CLINICAL STUDIES

Trace elements in human milk: Correlation with blood levels, inter-element correlations and changes in concentration during the first month of lactation

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Abstract

Using inductively coupled plasma mass spectrometry (ICP-MS) based analytical procedures, the concentration of several trace elements (Mn, As, Pb, Co, Ni, Cu, Zn and Se) was determined in human milk samples collected from a group of healthy lactating Portuguese women (\(n = 44\)), both on the 2nd day postpartum (i.e., colostrum; \(n = 34\)) and at 1 month postpartum (i.e., mature milk; \(n = 19\)). Blood samples (\(n = 44\)), collected on the 2nd day after parturition, were also analyzed for the same trace elements.

No major correlations were observed between the levels of the analyzed trace elements in blood and colostrum samples.

All the studied elements, except for Co, Pb and Ni, showed a significant trend for a decrease in concentration in milk during the first month of lactation. This trend was more pronounced for Zn and Se, whose levels decreased to approximately 23\% and 44\% of their initial mean concentration, respectively. With the exception of Co (\(r = 0.607\)) and Zn (\(r = 0.487\)), no significant correlations were observed when comparing the levels of each trace element between samples of colostrum and mature milk.

Several inter-element correlations were found within each type of milk sample. The most significant were: (i) Se vs Cu (\(r = 0.828\)) and Se vs Co (\(r = 0.605\)) in colostrum samples and (ii) Ni vs Pb (\(r = 0.756\)), Ni vs Mn (\(r = 0.743\)) and Se vs Co (\(r = 0.714\)) in mature milk samples. An inverse correlation between Zn and Se was also found in both types of milk sample; however, it only reached statistical significance for mature milk (\(r = -0.624\)).

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Keywords: Human milk; Trace elements; Temporal changes; Correlation with blood levels; Inter-element correlation

Introduction

Few other aspects of human feeding have the same biological relevance as feeding during the early stages of development, namely during pregnancy and the first postnatal months [1], and it is widely accepted that
human milk provides all the nutrients, including essential trace elements, that are required by the normal term newborn infant [2]. Conversely, human milk can also be a transfer medium for undesirable (toxic) elements from the mother to the infant. Despite of being a complex process where nutritional factors of the lactating woman interact with structural, hormonal and behavioral influences [3], for several oligoelements (e.g., Fe, Zn, Cu) it seems that the infant is well protected by maternal homeostatic processes [2,4,5]. Therefore, a mother’s excessive dietary intake or moderate deficiencies do not significantly alter the levels of these micronutrients in the mother’s milk and, consequently, in the infant’s supply [2]. For some elements, however, there is no such knowledge, which justifies the current interest in better understanding these homeostatic processes and whether they can compensate for adverse conditions suffered by the mother (e.g., inadequate uptake of trace elements), thus helping to identify infants at risk of deficiency [4].

In this work, the concentrations of the trace elements Mn, Pb, Co, Ni, Cu, Zn and Se were measured in colostrum (2nd day postpartum) and mature milk (1 month postpartum) samples, collected from a group of lactating Portuguese women. The same trace elements were also analyzed in blood samples collected on the 2nd day postpartum. The main objectives of this work were to study the relationship between the levels of the abovementioned trace elements in maternal milk and their corresponding levels in blood, to evaluate the changes in their concentrations in milk during the first month of lactation, and to detect potential inter-element correlation within each type of sample.

Materials and methods

Subjects

This study was conducted with pregnant women (n = 44) who attended Maternidade Júlio Dinis, Porto, Portugal, during November 2003. Women were consecutively invited to participate in the study and no other inclusion/exclusion criteria were defined except the individual consent to participate and the ability to supply at least 2 mL of milk on the 2nd day postpartum. The study was approved by the Ethical Committee of the Institution, and all the participating women gave their written informed consent. The ages of the women ranged between 18 and 40 years (mean = 28.3 years). Twenty-one (47.7%) were primiparous, 17 (38.6%) were biparous and 6 (13.6%) were triparous. Most of the women (86.4%; n = 38) had full-term deliveries. The remaining six women gave birth after 36, 35, 33, 32, 28 and 27 weeks of gestation, respectively.

