# **ESSENTIAL ELEMENTS IN MATURE HUMAN MILK**

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# Abstract

Human milk is recognised as the best source of essential elements for the infant in the first months of life. The objective of research was to determine the content of calcium, iron, magnesium, potassium and selenium in mature human milk. To evaluate possible relation with maternal (age, parity, breastfeeding pattern, dietary intake) and infant's (age, birth weight, sex) attributes. In the study participated 65 lactating women from Latvia (at least one month postpartum). Elements' content in human milk was analysed using ICP-MS (Agilent 7700x, Japan). Dietary data (72-hour food diary) were evaluated using the Fineli Food Composition Database (https://fineli.fi). Data analysis were carried out using IBM SPSS Statistics, version 22.0. Spearman's correlation, partial correlation and Mann-Whitney U test were used to evaluate how maternal & infant characteristics influence essential elements' content in human milk. Average content for calcium, magnesium and potassium in human milk was  $28.40\pm7.06$ ,  $3.85\pm0.64$  and  $59.60\pm8.29$  mg 100 mL<sup>-1</sup>, respectively. Iron content in 97% of samples (n=63) was below detection limit (<0.10 mg 100 mL<sup>-1</sup>). Selenium content in all samples was below detection limit (<0.002 mg 100 mL<sup>-1</sup>). Calcium content in human milk was affected by breastfeeding pattern – average content of  $30.51\pm7.31$  mg 100 mL<sup>-1</sup> for exclusive breastfeeding and  $25.41\pm5.43$  mg 100 mL<sup>-1</sup> for partial breastfeeding, p=0.003. Dietary intake of essential elements among participants was within recommendations. Our obtained values for calcium, magnesium and potassium in human milk. Were in accordance with other studies. Further evaluation should be done for the optimisation of the ICP-MS method for the detection of iron and selenium in human milk.

Keywords: human milk, essential elements

# Introduction

For the first six months of life, human milk is the most suitable source of essential elements (calcium, potassium, magnesium, iron, selenium) necessary for infant's growth and development (World Health Organization, 2019). These elements are transported from maternal blood to lactocytes and then secreted in milk. This mechanism is closely controlled by mammary epithelial cells to match infant's requirements (Montalbetti et al., 2014; Wysolmerski, 2010).

Calcium is needed for normal development of the skeletal system (bones, teeth) (Bae, Kratzsch, 2018). Calcium content in human milk varies around 20 to 30 mg 100 mL<sup>-1</sup> (EFSA, 2013). To maintain the necessary amount of calcium in milk, maternal bone resorption, intestinal absorption and renal retention of calcium increases. Yet, maternal diet has less impact on calcium level in milk (Bae, Kratzsch, 2018).

Magnesium supports normal muscle and nerve function and is a cofactor in several enzymatic reactions (de la Guardia, Garrigues, 2015; EFSA, 2013). Magnesium content in human milk broadly varies (from 15 to 64 mg 100 mL<sup>-1</sup>) (EFSA, 2013).

Potassium is the major cation in the human cells, therefore important in maintaining homeostasis. Its content in human milk is  $\sim$ 50 mg 100 mL<sup>-1</sup> (EFSA, 2013).

Iron is the oxygen carrier in the human body (de la Guardia, Garrigues, 2015; EFSA, 2013). The major source of iron during the first six months of life is infant's body stores accumulated during the prenatal period (Friel et al., 2018). Human milk contains a comparatively small amount of iron (0.04–0.10 mg 100 mL<sup>-1</sup>) but its bioavailability is high (20 to 50%) (EFSA, 2013; Hale, Hartmann, 2017). Both

sources of iron (body stores and human milk) ensure that infant's requirements for iron are satiated for the first six months of life (Friel at al., 2018; Hale, Hartmann, 2017; Montalbetti et al., 2014). After six months, it is recommended to ensure that an infant receives iron-containing complementary foods (Hale, Hartmann, 2017; World Health Organization, 2019). Maternal intake of iron does not affect iron content in human milk (Hale, Hartmann, 2017).

