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Cerium in human milk samples and its transfer from blood to milk: Is there an elevated nutritional risk for breast-fed babies?*

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Abstract: The general population is increasingly exposed to cerium (Ce), which is contained in industrial products or is present as nuclear Ce fission products. Some studies suggested a link between elevated Ce levels and endomyocardial fibrosis. Since breast milk is the optimal, and directly after birth, usually the sole nutrition for newborns, exposure of females to Ce and its transfer to infants by breast-feeding is of concern in neonate protection. Consequently, the transfer rate of Ce from blood to breast milk is of interest for elucidating the Ce exposure of infants. Biomonitoring of paired serum and breast milk samples provides such information about Ce transfer to human milk. Therefore, this study is aimed at clarification of the relationship between Ce in human milk and serum from respective mothers for elucidating Ce enrichment in human milk with possible nutritional risk for newborns. As a prerequisite a strictly quality-controlled Ce determination method applicable to very low Ce concentration was developed, and its figures of merit were determined and found to be sufficient for our purpose. It turned out that Ce concentration in milk from Munich (Germany) and Madrid (Spain) showed a median of 13 ng/L. Ce concentrations in serum were at limit of quantification (LOQ) 10 ng/L (Munich) or 21.6–70.3 ng/L (Madrid), suggesting a higher Ce intake in Madrid. No enrichment from blood to milk was seen, and no elevated nutritional risk for breast-fed babies from Ce was found. Ce in serum, but not in milk, could indicate environmental Ce.

Keywords: blood plasma; breast milk; cerium; inductively coupled plasma-mass spectrometry (ICP-MS); humans.

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INTRODUCTION

Cerium (Ce) is a rare-earth metal of the lanthanide series with about 68 mg/kg in the Earth's crust. Ce is applied in many industrial products (glass additives, lighters, carbon arc lamps, ceramics), it is an important component of catalytic converters, and it is used as a diesel fuel additive [1]. Human exposure to Ce is increasing as it is a main component of microfertilizers manufactured in China, which are also used in many other countries [2]. In medicine, Ce pharmaceuticals are applied for skin burns [3]. In addition, there are two radioactive isotopes of Ce, ^{144}Ce ($t_{½}$ = 284 days) and ^{141}Ce ($t_{½}$ = 32.5 days), which are important nuclear fission products contained in the effluents from nuclear power and reprocessing plants [4].

This steady rise of Ce in the environment through its wide industrial and agricultural applications, especially the use of Ce additives in diesel vehicles, could cause an increased risk of exposure to Ce in humans. The general population may be exposed mainly by oral uptake and by inhalation of fine particles probably from diesel vehicle exhausts. Studies of the daily Ce oral intake showed a rate of 5.6–8.6 μg Ce/d [5,6]. Higher intake values of 83–145 μg/d were suggested for farmers living near an ore deposit containing about 3 % of rare earths [7]. From animal experiments oral Ce uptake is considered to show low toxicity owing to a small intestinal absorption. Studies on the intestinal absorption of Ce citrate in humans confirmed a low Ce absorption of 1.1×10^{-3} [8]. However, the EPA report on Ce compounds reviewed studies suggesting a relationship between exposure to Ce in food and the development of endomyocardial fibrosis [9]. Recently, also an association between chronic Ce exposure and increased risk of acute myocardial infarction was argued [10]. Additionally, numerous cases have been reported of Ce-exposed workers who developed pneumoconiosis. This lung disease was associated with accumulation of Ce in the lungs after a prolonged occupational exposure to Ce-containing fumes or dust after regular use of carbon arc lamps [9]. Therefore, health effects after inhalation of Ce seemed to be possible but further validation is needed.

Exposure and incorporation of radioactive fission products after nuclear accidents has recently gained strong attention again. However, the knowledge of the biokinetics of radioactive Ce isotopes in humans is still not examined properly. Owing to scarcely available human data, the currently used biokinetic model for human Ce metabolism is mainly based on animal experiments and, by supposed analogy, on trivalent actinides [11]. Indeed, the newest experiments on Ce biokinetics in humans suggest a revision of the current biokinetic model of Ce [8]. Of peculiar interest for radiation protection is the transfer of Ce in human milk of breast-feeding mothers.

