Diurnal Variations in Qualitative Composition of Breast Milk in Women with Iron Deficiency

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Biorhythms of iron metabolism in healthy women and patients with iron deficiency were studied during lactation. Healthy nursing women were characterized by circadian variations in the concentrations of iron, α-tocopherol, and malonic dialdehyde in breast milk. Diurnal variations in iron concentration, antioxidant potential, and lipid peroxidation in breast milk depended on iron metabolism in nursing women. Iron deficiency was accompanied by a decrease in the concentrations of α-tocopherol and iron, increase in malonic dialdehyde content, and suppression of circadian variations in these parameters in breast milk.

Key Words: *biorhythms; lactation; iron deficiency; α-tocopherol; lipid peroxidation*

Breast feeding for 6 months or longer decreases the risk of iron deficiency (ID) in children over the 1st year of life. Bioavailability of this trace element in breast milk is 5-6 times higher than in milk substitutes [8]. However, progressive ID is observed even in breast-fed children of women with iron-deficiency anemia (IDA) [3]. Transport of this trace element from blood plasma to breast milk is an active process. The amount of iron in breast milk does not depend on maternal blood iron concentration [4,6,7]. Here we studied the dependence of breast milk iron concentration on iron metabolism in women.

MATERIALS AND METHODS

We examined 200 women at the 6th month of lactation (136 healthy nursing women, 36 women with latent ID (LID), and 28 women with IDA). The age of lactating women was 20-32 years.

Breast milk (50-ml portions) and cubital vein blood were sampled 4 times a day (6.00, 12.00, 18.00, and 24.00). Serum ferritin level was measured by enzyme immunoassay. The concentration of iron in blood plasma and breast milk was estimated by the diphenylphenanthroline method (Lachema). The intensity of lipid peroxidation (LPO) in blood plasma was determined by the concentration of malonic dialdehyde (MDA) [2]. α-Tocopherol concentration was measured as described elsewhere [5].

Disorders of iron metabolism were diagnosed by the chronobiological method. LID was verified by suppression of circadian variations in iron metabolism and decrease in the mean daily concentration of serum ferritin (below 30 μg/liter). It should be emphasized that the mean daily number of erythrocytes and blood hemoglobin level remained unchanged under these conditions. Desynchronosis of blood parameters and iron metabolism, as well as the decrease in the mean daily fractions of reserve, transport, and hemoglobin iron, served as the criteria of IDA.

The results were analyzed by Student's *t* test.

RESULTS

The mean daily concentration of iron and diurnal variations in iron metabolism in lactating women did not differ from those in healthy nonpregnant and nonlactating women of reproductive age. Blood iron concentration reached maximum in the morning and daytime, but was minimum in the evening and nighttime (Table 1). Studying the fractional composition of blood iron

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revealed a decrease in the mean daily concentration of Fe^{2+} or free iron (2.17±0.15 μmol/liter). These changes were accompanied by a significant decrease in plasma MDA concentration (Table 1). The increase in plasma antioxidant activity is typical of healthy nursing women. In these women the mean daily concentration of α-tocopherol increased by 1.5 times, while the MDA/α-tocopherol ratio decreased by 1.6 times. The increase in antioxidant activity during lactation is probably associated with specific features of hormonal status in this period (*e.g.*, antistress effect of prolactin) [1].

The mean daily concentration of iron in breast milk was 2-fold lower compared to blood iron level (Table 1). Synchronization of diurnal changes in the concentrations of iron and α-tocopherol in breast milk indicates that active consumption and transport of iron in blood plasma are mediated by transferrin receptors on breast epithelial cells during lactopoiesis and depend on the state of cell membranes, which is regulated by the ratio between LPO and antioxidant activity. The MDA/α-tocopherol ratio in breast milk was lower than in blood plasma. Our findings indicate that breast milk has high antioxidant activity.

Nursing women with LID were characterized by a decrease in the mean daily concentrations of serum ferritin (11.1±4.7 μg/liter) and serum iron and reduc-

TABLE 1. Diurnal Variations in Concentrations of Iron, MDA, and α-Tocopherol in Blood Plasma and Breast Milk (*M*±*m*)