Sample collection

All samples were collected into special metal-free (royal blue top) Vacutainer® tubes (BD, Franklin Lakes, NJ, USA). Breast milk samples (2–10 mL) were collected at 2 days (colostrum) and at approximately 1 month (29.5 ± 7.1 days) postpartum (mature milk). Using plastic disposable gloves, breast milk was expressed by hand directly into the tubes. Venous blood (approximately 5 mL) was collected at 2 days postpartum. Because most elements of the chosen panel (Co, Ni, Cu, Zn and Se) are usually determined in blood serum, but others are usually determined in whole blood (Mn, As and Pb), both kinds of samples were obtained for each woman. Whole blood samples were obtained through direct collection into Vacutainer® tubes containing disodium EDTA as anticoagulant. To obtain serum samples, blood was collected into Vacutainer® tubes with no anticoagulant. After coagulation and centrifugation, the serum was separated and stored into the same type of tubes. All the samples were kept in the refrigerator (4 °C) until their analysis by inductively coupled plasma mass spectrometry (ICP-MS).

Sample analysis

Trace elements determination was performed using a VG Elemental (Winsford, UK), PlasmaQuad 3 ICP-MS, equipped with a Meinhard® type A pneumatic concentric nebulizer, a quartz water-cooled impact-bead spray chamber, a standard quartz tube torch and nickel sample and skimmer cones. Both the spray chamber and sampling interface were cooled to 10 °C by circulating water. Argon of 99.9999% purity (Alphagaz 2™, supplied by Air Liquide, Maia, Portugal) was used as the plasma source. For sample introduction, a Gilson (Villiers le Bel, France) model M312 peristaltic pump was used. The main operating conditions for the ICP-MS determinations are indicated in Table 1. The elemental isotopes (m/z ratios) 55Mn, 75As, 208Pb, 59Co, 60Ni, 65Cu, 66Zn and 82Se (as analytical masses) and 48Sc, 89Y, 115In, 159Tb and 209Bi (as internal standards) were monitored. 85Kr and 77ArCl were also monitored to allow correction of instrumental isobaric interferences. The instrument was tuned daily for maximum signal sensitivity and stability using 115In as the target isotope. The detection limits were calculated as the concentration corresponding to 3 standard deviations of 10 repeated determinations of the blank. Expressed as µg/L in real samples, the typical detection limits achieved in routine operation during the samples analysis were: 0.02 (Mn); 0.01 (As); 0.03 (Pb); 0.01 (Co); 0.12 (Ni); 0.11 (Cu); 0.21 (Zn); 0.2 (Se).

Except for concentrated nitric acid, which was of Suprapur® grade and obtained from Merck...
Whole blood and serum analyses were performed according to the analytical procedures described in two “Application Notes” from the ICP-MS equipment manufacturer [6,7]. Whole blood was previously diluted (1+9) with a solution containing 1% (v/v) ammonium hydroxide, 0.01% (m/v) disodium EDTA and 0.1% (v/v) Triton X-100 (diluting solution for whole blood). Serum samples were previously diluted (1+9) with a solution containing 2% HNO₃ and 0.01% (v/v) Triton X-100 (diluting solution for serum). Milk samples were processed in the same way as serum samples, i.e., they were also previously diluted (1+9) with a 2% HNO₃ and 0.01% (v/v) Triton X-100 solution.

Calibrating solutions were prepared by dilution of a commercial multi-element aqueous solution (AccuTrace™ Reference Standard, ICP-MS 200.8-CAL1R-1; AccuStandard Inc., New Haven, CT, USA) using the abovementioned diluting solutions. Internal standards (Sc, Y, In, Tb and Bi) were added to the diluting solutions by dilution of a commercial aqueous solution (AccuTrace™ Reference Standard, ICP-MS 200.8-IS-1; AccuStandard Inc.), in order to attain a final concentration of 50 μg/L. Because the levels of Zn and Cu were much higher than those of the other analyzed elements, their concentration in the calibrating solutions (prepared as described above) was increased through the addition of a commercial standard stock solution of each element (Spectrosol®, BDH, Dorset, UK).

