

## Metabolism of Iron in Breast Milk of Women during Different Stage of Lactation

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### ABSTRACT:

Breast milk is the only source of nutrients that contains all the components of normal growth and development of children. That is why breastfeeding is so important for a healthy baby. In the dynamics of lactation, the analysis of iron, binding of iron, breastfeeding properties and quantitative indicators of lactoferrin have great scientific and practical importance.

**Keywords:** Colostrums, Intermittent Milk, Matured Milk, Iron, Lactoferrin, Lactoferrin Saturation Factor, Iron Binding Properties of Lactoferrin.

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## INTRODUCTION

It is well known that breast milk is the optimal biological fluid with all the necessary plastic nutrients (Erick M., 2018, Ballard O., 2013). Milk from breastfeeding women has long been the only source of nutrients, including energy, immune, micronutrients, vitamins and other components for normal growth and development in children (Arthur I. Eidelman, Richard J. Schanler, 2011, Baker R.D., Greer F.R., 2010). This is why breastfeeding is so important for ensuring healthy nutrition and full nutrition for children. Children who are breastfeeding have a high immunity level. For some reasons, they grow much stronger and healthier than their peers fed on artificial milk mixes (Bank J., 2014, Gulec S., Anderson G., Collins J., 2012).

Iron deficiency in women's breast milk increases the physiological anemia (Domellof M. et al., 2014, Lozoff B. et al., 2012). This is because iron metabolism in the first half of the life of the child

is quantitative and is formed by two structures, the iron reserve formed in the womb of the newborn and the iron absorbed by mother's milk. Thus, the notion of physiologic anemia arises from the amount of iron in the colostrum and the maximum amount of iron in the intermitten period as a unit of measurement at the beginning of lactation, which is related to the occurrence of the symptoms of breastfeeding and physiological anemia. Formation of a nursing woman corresponds to an increase in the hemoglobin levels in the chronologically newborn's erythropoiesis. During this period, erythropoiesis is more dependent on the absorption of exogenous iron in breast milk (Cai C., Eck P., Friel J.K., 2017, Lonnerdal B., 2017, Cai C., Granger M., Eck P., Friel J., 2017). During this long chronological period, iron metabolism in the first six months of life is directly dependent on the amount of iron in the mother's milk. The need for iron during this period is 30% due to the use of iron in milk and 70% due to the reuse of iron in the red blood cells.

Comparative analysis of iron and other parameters of breast milk during lactation of nursing women, who have normal hemoglobin and living in different regions.

## MATERIAL AND METHODS

The analysis of iron, lactoferrin binding in breast milk of women in breastfeeding, iron binding properties of lactoferrin and lactoferrin saturation coefficient for analysis of lactation during lactation (post-delivery), intermittent milk (5 days) and ripened milk (30 days later) samples were analyzed. Of the 40 women selected, the average age was 24 years in Tashkent region and 22 in the Samarkand region.

Iron content of breast milk was analyzed by atomic absorption spectrophotometry. The ion exchange chromatography was used to separate lactoferrin from milk.

## RESULTS AND DISCUSSION

Another important issue in examining the iron content of breast milk in women during lactation is the impact of biogeochemical, socio-economic, environmental and other conditions on various ethnogeographic subpopulations (Lönnerdal B., 2017, Nagin M., 2009).

In this regard, one of the objectives of our research is to investigate the classification of iron metabolism in breast milk in subpopulations of breastfeeding women in Tashkent and Samarkand regions on the above factors. To achieve these objectives, 40 colostrums, intermittent and matured lactating women were selected, and their iron content, iron binding, and lactoferrin levels were analyzed by comparisons of women in the two regions. The experiments were performed on conventional healthy lactating women with a total hemoglobin index of more than 110.0 g/l. The comparison results are presented in Table 1.

**Table 1: Comparative analysis of iron status in breast milk of women living in Tashkent and Samarkand region**

Indicators	Tashkent			Samarkand		
	Colostrums	Intermittent	Ripened milk	Colostrums	Intermittent	Ripened milk
Fe, mkmol/l	15.5 ± 0.8	14.1 ± 0.69	9.3 ± 0.21	10.8 ± 0.21*	9.7 ± 0.27*	6.7 ± 0.32*
TBX, mkmol/l	132.8 ± 2.7	120.7 ± 3.8	115.1 ± 4.39	102.7 ± 3.4*	100.4 ± 3.9*	78.6 ± 1.3*
Lf, g/l	6.13 ± 0.18	6.03 ± 0.07	4.92 ± 0.05	5.23 ± 0.13*	5.25 ± 0.09*	4.90 ± 0.12
Lf, %	10.5 ± 0.62	9.5 ± 0.42	7.7 ± 0.22	8.3 ± 0.28*	7.6 ± 0.26*	6.0 ± 0.38*

