However, while the decline in lactoferrin tended to be different between groups ($P = 0.078$), changes in the lactoferrin to protein ratio were significantly different ($P = 0.016$). The relative contribution of lactoferrin to total protein in the supplemented group remained similar to that in colostrum during the study, (about 20%) while in the non-supplemented mothers this proportion declined to 13% in this period.

Mature milk (90 to 100 days) of the supplemented women *(Table 2)* had higher total iron-binding capacity ($P = 0.032$) and higher lactoferrin to protein ratios ($P = 0.034$) when compared with the non-supplemented women. Lactoferrin levels were also higher in the supplemented group, although the difference did not reach significance ($P = 0.059$). Fe:LF ratios in milk were not significantly different between both groups of women at the end of the study.

Variability and total range of milk components *(Table 2; Figure 1)* were similar in both groups at the beginning of the study, but variability of composition was higher in the supplemented group as lactation progressed, particularly at 90 to 100 days. Although half of the women in this group consistently presented two or more components in milk of

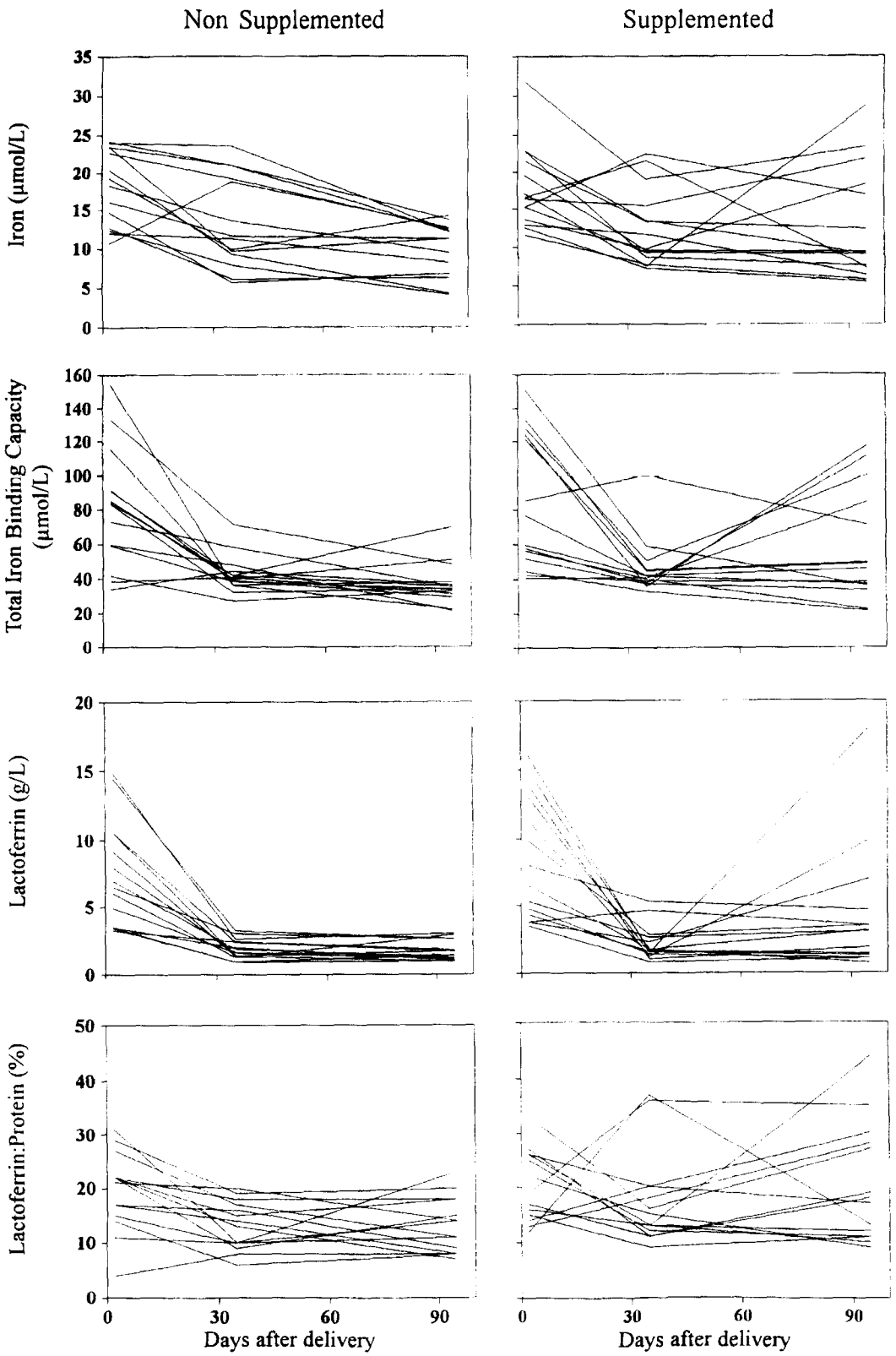

Figure 1 Individual variations of milk components during the study in the supplemented and non-supplemented lactating women.

90 to 100 days above the highest value observed in the non-supplemented group in the same period, the other had milk composition within the range observed for the non-supplemented women.

Correlations in colostrum and mature milk between iron-related components in both groups are shown in *Table 3*. Correlations were similar in these groups in the stage of colostrum, being particularly strong between lactoferrin and total iron-binding capacity (correlation coefficients higher than 0.80). However, correlations differed between groups at other stages of lactation. Correlations at 90 to 100 days after delivery were significant between iron and total iron-binding capacity, iron and lactoferrin, iron in whey fraction (molecular weight > 25000) and lactoferrin, and total iron and whey iron, only in the supplemented group, with correlation coefficients between 0.77 and 0.92. Correlation between lactoferrin and total iron-binding capacity was significant in both groups at this stage, with similar correlation coefficients (about 0.7).

Considering the correlations between milk components and maternal hematological indices in all women at the beginning and end of the study, significance was observed between total iron-binding capacity in milk and plasma transferrin saturation ($r = 0.443$; $P = 0.018$) and between the lactoferrin to protein ratio in milk and plasma transferrin saturation ($r = 0.376$; $P = 0.049$), both at the end of the study.

Iron-related milk components at the end of the study are presented in *Table 4* according to stratification of maternal hematological indices irrespective of the use of iron supplement. Significant differences were observed for total iron-binding capacity in milk ($P = 0.004$) and for the lactoferrin to protein ratio in milk ($P = 0.033$), both according to levels of plasma transferrin saturation.

Table 3 Correlations between iron-related components in colostrum and mature milk of the supplemented and non-supplemented lactating women

	Colostrum		Mature milk			
			30–40 days		90–100 days	
Related variables	*r*	*P*	*r*	*P*	*r*	*P*
Iron and lactoferrin						
Group NS	0.692	0.009	0.287	0.329	−0.082	0.780
Group S	0.686	0.010	0.281	0.016	0.872	<0.001
Iron and total iron-binding capacity						
Group NS	0.626	0.017	0.064	0.828	−0.103	0.727
Group S	0.504	0.049	0.480	0.083	0.771	0.001
Iron and whey iron*						
Group NS	—		—		0.298	0.347
Group S	—		—		0.902	<0.001
Lactoferrin and total iron-binding capacity						
Group NS	0.825	<0.001	0.524	0.054	0.664	0.010
Group S	0.850	<0.001	0.650	0.012	0.771	<0.01
Lactoferrin and whey iron*						
Group NS	—		—		0.504	0.095
Group S	—		—		0.921	<0.001

r = correlation coefficient; *P* = probability level.
Group NS and Group S: as described in Table 1.
*Iron in the whey fraction of high molecular weight components (>25,000 daltons).