Diluting solutions were used for blank subtraction. A 2% HNO₃ and 0.05% (v/v) Triton X-100 solution was used in the washing steps. Quality control samples, Seronorm™ Trace Elements Serum, Levels 1 and 2; Seronorm™ Trace Elements Whole Blood, Level 2 (both from Sero As, Billingstad, Norway); and BCR-150 (spiked skimmed milk powder), were used.

All the samples (after adequate dilution) were analyzed twice (two determinations in the same analytical run). For “non-concordant” results (RSD% >5%) another two determinations were performed. Samples with “abnormal” results (i.e., outside the typical range) were re-analyzed after a new dilution, in order to exclude the possibility of contamination during this step of the analytical procedure. The precision achieved was typically good. For example, for Mn in colostrum (Table 3, first column), the RSD% values for the two determinations performed on each sample ranged from 0.01% (minimum) to 6.7% (maximum), with a mean value of 1.3% and a standard deviation of 1.4%. Similar performance was obtained for the other elements and for the other types of sample (whole blood, serum, mature milk). As acceptance criteria for the analytical results it was imposed that the results for the quality control samples should be within the 95% confidence interval indicated by the supplier. Special care was taken to avoid contamination during sample preparation. Adequately acid-washed plastic material was used throughout.

### Statistical evaluation

Statistical analysis of the data was performed using both Microsoft® Office Excel® (Microsoft Corporation, Redmond, WA, USA) and Minitab® 10Xtra (Minitab Inc., State College, PA, USA) statistical software. The chosen confidence level was 95%. Univariate statistical methods were used to calculate descriptive statistical parameters (mean, median, standard deviation, minimum, maximum, 1st quartile and 3rd quartile) for each element within each kind of sample: blood, colostrum and mature milk. The Kolmogorov–Smirnov normality test was applied to evaluate the distribution of each data set. The F-test (for results with a normal distribution) or the Levene’s test (for results with a non-normal distribution) was used to compare the variance of the samples. The two-sample t-test (for results with a normal distribution or results with a non-normal distribution and unequal variances) or the Mann–Whitney test (for results with a non-normal distribution and equal variances) were performed for comparison of means.

### Results and discussion

The results of the analysis of blood, colostrum and mature milk samples are summarized in Tables 2–4, respectively. The main aspects from each table are highlighted below.
**Trace elements in blood**

Data from Table 2 show that the concentration of all the analyzed essential elements in whole blood fell within the “reference” ranges for this population. Additionally, the values obtained for the toxic elements, Pb and As, were very low, which is also a good indication of maternal status. Copper levels in serum, which ranged from 2087 to 2302 μg/L, were “normal” for pregnant women. Taylor [8] indicates a reference range of 1.7–2.5 mg/L for the time period from 16 weeks of gestation until term, and Krachler et al. [9] found