Explanation: \* -  $P < 0.001$ ;  $n = 40$

In both subpopulations, there was some variation in iron content in colostrum. Iron content in the colostrum of breastfeeding women in Tashkent region was  $15.5 \pm 0.8 \mu\text{mol/l}$  ( $0.86 \pm 0.04 \mu\text{g/ml}$ ), with a dispersion rate ranging from  $10.4 \mu\text{mol/l}$  (min) to  $24 \mu\text{mol/l}$  (max). Was found to be up to  $4 \mu\text{mol/l}$  (max). In breastfeeding women in the Samarkand region, this distribution is  $10.8 \pm 0.21 \mu\text{mol/l}$  ( $0.60 \pm 0.01 \mu\text{g/ml}$ ), respectively, and the dispersion range is  $8.9 \mu\text{mol/l}$  (min) to  $12.3 \mu\text{mol/l}$  (min). Significant differences in iron binding properties of lactoferrin are also found in milk (see Table 1). Iron intake of lactating women in Tashkent region was  $132.8 \pm 2.7 \mu\text{mol/l}$ , while in

Samarkand region this indicator did not exceed  $102.7 \pm 3.4 \mu\text{mol/l}$ . This may be explained by the fact that in the Samarkand subpopulation, the iron content of proteins, lipids and other low-molecular components in colostrum is low.

The iron-binding protein lactoferrin was found to be  $6.13 \pm 0.18 \text{ g/l}$  in lactating women in the Tashkent region, and in the mothers of the Samarkand region it was  $5.23 \pm 0.13 \text{ g/l}$ . This confirms the relatively low iron-binding factor in the colostrum of women in Samarkand Region. The iron content of lactoferrin is found to be  $10.5 \pm 0.62\%$  in lactating women in Tashkent

and in breastfeeding women in Samarkand province, it does not exceed  $8.3 \pm 0.28\%$ .

It is also noted that in milk of the intermittent period the above indicators differ by region. The iron content of milk during this period was  $14.1 \pm 0.69 \mu\text{mol/l}$  ( $0.78 \pm 0.04 \mu\text{g/ml}$ ) in Tashkent region, and  $9.7 \pm 0.27 \mu\text{mol/l}$  in Samarkand region.  $0.02 \mu\text{g/mL}$  ( $p < 0.001$  in both cases). Milk from women in the Samarkand region has low iron binding properties, i.e.  $100.4 \pm 3.9 \mu\text{mol/l}$  ( $5.58 \pm 0.22 \mu\text{g/ml}$ ), while women in Tashkent region have a  $120.7 \pm 3.8 \mu\text{mol/l}$  ( $6.71 \pm 0.21 \mu\text{g/ml}$ ).

It was also found that the lactoferrin content in lactating milk in women in the Samarkand region is much lower than that of women in the Tashkent region, i.e.  $5.25 \pm 0.09 \text{ g/l}$ . In the Tashkent region, the figure was  $6.03 \pm 0.07 \text{ g/l}$ . Similar differences were also observed with iron saturation levels of lactoferrin ( $r < 0.001$  at  $7.6 \pm 0.26\%$  in Samarkand and  $9.5 \pm 0.42\%$  in Tashkent).

There was a difference in the comparative analysis of iron metabolism rates in the milk of women living in both subpopulations. For example, in the Tashkent region, the level of iron in women's milk is  $9.3 \pm 0.21 \mu\text{mol/l}$  ( $0.52 \pm 0.10 \mu\text{g/ml}$ ), while women in the Samarkand region are  $6.7 \pm 0.32 \mu\text{mol/l}$  ( $0.37 \pm 0.18 \mu\text{g/ml}$ ).

Iron binding properties were found to be 1.5 times higher for women in Tashkent region than women in Samarkand province ( $115.1 \pm 4.39 \mu\text{mol/l}$  ( $6.39 \pm 0.24 \mu\text{g/ml}$ ) and  $78.6 \pm 1$ , respectively.  $3 \text{ mg/l}$  ( $4.37 \pm 0.07 \text{ mg/ml}$ ,  $p < 0.001$ ) It was found that lactoferrin in mature milk is higher in breastfeeding women in Tashkent region with an average of  $4.92 \pm 0.05 \text{ g/l}$ , Lactoferrin is found to be  $7.7 \pm 0.12 \text{ g/l}$  in breastfeeding women in the Samarkand region.  $0.22\%$  and  $6.0 \pm 0.38\%$  ( $p < 0.001$ ).

Thus, chronobiological changes in iron levels in nursing women have been shown to have a specific classification of the subpopulations that have been investigated in the metabolism of iron-binding properties of lactoferrin during different stages of lactation.