Glutathione peroxidase is a selenium-containing antioxidant enzyme that protects the body against oxidative stress (EFSA, 2013). Selenium is also needed for the synthesis of thyroid hormones and proper functioning of the immune system (Rayman, 2000). Selenium content in human milk is affected by maternal intake of selenium and it significantly varies among women in Europe - from 0.0003 to 0.0084 mg 100 mL<sup>-1</sup> with an average content of 0.003 mg 100 mL<sup>-1</sup> (EFSA, 2013; Hale, Hartmann, 2017). In Latvia's neighbouring country - Estonia, low selenium level in mature human milk samples has been observed (0.0008–0.0029 mg 100 mL<sup>-1</sup>) (Kantola et al., 1997). Zachara and Pilecki (2001) have pointed out that infants in Poland also could receive a lower intake of selenium than recommended. It is potentially due to low selenium content in food products and therefore in the maternal diet (Zachara Pilecki, 2001). Soil fertilisation with sodium selenite in Finland have resulted in an increased intake of selenium among lactating women and therefore in a rise of selenium level in serum and milk, respectively (Kantola et al., 1997). Soils in Latvia are low in bioavailable selenium, therefore food products also could contain a low amount of selenium (Duma et al., 2011).

European Food Safety Authority's (EFSA) opinion as well as recommended daily intakes set by the Ministry of Health of the Republic of Latvia on the dietary intake of essential elements for infants and toddlers are compiled in Table 1 (EFSA, 2013; Recommended total energy and nutrient intake among Latvian population, 2017).

Table 1

Dietary intake of essential elements for the infants and toddlers

Element	Adequate intake <sup>1</sup>	Recommended daily intake <sup>2</sup>
	mg per day	mg per day
	400 <sup>1</sup>	n.i. <sup>3</sup>
D (	(0 to>6 months old) 800 <sup>1</sup>	(0 to 6 months old) $1100^{2}$
Potassium	(6 to<12 months old) 800 <sup>1</sup>	(7 to 12 months old) 1600 <sup>2</sup>
	(12 to<36 months old)	(1 to 3 years old)
	200 <sup>1</sup>	n.i. <sup>3</sup>
01:	(0  to>6 months old) $400^{-1}$	(0 to 6 months old) $550^{2}$
Calcium	(6 to<12 months old) 600 <sup>1</sup>	(7 to 12 months old) 600 <sup>2</sup>
	(12 to<36 months old)	(1 to 3 years old)
	25 <sup>1</sup>	n.i. <sup>3</sup>
	(0  to  >6  months old) 80 <sup>1</sup>	(0 to 6 months old) $60^2$
Magnesium	(6 to <12 months old) $85^{-1}$	(7  to  12  months old) 100 <sup>2</sup>
	(12  to  < 36  months old)	(1 to 3 years old)
	0.3 1,4	n.i. <sup>3</sup>
Ŧ	(0 to>6 months old) $8^{1}$	(0  to  6  months) 8 <sup>2</sup>
Iron	(6 to<12 months old) $8^{1}$	(7  to  12  months) 8 <sup>2</sup>
	(12 to<36 months old)	(1 to 3 years)
	0.0125 1	n.i. <sup>3</sup>
Selenium	(0  to  >6  months old)	(0 to 6 months old)
	0.0150 1	0.0150 <sup>2</sup>
Selement	(6 to<12 months old) 0.0200 <sup>1</sup>	(7 to 12 months old) 0.0200 <sup>2</sup>
	(12 to<36 months old)	(1 to 3 years old)

<sup>1</sup> – EFSA, 2013

 $^2$  – Recommended total energy and nutrient intake among Latvian population, 2017

 $^{3}$  – no information

<sup>4</sup> - breastfed infants

Different methods have been used for the detection of elements in human milk like atomic absorption spectrometry (AAE), flame atomic emission spectrometry (FAES), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is the most suitable method to measure elements in human milk due to its ability to simultaneously measure several elements even if they are found in trace amounts (de la Guardia, Garrigues, 2015; Levi et al., 2018).