**Table 2. Results (μg/L) of blood samples analysis** (collection at the 2nd day post-partum; n = 44)

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Element (μg/L)</th>
<th>Mn</th>
<th>As</th>
<th>Pb</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td>12.1</td>
<td>3.1</td>
<td>20.1</td>
<td>0.34</td>
<td>4.0</td>
<td>2195</td>
<td>708</td>
<td>59.5</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>11.4</td>
<td>2.9</td>
<td>19.1</td>
<td>0.33</td>
<td>3.1</td>
<td>2208</td>
<td>706</td>
<td>62.2</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>3.4</td>
<td>1.4</td>
<td>7.0</td>
<td>0.10</td>
<td>5.2</td>
<td>354</td>
<td>134</td>
<td>16.6</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td>11.0–13.1</td>
<td>2.7–3.6</td>
<td>17.9–22.2</td>
<td>0.31–0.037</td>
<td>2.4–5.6</td>
<td>2087–2302</td>
<td>667–749</td>
<td>54.5–64.6</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>6.5</td>
<td>1.3</td>
<td>7.9</td>
<td>0.23</td>
<td>0.8</td>
<td>1484</td>
<td>413</td>
<td>19.6</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>24.5</td>
<td>8.9</td>
<td>38.6</td>
<td>0.66</td>
<td>35.5</td>
<td>2952</td>
<td>1038</td>
<td>93.7</td>
</tr>
<tr>
<td>1st quartile</td>
<td></td>
<td>9.6</td>
<td>2.2</td>
<td>14.2</td>
<td>0.27</td>
<td>2.6</td>
<td>1942</td>
<td>612</td>
<td>46.7</td>
</tr>
<tr>
<td>3rd quartile</td>
<td></td>
<td>14.0</td>
<td>3.9</td>
<td>24.5</td>
<td>0.38</td>
<td>3.6</td>
<td>2432</td>
<td>791</td>
<td>70.6</td>
</tr>
<tr>
<td>P(normality) (N)</td>
<td></td>
<td>&gt;0.15</td>
<td>&lt;0.01</td>
<td>&gt;0.15</td>
<td>0.094</td>
<td>&lt;0.01</td>
<td>&gt;0.15</td>
<td>&gt;0.15</td>
<td>&gt;0.15</td>
</tr>
<tr>
<td>Ref. values</td>
<td></td>
<td>4.2–16.5</td>
<td>0.1–0.4</td>
<td>0.6–7.5</td>
<td>700–1400</td>
<td>750–2900</td>
<td>23–190</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aMn, As and Pb were measured in whole blood samples; Co, Ni, Cu, Zn and Se were measured in blood serum samples.
From Taylor [8].
dReference values for pregnant women (16 weeks-term).

**Table 3. Results (μg/L) of analysis of colostrum samples (collection at the 2nd day postpartum; n = 34) and some published values**

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Element (μg/L)</th>
<th>Mn</th>
<th>As</th>
<th>Pb</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td>7.7</td>
<td>7.8</td>
<td>1.55</td>
<td>0.69</td>
<td>7.6</td>
<td>760</td>
<td>12137</td>
<td>72.2</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>6.6</td>
<td>7.6</td>
<td>1.17</td>
<td>0.55</td>
<td>4.6</td>
<td>632</td>
<td>11150</td>
<td>58.6</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>5.0</td>
<td>2.2</td>
<td>1.38</td>
<td>0.36</td>
<td>7.9</td>
<td>484</td>
<td>4714</td>
<td>36.4</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td>6.0–9.5</td>
<td>7.0–8.6</td>
<td>1.07–2.04</td>
<td>0.57–0.82</td>
<td>4.8–10.4</td>
<td>591–929</td>
<td>10,491–13,782</td>
<td>59.5–85.0</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>2.2</td>
<td>3.6</td>
<td>0.06</td>
<td>0.30</td>
<td>1.0</td>
<td>186</td>
<td>1869</td>
<td>34.2</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>27.5</td>
<td>14.0</td>
<td>5.43</td>
<td>1.72</td>
<td>35.8</td>
<td>2628</td>
<td>22,050</td>
<td>187.6</td>
</tr>
<tr>
<td>Q1</td>
<td></td>
<td>4.2</td>
<td>6.2</td>
<td>0.55</td>
<td>0.46</td>
<td>2.7</td>
<td>483</td>
<td>9705</td>
<td>45.3</td>
</tr>
<tr>
<td>Q3</td>
<td></td>
<td>9.7</td>
<td>8.8</td>
<td>1.96</td>
<td>0.89</td>
<td>10.2</td>
<td>956</td>
<td>15,233</td>
<td>90.6</td>
</tr>
<tr>
<td>P(normality) (N)</td>
<td></td>
<td>0.041</td>
<td>0.081</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&gt;0.15</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

