

# Breast Milk Micronutrients in Lactating Mothers from Ribeirão Preto (SP), Brazil

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## Abstract

Breast milk is the ideal food for infants and should be consumed as the exclusive food for the first six months and then be complemented up to two years of age. The aim of this study is to determine breast milk's content of macro and micronutrient in lactating mothers from the Ribeirão Preto (SP) region, in Brazil. A cross-sectional study was conducted with breast milk samples of 70 mothers from the city of Ribeirão Preto (SP). The samples were analyzed in order to determine iron, copper, zinc, calcium and magnesium concentrations. They were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The study was approved by the Research Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, and all subjects gave written informed consent to participate. The current study revealed that all 70 lactating mothers were healthy and delivered babies more than 38 weeks gestation; 71.4% of them were younger than 30 years and 47.1% were primiparae. The iron, copper, zinc, calcium and magnesium concentrations detected in milk were  $2.7 \pm 0.4$ ,  $0.4 \pm 0.02$ ,  $3.6 \pm 0.2$ ,  $142 \pm 21.6$  and  $39.8 \pm 4.2$  mg/L, respectively. Therefore, it can be concluded that in the present sample, except for calcium, all other mineral concentrations were adequate for the infant's requirements according to the Dietary Reference Intake (DRI).

## Keywords

Human Milk, Breastfeeding, Micronutrients, Micronutrients, ICP-MS

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## 1. Introduction

Breast milk is the ideal food for infants and should be consumed as the exclusive food for the first six months

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and then be complemented up to two years of age. This is a living food of dynamic composition which contains all nutrients necessary for growth and development of the lactating baby, in addition to offering protective elements against infections, allergies and chronic-degenerative diseases [1].

The composition of maternal milk is frequently modified according to the time of day, duration of feeding and time of lactation, as well as the parity and nutritional status of the mother, being also influenced by genetic and environmental conditions [1] [2]. Regarding the volume produced, the estimate is that intake will increase, on average, from 600 ml/day during the first four weeks of life to 820 ml/day in the fourth month of life [3] [4].

Minerals are very important elements for the organism since they are related to numerous enzymatic systems, acting on immunological function and gene expression, in addition to playing an obvious role in growth and development. During the first months of life, breast milk is the only source that satisfies the requirements of the nursing baby. Among the main minerals of interest for human health are iron, calcium, zinc, magnesium, and copper. Deficiency of these minerals can cause many problems for the children, specially retarded physical growth and cognitive performance, poor bone development and changes in the transmission of nerve impulses, with impaired immunological function and changes in enzymatic systems, myelination and hemoglobin [5]-[7].

The mammary gland is a highly differentiated structure functioning by means of endocrine and neurohormonal controls, which prepares itself during pregnancy to provide a product of excellent quality for the infant. Some mechanisms regulating the concentrations of minerals are found in the gland, acting both on the uptake of mineral from serum and on the synthesis and secretion of milk by the cells, leading to wide variations in the composition and volume of milk [8] [9].

In view of the importance of maternal milk for children, exclusive breastfeeding should be encouraged during the first six months of life. It is also very important and necessary to determine the mineral content of mothers' milk in order to provide a supply of all the elements necessary for the growth and development of nursing infants [10].

In this context, the objective of the present study was to determine the iron, zinc, copper, magnesium and calcium content in the breast milk of mothers whose babies are seen at a primary care service for mothers and infants in the city of Ribeirão Preto (SP).

## 2. Methodology

a) Experimental design: a cross-sectional, observational and descriptive study was conducted from April 1 to September 30, 2011 on 70 healthy women (minimum age = 16 years, maximum age = 39 years) who were exclusively breastfeeding their babies, younger than 6 months. All infants were enrolled in the Puericulture Program of a primary health care unit for mothers and infants in the city of Ribeirão Preto, State of São Paulo, Brazil. Each participant offered a 5 ml sample of milk collected manually from a breast previously cleaned with cotton wool and distilled water. The milk sample was transferred to a Falcon<sup>®</sup> BD polyethylene tube and stored at -20°C, being later thawed at room temperature for triplicate analysis. All analyzes were performed after completion of all 70 samples in the laboratories of the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP). The study was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, USP. The mothers were informed about the purpose of the study and written consent was obtained from all participants.

b) Macro and micronutrients determination by ICP-MS

Mineral concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, Perkin Elmer Sciex, Shelton, IL, USA) with the samples being submitted to microwave-assisted acid digestion in a closed system. The analyses were carried out using an ICP spectrometer equipped with a reaction cell (DRC-ICP-MS, ELAN DRCII, PerkinElmer, SCIEX, Norwalk, CT, USA) operating with ultrapure argon (99.999%, Praxair, Brazil). The sample introduction system consisted of a quartz cyclonic spray chamber and a Meinhard<sup>®</sup> nebulizer connected by Tygon<sup>®</sup> tubes to the peristaltic pump of the ICP-MS (adjusted for 20 rpm). The ICP-MS operated with a sampling cone and Pt skimmer from PerkinElmer.