The majority of essential elements in human milk are independent of maternal intake, nutritional requirements for certain nutrients during lactation raises (Hale, Hartmann, 2017; Segura et al., 2016). Recommended intake of potassium, magnesium and iron among lactating women is the same as for non-lactating but guidelines developed by the Ministry of Health of the Republic of Latvia suggest that intake of calcium and selenium should be increased during lactation (see Table 2) (Recommended total energy and nutrient intake among Latvian population, 2017).

Table 2

Guidelines for daily essential elements' intake among women in Latvia<sup>1</sup>

Element	Non-lactating women	Lactating women
	mg per day	mg per day
Potassium	3100	3100
Calcium	800	900
Magnesium	280	280
Iron	15	15
Selenium	0.05	0.06

 $\overline{1}$  – Recommended total energy and nutrient intake among Latvian population, 2017

Previously essential elements' content hasn't been sufficiently analysed in Latvia. Therefore, the aim of this study was to determine the content of calcium, iron, magnesium, potassium and selenium in mature human milk and to evaluate possible relation with maternal (age, parity, breastfeeding pattern, dietary intake) and infant's (age, birth weight, sex) attributes.

#### **Materials and Methods**

This cross-sectional study was conducted from November 2016 to December 2017. Overall, 65 lactating women from Latvia (at least one month postpartum) took part in the study. Maternal (age, parity, breastfeeding pattern) and infant's (age, birth weight, sex) characteristics were compiled using a questionnaire and are summarized in Table 3.

 Table 3

 Characteristics of the participants (n=65)

		. ,
Characteristic	Average±SD <sup>1</sup>	Range
	Maternal	
Age, years	31±4	23-39
Parity	2±1	1–4
	Infants & toddlers	

Age, months	<u>6</u> ±4	2-21
Sex	31 girls, 34 boys	
Birth weight, kg	3.51±0.59	1.60-5.36
Breastfeeding pattern	n according to infan	ts & toddlers age
Exclusively	36 infants (1 to 6 months old)	
breastfed	2 infants (6 to 12 months old)	
Partially breastfed	4 infants (1 to 6 r 19 infants (6 to 1 4 toddlers (>12 n	nonths old) 2 months old) nonths old)

<sup>1</sup> – standard deviation

Participating women filled a 72-hour food diary and obtained a pooled human milk sample in the next 24 hours (~10 mL). Mothers were allowed to choose the most convenient method for milk expression – 18 participants used manual method (by hand),

38 participants used breast pump but 9 participants combined both methods.

Participants expressed few millimetres of milk after the breastfeeding session. Sampling capacity was not specified but the pooled sample had to include morning, mid-day and evening feedings' milk. Milk was collected into non-sterile propylene container (Plastiques Gosselin, France). Participants were instructed to keep samples refrigerated ( $+4\pm2$  °C) during sampling and afterwards place the container into a freezer (-18 °C). Then samples were transported to the laboratory (using a cooler with ice packs) and kept frozen until the analysis (-18 $\pm$ 3 °C).

Elements' (<sup>39</sup>K, <sup>43</sup>Ca, <sup>24</sup>Mg, <sup>56</sup>Fe, <sup>78</sup>Se) content in human milk was analysed using ICP-MS, according to manufacturer's (Agilent 7700x, Japan) instructions.

Dietary data (a 72-hour food diary) were evaluated using the Fineli Food Composition Database (https://fineli.fi) and exported to the MS Excel 2013. Unfortunately, we were unable to use the Latvian Food Composition Database to calculate essential elements' intake among participants. Taking into account that in the 1990s, soils in Finland were fertilised with sodium selenite (Kantola et al., 1997), food products from Finland should contain higher amounts of selenium than products from Latvia and our calculated results for selenium intake among participants from Fineli Food Composition Database could be overestimated.

Approximately one-third of the participants (n=20) noted the use of nutritional supplements containing at least one of the evaluated elements. Information about essential elements? in nutritional content from supplements was taken the Food and Veterinary Service's Register of Nutritional (https://registri.pvd.gov.lv/ub) Supplements and counted to the data calculated from the Fineli Food Composition Database.