aPresented as range or mean±S.D. Adopted from: aWasowicz et al. [14] (collection at the 4th day postpartum); bArnaud and Favier [18] (collection at the 2nd day postpartum); cRossipal and Krachler [16] (collection at 1–3 days postpartum); dHannan et al. [30] (collection at day zero); eLeotsinidis et al. [19] (collection at the 3rd day postpartum); fRoekens et al. [20]; gGundacker et al. [31] (collection between 2 and 14 days postpartum); hTuran et al. [32] (collection at 2 days postpartum).
maternal serum levels to be twice the concentration found in non-pregnant healthy adult women, which is commonly accepted to be in the 0.7–1.3 mg/L range [8]. Zinc values fell into the lower limit of the reference range for the overall population. However, it has been reported that pregnant women have serum Zn levels lower than non-pregnant healthy women [8,10,11]. According to Tamura et al. [12] there is a decline in plasma Zn concentration during pregnancy that begins in the first trimester and continues until about 22 weeks of gestation. These authors reported a decline from approximately 785±140 µg/L, at less than 12 weeks of gestation, to approximately 634±144 µg/L after 20 weeks. The values obtained for Zn in serum in this study (708.3±134.4 µg/L) can be regarded as comparable to those previously reported.

In contrast, manganese levels fell into the upper limit of the reference range for the overall population, but this has also been previously described. In fact, it seems that a change in manganese status occurs during pregnancy. Tholin et al. [13] found a significant increase in Mn levels from a median value of 8.5 µg/L (range: 4.3–19.8 µg/L) in the 1st trimester to 10.5 µg/L (range: 5.4–22.4 µg/L) in the 2nd trimester and to 12.6 µg/L (7.3–26.4 µg/L) in the 3rd trimester of pregnancy. The results of the present study (median: 11.4 µg/L; range: 6.5–24.5 µg/L) are very similar to those found by Tholin et al. in the 3rd trimester.

The results of selenium determination in serum were within the typical reference range for this element. The values found in the present study were significantly higher than those published by Wasowicz et al. [14] in lactating women. These authors reported a Se concentration in plasma of approximately 34.9±11.8 µg/L during the first 4 days of lactation (with an increase to 54.3±14.6 µg/L at 10–30 days postpartum); however it is well known that serum/plasma Se levels are dependent on dietary intake and may vary considerably in different countries [8].

### Trace elements in colostrum

The first milk sample collection (colostrum) was performed on the 2nd day postpartum, at the same time as the collection of blood samples. Table 3 presents a summary of the results obtained. In contrast to blood, there are no well-established “normal values” for trace elements in colostrum, so interpretation was made essentially by comparing the obtained results with those reported in the available literature.

Copper levels in colostrum were near the lower limit of the normal range for serum (mean: 760.0 µg/L; median: 632.3 µg/L). In contrast, zinc values appeared to be elevated to high levels in colostrum (IC95% = 10.5–13.8 mg/L) when compared with serum...

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**Table 4.** Results (µg/L) of analysis of mature milk samples (collection at 29.5±7.1 days postpartum; n = 19) and some published values

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Element (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn</td>
</tr>
<tr>
<td>Mean</td>
<td>4.9</td>
</tr>
<tr>
<td>Median</td>
<td>4.6</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>4.0–5.7</td>
</tr>
<tr>
<td>Minimum</td>
<td>2.2</td>
</tr>
<tr>
<td>Maximum</td>
<td>9.3</td>
</tr>
<tr>
<td>Q1</td>
<td>3.7</td>
</tr>
<tr>
<td>Q3</td>
<td>5.6</td>
</tr>
<tr>
<td>P (normality)</td>
<td>&gt;0.15</td>
</tr>
<tr>
<td>(N)</td>
<td>(N)</td>
</tr>
<tr>
<td>a</td>
<td>3.2–39.5</td>
</tr>
<tr>
<td>b</td>
<td>3.13±2.0</td>
</tr>
<tr>
<td>c</td>
<td>0.2±108</td>
</tr>
<tr>
<td>d</td>
<td>340±190#</td>
</tr>
<tr>
<td>e</td>
<td>17±11</td>
</tr>
<tr>
<td>f</td>
<td>(3–41)</td>
</tr>
</tbody>
</table>