Human breast milk is the optimal nutrition for the newborn, containing appropriate amounts of carbohydrate, protein, lipids, and micronutrients as well as essential trace elements [12,13], but it may contain heavy metals and other pollutants related to environmental contamination and the mother's diet [14,15]. In addition, the exposure of a female to radionuclides and their possible transfer to the infant during pregnancy or breast-feeding is of special concern in radiation protection for the public. Radiation doses to the infants through breast-feeding after maternal exposure from food depend on the direct transfer from the mother's blood to the breast tissue and milk. Since there are still inconsistencies in the current biokinetic behavior and specifically in the Ce transfer rates to breast milk, there is a strong requirement for further validation of biokinetic parameters. Biomonitoring of paired blood and breast milk is a preferential way to provide information about the Ce body burden of women as well as the exposure of infants.

To the authors' best knowledge, to date only two studies [5,16] are known aside from our own recent paper [17], where Ce in human milk was determined. In one of the studies [5], the transfer factor of Ce from food of the mothers to their milk was calculated. Of special concern were the Ce concentrations reported for breast milk as they were considerably higher than those in human blood plasma [18] reported (however) from a different study, possibly indicating enrichment from plasma to milk. With respect to radioactive Ce isotopes, this could be a matter of concern.

Consequently, the aim of this study was to establish a strictly quality-controlled Ce determination at very low concentration levels, based on a simple method, having as few as possible pre-analytical steps. Applying this method, reliable Ce values in serum, plasma, and milk were achieved for evaluating the relationship between Ce in human milk and blood plasma or serum. Finally, a possible Ce enrichment in human milk with possible elevated (nutritional) risk for newborns was assessed.

This conference contribution is based on data that were partly published earlier in reference [17].

MATERIALS AND METHODS

Subjects

In a preliminary study conducted from April to September 2007, 10 healthy lactating mothers (age 29–40 y) living in the greater Munich area (Germany) voluntarily donated some breast milk. This study was repeated and expanded between August 2008 to January 2009, where breast milk and also blood samples were collected from another 32 mothers (age 22–41 y, median = 33 y). In parallel, 26 Spanish mothers from the greater Madrid area (age 26–44 y, median = 33 y) were asked to donate paired samples of blood and breast milk; these collections were performed in March and May 2009, each 4 weeks postpartum. All mothers were informed about the aim of the study, and written informed consent was obtained from the mothers before the collection started. Each subject obtained an identification code (CeMu0-41 for Munich, and CeMa42-67 for Madrid) and had to fill out a personal questionaire. Several mothers from the Munich area donated samples at various stages of their lactation. Altogether, breast milk samples from 5 days to 51 weeks postpartum were collected in this study. In order to reduce the risk of contamination, the area around the nipple was carefully cleaned with ultrapure water and a lint-free, soft Kimwipe tissue, and breast milk was extracted by gentle manual pressing on the breast into polyethylene bottles. Spanish mothers collected milk as a mix of milk before and after feeding (after discarding the first drops), whereas German mothers collected independently on feeding into pre-cleaned (5 % nitric acid suprapur) and rinsed (Milli-Q water) polyethylene containers. All breast milk samples were stored at –20 °C until analysis. Blood samples were collected from the German mothers once from 1 day before birth to 51 weeks postpartum. At least one blood sample was collected on the same day of a breast milk sampling.

Chemicals and reagents

Chemicals and reagents used were of suprapure grade. Certified Ce and Ir stock standards (both dissolved in HNO_3, each 1000 mg/L) were purchased from CPI (Santa Rosa, USA). Dilutions of standards and serum or plasma samples were made with deionized water (18.2 MΩ cm) prepared by a Milli-Q system (Millipore, Bedford, MA, USA). HNO_3 was bought from Merck, Darmstadt, in *pro analysis* quality and sub-boiling distilled before use. Ar_{liq} was purchased from Air Liquide, Gröbenzell, Germany.