It is important to determine the iron binding ability or buffer capacity of milk in healthy breastfeeding women. From the literature and our own research, comparative analysis of iron and lactoferrin in the milk was conducted in order to accurately explain the biologic fluids circulating round the daily, undoubtedly due to the fact that biological fluids circulate round the daily. For this, milk samples were collected and analyzed from healthy nursing women at different times of the day, including 8 am in the morning, 12 pm in the afternoon and 20 pm in the evening. The results are presented in Table 2.

**Table 2: Average amount of iron in women's milk at different times of day (n = 40)**

Indicators	Morning	Daytime	Evening
Fe $\mu\text{mol/l}$	4.26	11.1	6.31
	11.9	20.5	15.4
	$8.47 \pm 0.63$	$14.3 \pm 0.82$	$11.3 \pm 0.61$
Lf $\text{g/l}$	4.22	5.60	4.85
	5.11	6.35	5.68
	$8.65 \pm 0.50$	$5.98 \pm 0.08$	$5.24 \pm 0.43$

The serum content of hemoglobin in nursing women was  $113.0 \pm 0.6 \text{ g/l}$ . The maximum iron concentration in the daytime is  $14.3 \pm 0.82 \mu\text{mol/l}$  ( $0.79 \pm 0.05 \mu\text{g/ml}$ ), with a spreading dispersion of  $11.1 \mu\text{mol/l}$   $20.5 \mu\text{mol/l}$  (max). Low iron concentrations in the morning at  $8.47 \pm 0.63 \mu\text{mol/l}$  ( $0.47 \pm 0.04 \mu\text{g/ml}$ ), with dispersion

from  $4.26 \mu\text{mol/l}$  (min) to  $11.9 \mu\text{mol/l}$  (max). In the evening, the mean iron content is  $11.3 \pm 0.61 \mu\text{mol/l}$  ( $0.63 \pm 0.03 \mu\text{g/ml}$ ), and the dispersion ranges from  $6.31 \mu\text{mol/l}$  (min) to  $15.4 \mu\text{mol/l}$  (max). It was found to be less than in the daytime and more than in the morning.

The amount of lactoferrin in milk is  $5.98 \pm 0.08$  g/l in the daytime, with a dispersion ranging from 5.60 g/l (min) to 6.35 g/l (max) and the minimum lactoferrin content is 4.65 in the morning.  $\pm 0.50$  g/l, the dispersion was found to range from 4.22 g/l (min) to 5.11 g/l (max). In the evening, the mean dispersion of  $5.24 \pm 0.43$  g/l was proved to be 4.85 g/l (min) to 5.68 g/l (max). Consequently, a study of the daily variation of iron and lactoferrin in women's

breast milk revealed the highest possible concentration of urine samples at that time. In this way, the biometric error rate is minimized. During our research, we found that the iron content of breast milk of women varies with the maturity period of milk.

Table 3 provides a comparative analysis of the correlation of iron in breast milk with the dynamics of milk maturity dynamics.

**Table 3: Changes in iron content in total lactation at different stages of lactation (n = 40)**

Lactation periods	The amount of milk produced, ml	The amount of iron in milk mkmol/l	Total amount of iron, mg
Day 1	20	11.1 24.4 $15.5 \pm 0.8$	0.0172
Day 10	500	8.7 19.0 $14.1 \pm 0.69$	0.39
Day 30	900	8.1 11.1 $9.3 \pm 0.21$	0.47

Thus, the hemopoietic iron trace in breast milk is relatively small. In addition, milk concentration decreases by the time milk is released. However, the problem arises as to whether such fluctuations of iron adequately meet the newborn's demand for this biomaterial as the only exogenous nutrient from the outside.

As shown in Table 3, iron reduction in the milk of healthy nursing women does not affect infant iron availability.

In the course of the study, samples of primary colostrum, intermittent and matured milk were analyzed. The content of iron in colostrum was  $15.5 \pm 0.8$   $\mu\text{mol/l}$  ( $0.86$   $\mu\text{g/ml}$ ), with dispersion variability ranging from  $11.1$   $\mu\text{mol/l}$  (min) to  $24.4$   $\mu\text{mol/l}$  (max). The hemoglobin content was found to be  $115.0 \pm 1.4$  g/l on average, and the dispersion rate ranged from  $110.0$  g/l (min) to  $124.0$  g/l (max). Therefore, if during this period colostrum produced 20 ml on average, total iron content would be 0.0172 mg.

Milk has a very high iron binding ability when it is tested for its ability to bind milk components during this biometallic time, or when the buffer capacity of the breast milk is high, which is an important source of iron in breast milk. The content of iron binder in the analyzed colostrum was  $132.8 \pm 2.5$   $\mu\text{mol/l}$ . Lactoferrin, the iron-binding protein in breast milk, is the main specific ironbinding protein. Therefore, the amount of this protein in the colostrum is very important. The amount of lactoferrin in colostrum was  $6.13 \pm 0.18$  g/l. The iron content of lactoferrin was found to be  $10.5 \pm 0.62\%$  on average.