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1Presented as range or mean±S.D. Adopted from: "Parr et al. [27] (collection at approximately 3 months postpartum); Leotsinidis et al. [19] (collection at the 14th day postpartum); Hannan et al. [30] (collection at day 20); Yamawaki et al. [33] (* collection at 11–20 days postpartum; # collection at 21–89 days postpartum); Roekens et al. [20]."
samples. This means that, while copper levels are very high in the mother’s blood and much lower in colostrum (only about 35% of the blood levels), the situation is reversed for zinc: it is decreased in maternal blood and present at very high concentrations in colostrum (approximately 17 times higher than in blood). This can be regarded as a consequence of the important physiological functions of zinc: because it is essential for the development of the newborn, the mother excretes large quantities of this element in the first milk, in order to adequately supply the newborn infant [5,15].

Taking into consideration the well known decrease in milk zinc levels during lactation, especially in the first days after parturition [15], the values obtained for zinc in this study (12.1±4.7 mg/L) should be compared only with those obtained from samples collected in the same sampling period [16]. The obtained results are very similar to those published by Casey et al. [17] for colostrum samples, which were also obtained on the 2nd day postpartum (11.5±4.7 mg/L). These authors also reported a concentration of 0.6±0.12 mg/L for Cu on the 5th day and a concentration of 5.4±1.6 μg/L for Mn on the 1st day postpartum, which can be considered to be of the same order of magnitude as the values obtained in this study. Arnaud and Favier [18], in a more recent study with a French population, also obtained similar values for Zn in 82 colostrum samples collected on the 2nd day postpartum (12.0±4.6 mg/L). These authors reported a Mn concentration on the 2nd day postpartum significantly higher than the value found in this study (12.0±5.6 vs 7.7±1.8 μg/L, respectively), but lower concentrations have also been reported [16,19]. Compared with data reported in the literature [20–22], the Se levels found in this study were higher than most of the reported values. However, geographical variation in the Se content of human milk is known to occur, and higher values, similar to those obtained in this study, have also been reported in other countries [22].

Using box-and-whiskers plots for easier comparison (Fig. 1) it can be seen that As, Co, Ni and Se were higher in colostrum than in blood; the opposite occurred for Mn and Pb. For Se, there was greater variability of the results obtained for colostrum samples (S.D. = 36.43; IC95% = 59.53–84.95 μg/L) compared with the results from blood samples (S.D. = 16.57; IC95% = 54.49–64.56 μg/L), which was confirmed by an F-test (z = 5%).

Significant correlations [r_{tab} (0.05,32) = 0.339] between the analyzed trace elements were found in colostrum samples for Se vs Cu (r = 0.828), Se vs Co (r = 0.605), Cu vs Ni (r = 0.482), Zn vs Mn (r = 0.410), Co vs Pb (r = 0.402), Cu vs Co (r = 0.368), Se vs Ni (r = 0.356) and Ni vs Co (r = 0.341). It is interesting to note that a significant positive correlation between Se and Cu has already been described by Perrone et al. [23]. These authors also found an inverse correlation between Se and Zn. However, in this study, such correlation did not reach statistical significance (r = −0.282).

The occurrence of these correlations between trace elements probably reflects similar mechanisms in their passage from plasma to milk in the mammary gland, but more information is needed to substantiate this assumption.

**Correlation of trace elements in colostrum (2nd day postpartum) vs trace elements in blood (2nd day postpartum)** – To test for a potential relationship between the levels of each trace element in colostrum and blood, Pearson correlation coefficients (r) were calculated. No significant correlations were found for all the analyzed trace elements [for Cu, the obtained value (r = 0.244) was close to the tabulated value: r(0.05,66) = 0.239].

With regard to essential trace elements (e.g., Fe, Zn, Cu), it is generally accepted that the infant is well protected by maternal homeostatic processes [2]. The mammary gland appears to have developed mechanisms to regulate the concentrations of these elements in milk in order to adequately supply the breast-fed infant independently of maternal status [4]. It is assumed that the passage of these trace elements from blood to milk does not occur by passive diffusion but by regulated transport through the mammary gland epithelium [5]. Therefore, no positive correlations between the levels in mother’s blood and milk are expected [5].