Time of day, parameter	Blood plasma, μmol/liter			Breast milk, μmol/liter		
	iron	MDA	α-tocopherol	iron	MDA	α-tocopherol
Healthy nursing women 06:00	12.5±0.7	5.2±0.2	0.40±0.02*	4.0±0.8*	3.1±0.2	0.20±0.03*
12:00	18.8±0.6*	5.1±0.1*	0.52±0.03	7.1±0.6	3.07±0.20*	0.33±0.04
18:00	14.1±0.6	7.6±0.2	0.69±0.04*	8.8±0.6*	3.6±0.3	0.58±0.05*
24:00	10.0±0.8*	10.2±0.3*	0.57±0.06	5.5±0.7	6.19±0.40*	0.46±0.07
Mean daily level	13.6±1.3	7.2±0.3	0.55±0.07	6.4±0.5	3.99±0.40	0.36±0.08
Amplitude, % of mesor	2.60±0.40 (19.1)	2.6±0.4 (36.1)	0.15±0.04 (27.2)	2.2±0.8 (34)	1.56±0.40 (39)	0.19±0.05 (52.7)
Acrophase, hours (95% confidence interval)	12.32 (11.15; 14.47)	4.15 (22.15; 05.25)	18.05 (17.02; 19.50)	17.52 (17.15; 20.27)	3.17 (22.32; 04.12)	18.35 (17.22; 20.10)
Lactating women with LID 06:00	9.8±1.0	8.8±0.4	0.30±0.04	4.9±0.9	7.5±0.6	0.15±0.04
12:00	11.4±1.3	9.1±0.5	0.32±0.03	5.5±1.1	8.0±0.5	0.17±0.03
18:00	11.1±0.9	8.0±0.3	0.,5±0.04	5.7±0.9	6.4±0.4	0.19±0.04
24:00	10.1±0.8	7.3±0.3	0.4±0.05	3.1±0.8	6.9±0.4	0.16±0.05
Mean daily level	10.6±1.3	8.4±0.6	0.32±0.06	4.8±0.9	7.3±0.6*	0.17±0.06
Amplitude, % of mesor	1.8±0.7 (7.4)	0.90±0.05 (10.7)	0.030±0.002 (9.3)	1.3±0.9 (27.3)	0.8±0.5 (10.9)	0.05±0.02 (29.4)
Acrophase, hours (95% confidence interval)	Absence of rhythm					
Lactating women with IDA 06:00	9.9±0.4	11.2±0.5	0.16±0.03	3.9±0.4	9.1±0.6	0.10±0.03
12:00	10.9±0.8	11.4±0.4	0.21±0.04	4.7±0.8	9.3±0.5	0.12±0.04
18:00	10.8±0.8	13.3±0.3	0.23±0.03	4.8±0.8	11.2±0.5	0.15±0.03
24:00	9.2±0.6	12.5±0.5	0.20±0.05	3.0±0.6	10.4±0.7	0.11±0.05
Mean daily level	10.2±1.2	12.1±0.5	0.20±0.07	4.1±0.8	10.0±0.8	0.12±0.07*
Amplitude, % of mesor	1.2±0.7 (11.7)	0.9±0.7 (7.4)	0.035±0.002 (17.5)	0.9±0.7 (21.9)	1.0±0.7 (10)	0.025±0.020 (20.8)
Acrophase, hours (95% confidence interval)	Absence of rhythm					

Note. Percentage of mesor is shown in brackets. *$p<0.05$ compared to the mean daily level.

tion of transferrin iron saturation (14.0±0.5%). However, the total iron-binding activity of blood plasma increased to 75.3±4.3 μmol/liter. These changes reflect exhaustion of the reserve and transport fractions of iron.

Diurnal changes in the intensity of LPO and nonenzymatic antioxidant activity suggest that the concentrations of MDA and α-tocopherol in blood plasma do not have a regular circadian rhythm under conditions of ID (Table 1). The mean daily concentration of α-tocopherol in blood plasma decreased in women with ID. MDA concentration in these subjects was higher than in healthy nursing women. An imbalance between the intensity of LPO and antioxidant activity is confirmed by a progressive increase in the MDA/α-tocopherol ratio in blood plasma. The MDA/α-tocopherol ratio in patients with LID and IDA and healthy nursing women was 26, 60.5, and 13, respectively. These data indicate that ID is accompanied by activation of LPO and exhaustion of the nonenzymatic antioxidant protection system. The imbalance between LPO and antioxidant activity is most pronounced in women with IDA (Table 1).

Disorders of iron metabolism and imbalance between LPO and antioxidant activity in nursing women with ID led to changes in the qualitative composition of breast milk. The mean daily concentration of iron in breast milk from women with LID was lower than in healthy nursing women (statistically insignificant). Iron concentration in breast milk significantly decreased only in women with IDA. These data indicate that the mechanisms for iron transport in breast milk are preserved in women with LID, but impaired during IDA. The mean daily concentration of α-tocopherol in breast milk from women with ID was lower than in healthy women. We revealed an increase in MDA concentration in women with ID (Table 1). The MDA/α-tocopherol ratio in breast milk from women with LID and IDA increased by 2 and 8 times, respectively. These changes reflect an imbalance between LPO and antioxidant protection. Excessive activation of LPO and exhaustion of the antioxidant system during IDA probably destabilize cell membranes and impair transport function of receptors on breast epithelial cells. These receptors are involved in accumulation and transport of iron from blood plasma to breast milk.

Our study showed that disorders of iron metabolism in nursing women are accompanied by the reduction of nonenzymatic antioxidant activity in breast milk, suppression of diurnal variations, and decrease in the mean daily concentration of iron in breast milk. The observed changes can lead to a decrease in bioavailability of this trace element from breast milk for children.

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