The milk samples (100 - 250 mg) were digested with 4 ml 14 M nitric acid + 2 ml 30% hydrogen peroxide. The reference material NIST 8435—Whole Milk Powder and blood reference materials of the Canadian National Institute of Public Health and of the New York Health Department were used to determine the accuracy and precision of the method.

c) Questionnaire: the mothers also answered a questionnaire concerning sociodemographic aspects such as age (<20, 20 to 30, and >30 years), educational level, number of children, current health status, use of medications, vitamins or food supplements and habits such as smoking and the consumption of alcoholic drinks.

d) Reagents and solutions: high purity deionized water (resistivity:  $18.2 \text{ M}\Omega\text{cm}^{-1}$ ) for the preparation of samples and dilutions was obtained using a Milli-Q water purifying system (Millipore RiOs-DI TM, Bedford, MA, USA). All reagents used were analytical grade except  $\text{HNO}_3$ , which was sub-distilled with a quartz distiller (Kürner Analysentechnik) before use. The solutions were prepared in a class 1000 clean room under a laminar flow hood. Rhodium (1000 mg/L) and multielement solutions (10 mg/L) were purchased from PerkinElmer (Shelton, CT, USA). Triton® X-100 was purchased from Sigma-Aldrich (St. Louis, MO, USA). All materials were first decontaminated in 15% (v/v)  $\text{HNO}_3$  for 24 h, washed five times with Milli-Q water, and dried under a laminar flow hood before use.

e) Statistical analyzes: descriptive analysis to check the distribution of the data, estimates of frequency, percentages and measures of central tendency and standard deviation was performed. The statistical package used was SPSS 10.0.

### 3. Results and Discussion

Breast milk samples from 70 mothers of babies born at term were analyzed. The mothers reported to be healthy and not to take medications, vitamins or nutritional supplements; 71.4% of them were younger than 30 years, 47.1% were primiparous, 74.3% were nonsmokers, and all denied consumption of alcoholic drinks, as presented in **Table 1**. Regarding the age distribution of the nursing infants, there was a predominance of less than 1 month of life (31.4%), while 20% were 1 to 2 months old and 17.1% were 3 to 4 months old.

Mean, median, maximum and minimum concentrations (mg/L) and 95% confidence intervals of macro and micronutrients of the lactating women, as well as the minimum daily requirements of these minerals according to the Dietary Reference Intake (DRI) of the Institute of Medicine [11] are shown in **Table 2**.

During the first semester of life, infants in good condition have a high weight gain and growth rate, doubling their birth weight by about the 5th month of life. To this end, they must obtain from their food adequate quantities of all nutrients under the proper conditions of utilization. Among the references most extensively used for this evaluation are the DRI tables elaborated by the Institute of Medicine of the National Academy of Sciences of the United States and used all over the world for several years [11]. In case of exclusively breastfed babies,

**Table 1.** Distribution of the sociodemographic variables of the 70 lactating mothers. Ribeirão Preto, 2011.

Maternal variables	Number of mothers	Frequency (%)
Age (years)		
Up to 20	14	20.0
20 to 30	36	51.4
>30	20	28.6
Number of children		
1	33	47.1
2	14	20.0
3	14	20.0
$\geq 4$	9	12.9
Smoking habit		
No	52	74.3
Yes	18	25.7
Alcoholic drink consumption		
No	70	100.0

**Table 2.** Mean, median, maximum and minimum concentrations (mg/L), 95% confidence interval and daily requirements of calcium, copper, iron, magnesium and zinc. Ribeirão Preto, 2011.

	minimum	maximum	mean $\pm$ SD	median	95% CI	Daily requirements (mg)*
Calcium	90.85	188.3	142.3 $\pm$ 21.6	144.2	138.1 - 148.2	210
Copper	0.15	1.37	0.4 $\pm$ 0.02	0.42	0.396 - 0.404	0.20
Iron	1.97	3.69	2.7 $\pm$ 0.4	2.65	2.61 - 2.79	0.27
Magnesium	20.40	52.1	39.8 $\pm$ 4.2	34.96	38.8 - 40.7	30.0
Zinc	2.19	9.88	3.6 $\pm$ 0.2	2.50	3.55 - 3.64	2.0

SD = standard deviation; CI = confidence interval; \*Dietary Reference Intake.

the quantities of the elements listed in the tables must be contained in the daily volume of maternal milk ingested by the infants, which is very difficult to quantify. In general, a lactating woman in good health can produce 640 to 820 mL of milk per day, an amount that fully satisfies most of the babies born at term during this period of life [12].