With respect to toxic and non-essential trace elements (e.g., Pb, As, Cd, Hg, Cs, Li, Sr), the literature data are contradictory: significant positive correlations, which suggest a passive diffusion mechanism (i.e., following concentration gradients) for their transport into milk, and non-significant correlations have both been reported. For Pb, for example, Gulson et al. [24] summarized several studies in which a significant correlation between the levels in maternal blood and breast milk was observed but also a number of studies in which no correlation or a poor correlation was observed. It is probable that the relationship depends on the actual blood levels of the toxic trace element. Gulson et al. [24] recognized that selected studies appear to show a linear relationship between breast milk and maternal whole blood in subjects with blood levels ranging from 20 to 340 μg/L. For lower concentrations, similar to those of the present study, this relationship seems not to exist. Oskarsson et al. [25] reported that, generally, there is a low level of transfer of toxic metals through milk when maternal exposure levels are low. These authors referred to a study in Sweden where, in general, the levels of toxic metals were low, and for Pb and Cd there was no significant correlation between the levels in milk and in blood. The reported Pb levels in blood were 32±8 μg/L, similar to those obtained here.
Recently, Sharma and Pervez [26], who compared three groups of lactating women (\(n = 120\)) from Central India, found positive correlations between the blood and milk concentrations of several toxic elements (Mn, Hg, Cd, Pb and As) in workers at a steel plant (group I; \(n = 40\)) and other residents of the steel plant township area (group II; \(n = 45\)). However, such a relationship was not obtained for women living at uncontaminated areas (more than 100 km away from the industrial unit; group III; \(n = 35\)).

![Box and whiskers plots](image)

**Fig. 1.** Box and whiskers plots depicting the concentration of the selected trace elements in colostrum (1), mature milk (2) and blood samples (3). [The ends of the vertical lines represent the smallest non-outlier value (bottom) and the highest non-outlier value (top). The box is limited by the first quartile (bottom) and the third quartile (top). The horizontal line inside the box represents the median (second quartile). The asterisks indicate the presence of values that were considered "outliers", i.e., values that lie more than 1.5 \( \times \) IQR lower than the first quartile or 1.5 \( \times \) IQR higher than the third quartile (note: IQR = interquartile range)].

**Trace elements in mature milk**

As mentioned before, a second milk collection was performed at approximately 1 month (29.5 ± 7.1 days) postpartum. The results obtained are summarized in Table 4. Using literature data as a reference, it can be concluded that the values obtained in this study generally fell within the published ranges. Our results for Zn and Se were near the upper limit of the range obtained in the international collaborative WHO/IAEA
study on trace element levels in milk [27], and the mean level of Cu was higher than the published limit. However, it must be noted that in the WHO/IAEA study the samples were collected at approximately 3 months postpartum. The literature data summarized by Benemariya et al. [22] for milk collected at 1 month showed mean concentrations ranging from 0.22 to 0.44 mg/L for Cu and from 2.0 to 4.1 mg/L for Zn; the values obtained in this study were higher for Cu but similar for Zn.

Fig. 2 shows the evolution of trace element levels between the first (2nd day postpartum) and the second (1 month postpartum) milk collections. As can be seen, the measured levels of trace elements tended to decline during the first month of lactation. The decrease was especially significant for Zn (which declined from a mean value of approximately 12.1 to 2.8 mg/L), followed by Se (which declined from 72.2 to 32.1 μg/L). The exceptions were Pb and Ni (the decrease in these elements did not reach statistical significance) and Co (which showed a tendency for increased levels in mature milk).