In a subsequent experiment, we analyzed the correlation between iron content and hemoglobin in the colostrum of nursing women with normal hemoglobin. The results of this experiment are presented in Table 4. The data presented in the table show that there is a very positive correlation between the iron content of colostrum and the lactoferrin iron saturation coefficient (PT), that is, the change in their quantity.

Table 4: The correlation coefficient between the iron levels in colostrums

	Fe	IBPL	Lf	LSF
Fe	-	- 0.212	+0.02	+0.852
IBPL	-0.212	-	-0.351	+0.185
Lf	+0.02	-0.351	-	-0.286
LSF	+0.852	+0.185	-0.286	-

Iron saturation level of lactoferrin is also low when colostrum is low in iron. Inverse relationship between them ( $r = -0.286$ ), that is, high concentrations of lactoferrin in milk produce low iron concentrations, and iron saturation levels remain low.

Significant changes in iron levels also occur during the interval in breast milk. The average iron content in milk during this period was  $14.1 \pm 0.69 \mu\text{mol/l}$  and the dispersion ranged from  $8.7 \mu\text{mol/l}$  (min) to  $19.0 \mu\text{mol/l}$  (max). If the average daily amount of milk in the intermittent period is 500 ml, the total iron content is 0.39 mg. Comparing the iron binding properties of the intermittent milk with that of the colostrum during the period, it was found to be  $120.7 \pm 3.8 \mu\text{mol/l}$ , respectively. In the intermittent period of iron-binding protein lactoferrin it was observed to be  $6.03 \pm 0.07 \text{ g/l}$ , and iron saturation was  $9.5 \pm 0.42\%$ .

In our next study, we analyzed the iron content of breast milk from nursing mothers with normal hemoglobin. The mean iron content in breast milk of nursing mothers was  $9.3 \pm 0.21 \mu\text{mol/l}$ , with a dispersion ranging from  $8.1 \mu\text{mol/l}$  (min) to  $11.1 \mu\text{mol/l}$  (max). Then 900 ml of daily milk was calculated to contain 0.47 mg of iron. It was found that the iron binding capacity of the mature breast milk was  $115.1 \pm 4.39 \mu\text{mol/l}$  on average. The amount of iron-binding lactoferrin protein in mature breast milk was  $4.92 \pm 0.05 \text{ g/l}$ , and the average iron content of the protein was  $7.7 \pm 0.22\%$ .

Changes in the composition of breast milk of healthy nursing women with regard to iron content, evolutionary changes in intermittent and transmitted milk are of a certain biological significance. Foreexample, the highest levels of iron occur during the initial lactation period, or colostrum. Because at this time the "newborn" physiological anemia has occurred in the

external circulatory system of the newborn, and the overall hemoglobin reduction is accelerated.

Due to the iron dynamics in healthy nursing mother's milk, it progresses towards the end of lactation. According to our analysis, iron metabolism in the first half of the life of the child has an effect on the amount of iron in mother's milk, that is, the process of iron metabolism in the baby during its antinatal period decreases. As a result, after six months the amount decreases to  $6.7 \pm 0.32 \mu\text{mol/l}$ . Consequently, by the end of the first half of the lactation, the iron content in the breast milk will decrease by about 30% compared to the colostrum, by 10% on average and by 8% by the end of the first month.

These data suggest that abnormal changes in the iron content of nursing women occur during the maturing period, ie there is a gradual decline in colostrum, intermediate periods, and matured milk. This means that there will be no sharp changes in iron in the first half of the year. From a biochemical point of view, iron is able to meet all the physiological needs of iron and the need for hemoglobin in the growing baby and to meet iron requirements during lactation in order to provide iron to breastfed babies.

According to our data, there was no direct correlation between erythropoiesis in healthy lactating women, namely, stored iron (serum ferritin) in the colostrum, intermittent, and matured milk. In addition to iron, the study of iron binding properties or milk storage also plays an important role in determining the iron in breast milk. The concept of this indicator is usually understood as the process of iron binding of specific and non-specific colostrum and other milk proteins (lactoferrin, lactalbumin), lipids and other low molecular components. Together, these data determine the degree of iron saturation of all components in biological fluid, ie. serum. However, such

information may be very important in identifying the role of iron in hemopoiesis in lactation. In practice, such information makes breastfeeding babies known as the "nutritional formula" the need for complementary feeding.

## CONCLUSION

Thus, chronobiological changes in iron levels in breastfeeding women, reduced lactation at different stages, and metabolism of iron-binding properties of lactoferrin during milk maturation have been shown to be specific subpopulations.

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