Discussion

This study was designed to evaluate specifically the effect of 3-month iron supplementation of nursing women on milk composition, excluding the possible complicating effects of maternal iron deficiency and socio-economic condition. The level of supplementation chosen was moderate, about 2.5 times higher than the habitual dietary iron intake of the women, so that the supplemented women ingested an average of 64 mg of daily iron during the experimental period of 90 days, compared with 14 mg per day in the non-supplemented group. All volunteers were healthy, gave birth to healthy infants, and maintained these conditions during the study. They also maintained adequate iron indices during the study, but at the end only the supplemented women had, on average, changes in most indices compatible with increased iron availability in their organisms, such as higher plasma transferrin saturation and higher (doubled) iron reserves as indicated by plasma ferritin levels *(Table 1)*. The increase in iron reserves of the supplemented lactating women may be desirable, especially for those that would become pregnant again in a short period of time.

This moderate 3-month iron supplementation did not increase, on average, the concentration of iron in milk *(Table 2)*. This result confirms the conclusions of previous non-controlled studies.[1,2] Maternal iron supplementation during lactation does not cause major changes in human milk iron content.

The regulation of iron incorporation into milk is still far from understood, but animal studies have shown that the entry in the mammary gland cells is not the limiting step. Sigman and Lönnerdal[19] observed in rats that a threefold increase in iron intake during pregnancy and lactation similar to the moderate supplementation used in our study produced an increase in the concentration and total number of transferrin receptors in the mammary gland cells, increasing the capacity of these cells for plasma iron uptake, although iron milk levels were not altered. The authors concluded that regulation of iron level in milk comes after the entry of iron into the mammary cell.

Although milk iron content was not affected, iron supplementation of the lactating women in our study significantly affected components related to iron in milk. Total iron-binding capacity, an indirect measurement of total iron ligands in milk, was higher in mature milk of the supplemented group, indicating that the extra iron in the maternal organism increases the total iron ligands secreted into milk. Lactoferrin is an important component of the total iron-binding capacity in human milk as observed in this *(Table 3)* and other studies.[20,21] In mature milk, lactoferrin level tended to be higher in the supplemented mothers, and lactoferrin contribution to total protein was higher in these women, maintaining a proportion similar to that of colostrum. Moreover, because the Fe:LF ratio was similar in the supplemented and non-supplemented women, the degree of lactoferrin saturation was maintained low in milk of these women. The mean Fe:LF ratios observed in the women of our study would correspond to a lactoferrin iron-saturation of 5.7% and 5.0%

Table 4 Iron-related milk components at the end of the study for different levels of maternal hematological indices

Hematological indices(**)	Milk components(*)				
	Iron (μmol/L)	Total iron-binding capacity (μmol/L)	Lactoferrin (g/L)	Lactoferrin:protein (%)	Iron:lactoferrin (μmol/g)
Hematocrit (vol. fraction)					
< 0.38	9.3 ± 4.2	43.7 ± 16.3	2.16 ± 1.03	19.3 ± 7.4	1.40 ± 0.68
0.38–0.415	12.5 ± 5.7	45.0 ± 22.6	3.26 ± 2.89	13.2 ± 5.4	1.33 ± 0.64
> 0.415	12.2 ± 5.9	56.8 ± 35.6	3.74 ± 2.54	19.4 ± 11.0	1.25 ± 0.46
Plasma iron (μmol/L)					
< 15.6	10.9 ± 7.1	40.6 ± 24.0	3.00 ± 2.34	14.7 ± 4.3	1.16 ± 0.43
15.6–27.4	10.0 ± 4.4	42.8 ± 18.6	2.12 ± 1.15	16.2 ± 8.9	1.55 ± 0.70
> 27.4	14.6 ± 4.6	63.6 ± 31.3	3.85 ± 2.25	19.6 ± 11.2	1.04 ± 0.38
Transferrin saturation (%)					
< 27.4	12.0 ± 7.1	40.1 ± 17.4[b]	2.86 ± 2.28	13.0 ± 4.8[a]	1.40 ± 0.09
27.4–45.6	9.3 ± 3.2	37.8 ± 11.6[b]	1.92 ± 0.90	14.9 ± 6.1[a]	1.54 ± 0.64
> 45.6	14.8 ± 5.9	74.3 ± 27.9[b]	4.39 ± 2.80	24.1 ± 8.0[a]	0.84 ± 0.25
Plasma ferritin (μg/L)					
< 32.5	9.5 ± 3.8	39.6 ± 6.4	2.14 ± 0.55	15.7 ± 2.8	1.34 ± 0.15
32.5–77.5	12.3 ± 6.4	45.3 ± 24.5	3.10 ± 2.25	15.1 ± 7.9	1.32 ± 0.18
> 77.5	11.5 ± 5.5	59.8 ± 34.7	3.75 ± 2.14	20.9 ± 12.0	1.34 ± 0.38