These observations are concordant with those of Krachler et al. [28] who, in a study of mothers during the 293 days postpartum, also observed a significant decline in Cu, Mn, Mo and Se levels, but not in Co, which, on the contrary, increased. The large decrease observed for Zn is similar to that described by Casey et al. [17] in a study of women during the first month of lactation. In the latter study a decrease in the level of Zn was found from 11.5 ± 4.7 mg/L on the 2nd day to 2.98 ± 0.78 mg/L at day 28 ± 3. According to the literature, this decrease is more pronounced in the first few days of lactation; the same authors, in a later work [29], reported values of only 4.7 ± 1.2 mg/L on the 7th day postpartum. In addition, Arnaud and Favier [18] reported a decline from 12.0 ± 4.6 mg/L on the 2nd day to 5.0 ± 1.4 mg/L on the 6th day, and Hannan et al. [30] reported that Zn levels decreased from 16.1 ± 2.7 mg/L at day 0 to 4.95 ± 1.3 mg/L at day 20 of lactation. The significant decrease in the concentration of selenium in milk during the first month of lactation is also well documented in the literature [20–22].

The decrease in the concentration of trace elements in human milk during the course of lactation has been considered to be a consequence of a decrease in the binding capacity of the milk (because of the decrease in the concentration of proteins and other ligands) [16]. In human milk, trace elements are mainly bound to proteins, and major proteins in the milk decrease during the first month of lactation. The increase observed for Co could be explained by an increase of its bioavailability for the mammary gland due to an increase of its concentration in blood either by mobilization from tissues or increased intestinal absorption [16]. Recently, the regulation of trace element homeostasis in milk has been studied by evaluating the molecular processes through which the mammary gland regulates the transport of trace elements into milk. These mechanisms include the uptake of metals into the secretory mammary epithelial cell and their subsequent secretion into the alveolar lumen of the mammary gland for sequestration in milk [15]. Using animal (rodent) models the authors found experimental evidence that the temporal changes in Zn, Cu and Fe concentrations in milk are regulated through coordinated changes in gene expression, protein levels and localization of several specific transporters.

As already reported for colostrum samples, significant correlations between several trace elements, demonstrated using Pearson’s correlation \[ r_{\text{tab}}(0.05,17) = 0.455 \], were also obtained in mature milk: Pb vs Ni \( (r = 0.756) \), Mn vs Ni \( (r = 0.743) \), Co vs Se \( (r = 0.714) \), Mn vs Pb \( (r = 0.677) \), Co vs Mn \( (r = 0.483) \), Se vs As \( (r = 0.478) \) and Co vs Pb \( (r = 0.478) \). The observation made by Perrone et al. [19] of the existence of an inverse correlation between Zn and Se was supported in these mature milk samples by a Pearson correlation coefficient of \( r = -0.624 \). Other negative correlations found were Zn vs Co \( (r = -0.597) \) and Zn vs As \( (r = -0.504) \).

**Correlation between trace elements in mature milk (1 month postpartum) and trace elements in colostrum (2nd day postpartum)** – In this additional evaluation, no significant linear correlation was found between the concentration of each trace element in colostrum and mature milk samples, with the exception of Co \( (r = 0.607) \) and Zn \( (r = 0.487) \) \[ r_{\text{tab}}(0.05,36) = 0.320 \]. This means that, despite the tendency of the mean level of each trace element to decrease between colostrum and mature milk samples, the individual tendency observed for each woman can be somewhat different.
Conclusion

In summary, our results for Mn, As, Pb, Co, Ni, Cu, Zn and Se in lactating Portuguese women were in agreement with those that have been reported by other authors for blood, colostrum and mature milk. In colostrum, the mean levels of Zn, Co, Ni, As and Se were higher than those obtained in blood; the opposite was found for Cu, Mn and Pb. No significant correlations were observed between colostrum and blood trace element levels, suggesting that the analyzed elements are subjected to a regulated transport from blood to milk.

Except for Co, Pb and Ni, a significant decrease in the concentration of the analyzed elements was observed between colostrum and mature milk samples. This decrease was more pronounced for Zn and Se. Significant positive correlations between several trace elements in both colostrum and mature milk and an inverse correlation between Zn and Se in mature milk were observed. The reliability of these inter-element interactions and their exact meaning requires further evaluation.

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References