Regarding the mean mineral concentrations obtained in the present study, it can be seen that, except for calcium, the remaining elements present in breast milk could satisfy the daily requirements of the infants during the first semester of life. However, if we consider the milk volume produced daily by the mother to be 800 mL, these ingested quantities would be insufficient for 18.5% (13/70) of the infants regarding copper, for 44.2% (31/70) regarding zinc and for 64.2% (45/70) regarding magnesium. Iron concentrations would be sufficient in all cases, while calcium concentrations would be below the daily requirements of the babies.

It is known that, in general, lactating women secrete 300 to 400 mg calcium per day [13]. In the present study, the mean calcium concentration detected in the milk samples was 142.3  $\pm$  21.6 mg/L, a value below the daily requirements of the infant. A similar result was obtained in Gambia in a study in which milk samples from 30 lactating women were analyzed at 3 months of lactation [14]. On the other hand, analyses of breast milk samples obtained during the first week of lactation in China [15] and in Ethiopia [16] revealed higher calcium concentrations than those detected here, *i.e.*, 300 mg/L and 758 mg/L, respectively. Studies conducted by Itriago *et al.* in Venezuela [17] and by Deng *et al.* in China [18] on mature milk samples detected lower calcium concentrations (244 mg/L and 280 mg/L, respectively). These results demonstrate that the presence of this mineral in milk undergoes variations, because calcium content in breast milk does not correspond to the concentration in maternal serum. Thus, calcium homeostasis in the maternal organism during lactation is a complex process destined to protect the nursing infant and regulated by the mammary gland, involving deposits of bone tissue [19].

A characteristic of maternal milk is the high bioavailability of iron and zinc even though the concentrations of these minerals are known to decrease during the first months of lactation [1] [20]. The iron concentration detected in the present study was 2.7  $\pm$  0.4 mg/L, above the daily recommended requirements for infants. Similar findings were obtained in studies conducted in the United States on 48 milk samples collected during the third month of lactation [21], and in studies conducted in Turkey during the second week of breastfeeding [22] and in Vietnam, in which iron concentrations exceeded the daily requirements of the infants [23].

The mean zinc concentration detected in the present study was 3.6  $\pm$  0.2 mg/L, also above the requirements of the infant. Likewise as observed for iron, zinc concentrations exceeded the requirements also among American and Turkish lactating women. Studies conducted in Libya and in Sweden analyzed mature milk samples and detected concentrations of 4.95  $\pm$  1.3 mg/L and 3.4  $\pm$  0.9 mg/L, respectively [10] [24]. In contrast, Fransson *et al.* [25], Mahdavi *et al.* [26], Orun *et al.* [27] and Winiarska-Mieczan [28] detected deficient zinc concentrations among Swedish, Iranian, Turkish and Polish lactating women, respectively. Adequate zinc and copper concentrations were detected in a Brazilian study in which 145 mature milk samples from lactating women aged 16 to 41 years were analyzed [29].

The mean copper concentration detected in the present study was 0.4  $\pm$  0.02 mg/L, practically double the daily requirements of the infants during this phase of life. Similar findings were obtained in a study conducted in China on 120 lactating primiparous during the second week of breastfeeding [15], and in Portugal [30], Iran [26] and Sweden [24] in milk samples collected after the third week of lactation. On the other hand, another study also conducted in China on 60 women aged 20 to 35 years during the second month of lactation detected low

copper concentrations, the same being observed among 95 Greek lactating women during the second week of breastfeeding [18] [31].

The magnesium concentration in human milk seems to be constant over the period of lactation [32]. While the mean magnesium concentration in the milk of mothers of Ribeirão Preto was  $39.8 \pm 4.2$  mg/L, a value above the daily requirements of the baby, Itriago *et al.* [17] in Venezuela and Bjorklund *et al.* [24] in Sweden and Morgano *et al.* [33] in Brazil detected lower levels of this metal in mature milk samples, underscoring the wide variability of the data obtained in various studies on the constituents of breast milk.

On the basis of the present study, despite the limitations due to the small number of samples and of lactating women residing in a determined geographical area, we may conclude that breast milk contains adequate quantities of iron, zinc, magnesium and copper and insufficient quantities of calcium. The wide variability of breast milk composition, especially in mineral content, suggests which are affected by genetic, socioeconomic and environmental influences, suggesting a relationship between the concentrations of these elements in milk and the nutritional status of the lactating woman [34]. Studies of this type can contribute to an increased practice of breastfeeding by identifying maternal nutritional deficits and permitting the provision of a complete food to the infant during its first months of life.

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