Sample preparation

Human milk samples

For most milk samples, two or more specimens were prepared for measurement. After thawing, samples were gently vortexed for re-homogenization and two 1 mL-aliquots from each sample were pipetted into precleaned quartz vessels of a Seif apparatus and mixed with 1 mL concentrated nitric acid (HNO_3, sub-boiling distilled) for subsequent pressure digestion (170 °C, 10 h). The clear digestion solution was filled up with Milli-Q-H_2O to the 10-mL mark. With respect to formerly published Ce values in milk reaching up to 150 ng/L [5], sample digests were diluted before measurement at the early on-set of the study (2007 campaign): 5 mL of each digest was first diluted 1:2 with Milli-Q-H_2O and

^{193}Ir was added as internal standard (final concentration 1 µg/L) before samples were measured for Ce using an inductively coupled plasma-mass spectrometer (ICP-MS; ELAN, DRC II, Perkin Elmer, Sciex Ontario). As this preliminary approach did not confirm such high Ce values, but values close to LOQ, digests from the follow-up 2009 campaign were measured directly without dilution (except addition of 20 µL ^{193}Ir as internal standard, final concentration: 1 µg/L). For quality control (QC), one remaining milk sample of 2007 was determined by the two different work-ups: the results were in agreement (16.2 ± 0.2 ng/L in 2007 vs. 16.5 ± 0.07 ng/L in 2009).

Human plasma/serum samples

In Munich, blood was sampled at the maternity medical practice using heparinized S-Monovettes® (Sarstedt, Germany). Samples were centrifuged and plasma removed; then the plasma samples were stored frozen until analysis. In Madrid, blood samples were drawn in the hospital (Madrid) using trace element-free Vacutainer® tubes (Becton Dickinson, USA); in this case, serum (and not plasma) was obtained as the residual material had to be utilized in another clinical trial. All biological samples from Madrid (milk and serum) were then shipped frozen to the analytical laboratory at the Helmholtz Zentrum München.

Both plasma and serum samples were thawed slowly and subsequently gently vortexed for re-homogenization. Aliquots of 500 µL were diluted 1/10 with Milli-Q-H_2O, and 20 µL ^{193}Ir were added (internal standard, final concentration 1 µg/L) before Ce measurement with ICP-MS.

Instrumental analysis

Instrumental analysis was performed on an ICP-MS ELAN DRC II from Perkin Elmer. Instrumental parameters are given in Table 1.

Table 1 Instrumental parameters of ICP-MS.

Instrument:	ELAN DRC II, Perkin Elmer (Sciex, Toronto, Canada)
RF power:	1250 W
Plasma gas:	15 L Ar/min (Air Liquide, Gröbenzell)
Nebulizer:	Meinhard (Glass Expansion)
Spray chamber:	Cyclon (Perkin Elmer)
Nebulizer gas:	0.85 L Ar/min, optimized daily
Isotope:	^{140}Ce Internal standard: ^{193}Ir, at 1 µg/L
Dwell time:	200 ms Replicates: 3; 6 sweeps per reading
Sample introduction:	Perimax peristaltic pump with "Antipulse-Head", Spetec, Erding.
Sample flow rate:	1.2 mL/min
Calibration:	8 point calibration (0–1000 ng/L): r^2 = 0.99993, which shows excellent linearity within the calibration range. No higher concentrations were checked as sample values were in the low ng/L range throughout.

These parameters were the optimal conditions for this instrument.

Analytical quality control

The analytical procedure was kept as simple as possible to avoid possible sources of contamination. Serum or plasma samples were just diluted with Milli-Q water, and milk samples were digested in a simple, but powerful digestion system without further manipulation. In addition, a rigid QC program was applied to the whole analytical procedure including the determination of blanks, digestion blanks, and control standards at 50 ng/L Ce directly before and after measurements of samples.

Additionally, a series of blank determinations of digestions, blank determinations of plastic and quartz ware filled with 5 % HNO_3 solution and allowed to stand for 60 min, serial and day-to-day precision at two concentrations each, as well as recovery experiments were performed.

In order to assess the comparability of serum and plasma samples Ce concentrations in both sample types, each taken from the same donor at the same time (n = 18), were analyzed. Further, both sample types (plasma and serum) were spiked with 5, 10, or 20 ng/L Ce (each concentration, n = 3) and recovery rates were calculated.