Data analysis were carried out using IBM SPSS Statistics, version 22.0. Spearman's correlation, partial correlation and Mann-Whitney U test were used to evaluate how maternal & infant characteristics influence essential elements' content in human milk (p<0.05).

Prior to the study, approval from Riga Stradiņš Ethic Committee was received (No. 4/28.7.2016). All the women signed written consent.

# **Results and Discussion**

This is one of the first reports determining essential elements' content in human milk along with the dietary assessment of essential elements' intake among women in Latvia. Average content for calcium, magnesium and potassium in human milk was  $28.40\pm7.06$ ,  $3.85\pm0.64$  and  $59.60\pm8.29$  mg 100 mL<sup>-1</sup>, respectively. Iron content in 97% of samples (n=63) was below detection limit (<0.10 mg 100 mL<sup>-1</sup>). Selenium content in all samples was below detection limit (<0.002 mg 100 mL<sup>-1</sup>) (see Table 4).

	Table 4
Essential elements' content in mature huma	n milk
according to infant's & toddler's age (n=	65)

Element	Age	Average±SD	Detection limit <sup>1</sup>
	months	mg 100 mL <sup>-1</sup>	mg 100 mL <sup>-1</sup>
Potassium	0 to 6	60.83±7.54	2
Totassium	≥6	57.48±9.06	2
Calcium	0 to 6	30.91±7.45	0.20
Calcium	≥6	25.31±5.66	0.20
Magnacium	0 to 6	3.96±0.67	2
Magnesium	≥6	3.69±0.59	2
Iron	0 to 6	RDI 2	0.10
IIOII	≥6	DDL	0.10
Selenium	0 to 6	BDI <sup>2</sup>	0.002
Sciemum	≥6	BDL	0.002

 $^{1}$  – based on 20 × standard deviation of method blanks, 2.5 mL analytical portion and 50 mL analytical solution.  $^{2}$  – below the detection limit

Our obtained average results for calcium (28.40 mg 100 mL<sup>-1</sup>), potassium (59.60 mg 100 mL<sup>-1</sup>) and magnesium content (3.85 mg 100 mL<sup>-1</sup>) in human milk was within range reported from other studies (16–35 mg 100 mL<sup>-1</sup> for calcium, 33–71 mg 100 mL<sup>-1</sup> for potassium and 1.6–5.0 mg 100 mL<sup>-1</sup> for magnesium) (de la Guardia, Garrigues, 2015).

Calcium content in human milk significantly correlated with potassium (r=0.471, p=0.000) and magnesium content (r=0.307, p=0.013). Calcium content in human milk was affected by breastfeeding pattern – average content of  $30.51\pm7.31$  mg 100 mL<sup>-1</sup> for exclusive breastfeeding and  $25.41\pm5.43$  mg 100 mL<sup>-1</sup> for partial breastfeeding, p=0.003. Other characteristics (maternal age, parity, infant's age, birth weight, sex) did not influence essential elements' content in milk (p>0.05).

World Health Organization recommends that infants should be breastfed till six months of age. After that, complementary foods should be introduced into an infant's diet but breastfeeding should be continued till two years of age and beyond (World Health Organization, 2019). To evaluate if exclusively breastfed infants till six months of age in Latvia receive sufficient intake of essential elements, we selected exclusively breastfed infants till six months of age among our participants (n=36), calculated average values for essential elements' content in human milk and multiplied it with the average amount of daily consumed milk (~780 mL, according to EFSA, 2013). Calculations were compared to the intake of essential elements which are considered adequate for the majority of infants according to EFSA (EFSA, 2013) (see Table 1). Results are summarized in Table 5 and show that exclusively breastfed infants in Latvia receive sufficient amounts of potassium, calcium and magnesium.

Partially breastfed infants from 7 to 12 months on average consume ~520 mL of human milk per day (World Health Organization, 1998). After six months of age, human milk is still an important source of essential elements, providing ~32% of magnesium, ~25% calcium and ~28% of the recommended daily intake of potassium (Table 5).