(*)Mean ± SD; total n = 28.
(**)Criteria for stratification were based on quartile values of each hematological index.
Significant differences in milk components by level of hematological index:
[a]$P < 0.05$.
[b]$P < 0.01$.

in the non-supplemented and supplemented women, respectively, within the expected range.[22] These results suggest that an adaptive mechanism operating in the mammary gland cells when more iron is available might be an increase in lactoferrin as a proportion of total protein secreted in milk, maintaining the low-iron saturation of this protein. This mechanism would preserve the important bacteriostatic role of lactoferrin in milk, preventing mastitis in the breast and protecting the infant intestine from bacterial over-growth.

In spite of the overall significant effects of iron supplementation on iron-related milk composition observed in our study, it should be noted that within the supplemented group the individual responses were quite variable *(Figure 1)*. This explains the stronger correlation between milk components in this group, because, in general, higher levels of iron, whey iron, lactoferrin, and total iron-binding capacity occurred simultaneously in these women.

The significant correlations observed at the end of the study between total iron-binding capacity and the lactoferrin to protein ratio in milk with plasma transferrin saturation and the significant differences in those iron-related milk components according to level of plasma transferrin saturation *(Table 4)*, suggest that the mammary gland may be sensitive to plasma transferrin saturation in humans. A high transferrin saturation may trigger responses in the mammary gland such as an increase in the secretion of total iron ligands into milk and in the proportion of protein secreted in milk as lactoferrin. Based on these results, it is possible to speculate that the use of iron supplements during lactation may increase the expression of transferrin receptors in the mammary gland in humans as it was observed in rats.[19]

A complementary aspect of our study was to evaluate the effect of iron supplementation during lactation on milk zinc levels. A competitive inhibition of zinc absorption by iron has been measured in humans, using inorganic salts and Fe:Zn ratios of 2:1 or greater.[23] Although the same effect is not evident when the same ratios are given as endogenous constituents of foods,[24] the use of iron supplements, such as in prenatal care, may be of concern in terms of zinc absorption. The level of iron supplements during pregnancy has been inversely related to plasma and urinary zinc levels,[25,26] and zinc absorption in pregnancy has been found to decrease by use of iron-folate supplements.[27] Therefore, the use of iron supplements by lactating women may affect milk zinc levels, especially if their dietary zinc intake is low, such as observed in our study. Average daily zinc intake in the women of our study was about 58% of the recommendation,[28] and the use of iron supplement increased the Fe:Zn molar ingested ratio from 1:1 to 6:1. However, in our study, milk zinc levels were not affected by these ratios, confirming other studies indicating that milk zinc levels are strongly regulated in spite of possible marginal maternal zinc status.[29]

Iron supplementation during lactation may be of value for the maternal organism even in non-anemic women. Our results show that a moderate iron supplementation of non-anemic nursing mothers for a period of 3 months increases the maternal iron reserves and is not harmful for the infant organism. On average, milk composition is not altered with supplementation in terms of iron and zinc levels, and the low iron-saturation of lactoferrin in milk is also maintained, thus preserving its important functions for the infant organism. However, after supplementation there is an increase in milk levels of total iron ligands and in the proportion of lactoferrin in total milk protein, probably as an adaptive response of the mammary gland to higher plasma transferrin saturation.

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