Table	5
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Calculated consumed amount of essential elements among participating infants (n=55)

Element	Intake via human milk	Recommendation
	mg per day	mg per day
Exclusively breastfed infants till 6 months of age (n=36)		
Potassium	474	400 1
Calcium	241	$200^{-1}$
Magnesium	31	25 <sup>1</sup>
Partially breas	stfed infants from 7 t	to 12 months of age
	(n=19)	
Potassium	307	1100 <sup>2</sup>
Calcium	137	550 <sup>2</sup>
Magnesium	19	60 <sup>2</sup>

1 - EFSA, 2013

 $^{2}$  – Recommended total energy and nutrient intake among Latvian population, 2017

While advancement in technologies allows us to determine trace amounts of elements in human milk, researchers are still faced with some difficulties. Currently, there is no suitable reference material to check the accuracy for obtained results in human milk. An acid digestion preparation was used prior to the ICP-MS analysis in our study. Levi et al. (2018) have observed that the concentration of selenium in the human milk samples were higher (~15%) with the alkali dilution method in contrast to the acid digestion but a higher iron concentration (~28%) in human milk were obtained using acid digestion method, respectively. Therefore, the selection of sample preparation method for human milk should be chosen according to the selected elements (Levi et al., 2018). Our obtained detection limits for iron and selenium were significantly higher compared to Björklund et al. (2012), who reported 100  $\mu$ g vs 0.16  $\mu$ g 100 mL<sup>-1</sup> for iron, 2  $\mu$ g vs  $0.06 \ \mu g \ 100 \ mL^{-1}$  for selenium. Although it should be noted that their limit of detection was based only on five blank samples while ours - on 20 blank samples.

Detection of selenium (<sup>78</sup>Se) and iron (<sup>56</sup>Fe) isotopes using ICP-MS could also be influenced by spectrometric interferences with other polyatomic ions, mostly argon ions (de la Guardia, Garrigues, 2015). Argon was used as a carrier gas for ICP-MS analysis in our study.

Further evaluation should be done for the optimisation of the ICP-MS method for the human milk analysis to decrease detection limits for iron and selenium isotopes. During lactation, mothers should consume a wellbalanced diet to restore nutrients lost due to milk synthesis (Hale, Hartmann, 2017). We asked our participants to complete a 72-hour food diary. Average daily intake of evaluated essential elements among participants was within recommended ranges (Table 6). Dietary potassium, calcium and magnesium intake did not influence the content of these elements in milk, respectively.

Table 6
Essential elements' intake among participating
women (n=65)

Element	Average intake±SD	<b>Recommended</b> daily intake <sup>1</sup>	
	mg per day	mg per day	
Potassium	3862.42±2001.55	3100	
Calcium	1002.25±570.13	900	
Magnesium	398.90±187.13	280	
Iron	16.92±15.16	15	
Selenium	$0.09 \pm 0.05$	0.06	

1 – Recommended total energy and nutrient intake among Latvian population, 2017

Correlation coefficients between dietary intake and content of elements in human milk were r=-0.041 (p=0.747) for potassium, r=-0.012 (p=0.924) for calcium, r=0.117 (p=0.355) for magnesium. This is consistent with the data from other studies (Bravi et al., 2016; Hale, Hartmann, 2017). Dietary intake of iron and selenium among participants was also within recommended ranges but obtained values of these elements in milk were below detection limit. Therefore, we could not calculate correlations between maternal intake of iron and selenium and content of these elements in milk, respectively.

# Conclusions

Exclusively breastfed infants till six months of age receive sufficient amount of potassium, calcium and magnesium. After the first half of year, human milk is still an important source of essential elements for the infant.

Dietary intake of evaluated essential elements among participating women was within recommendations developed by the Ministry of Health of the Republic of Latvia.

The Ministry of Health of the Republic of Latvia should develop recommendations regarding essential elements' intake for 0 to 6 months old infants.

Further evaluation should be done for the optimisation of the ICP-MS method for the detection of iron and selenium in human milk.

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