RESULTS AND DISCUSSION

Quality control results

Since it turned out in preliminary experiments that the concentrations in plasma and milk samples were rather low and close to LOQ, a rigid analytical QC was indispensable. The QC strategy included six steps:

1. A set of 20 sample vessels, filled with 5 % HNO_3 solution, were analyzed after 60 min resting time in order to check any contamination to be expected from vessels. All vessels showed values as low as instrumental blank values at 0.2–0.3 ng/L. No elevated values were monitored, indicating that no contamination from vessels should be expected.
2. A set of 10 blank digestions was performed before starting the sample analysis in order to check the scrupulous cleanliness of digestion apparatus and digestion vessels. Further, blank digestions were carried out regularly with each set of sample digestions. All digestion blanks showed values between 0.2 and 0.5 ng/L, proving that no contamination was introduced during the digestion procedure.
3. Serial precision (n = 10) was determined at ca. 1 ng/L by analysis of a digested milk sample (overall dilution 1/10) or of the same sample, but with 10 ng/L standard addition (target concentration ca. 11 ng/L), each 10 times in series. Measurements resulted in a mean (SD) of 1.01 (0.055) ng/L (RSD/serial precision = 5.4 % at ca. 1 ng/L), or after addition in a mean of 11.34 (0.24) ng/L (RSD/serial precision = 2.1 % at ca. 11 ng/L).
4. Day-to-day precision was determined by measuring control standards at 2 ng/L or aliquots of a digested milk sample (overall dilution 1/10; each n = 10). The 2-ng/L control standards showed a mean of 2.11 (±0.16) ng/L (RSD/day-to-day precision = 7.9 %), and the digested milk sample had a mean (SD) of 1.45 (±0.14) ng/L (RSD/day to day precision = 9.4 %).

   The detection limit (LOD, blank + 3 SD) was 5.8 ng/L Ce, and the limit of quantification (LOQ, blank + 10 SD) was 10 ng/L Ce for the measurements in 2007, and 2 ng/L (LOD) or 5 ng/L (LOQ) in 2009, each related to native (non-diluted) breast milk samples. The improved LOD and LOQ in 2009 is explained by a methodical change where milk digests were measured without dilution in 2009, but 1:2 diluted in 2007.
5. In general, accuracy should be determined by analyzing a certified reference material similar to the samples with respect to matrix composition and analyte concentration. However, no reference material is available for Ce at such low concentration in biological matrices. Therefore, accuracy was estimated by recovery experiments. During the 2007 campaign three samples were analyzed before and after spiking (sample CeMu1 + 30 ng/L, sample CeMu2 + 15 ng/L, sample CeMu5 + 10 ng/L) and recovery rates were calculated. Recovery was 96.9 ± 3.2 %. During the 2009 campaign, aliquots of milk sample digests were analyzed 5 (CeMu24) or 10 (CeMu30) times before and after the addition of 1 ng/L (CeMu24) or 10 ng/L (CeMu30). The recovery of the 1 ng/L addition was determined at 106 %, that of the 10 ng/L addition at 102 %. These QC results proved that the complete method including digestion and analysis was suitable for the analysis of the human milk and plasma/serum samples.

6. Finally, the comparability of serum and plasma was checked by analyzing in total 18 paired serum/plasma samples from the same (German) donors each. Table 2 shows the respective values, many of them being below the LOQ for serum/plasma samples at 10 ng/L. Only insignificant differences in Ce concentrations of plasma and related serum samples were seen, which can be easily assigned to instrumental variation at such low concentrations. Recovery rates of Ce spikes to serum or plasma were close to 100 % for both sample types and applied concentrations.

Table 2 Comparison of Ce concentrations in paired blood plasma and serum of donors from the Munich area. No statistically significant differences are monitored between serum and plasma. Values given in ng/L.

Donor	Serum	Plasma
A	13.3	11.3
B	16.9	13.9
C	11.1	10.1
D	14.4	14.2
E	10.4	<10
F	<10	<10
G	14.5	13.8
H	17.1	17
I	<10	<10
J	<10	<10
K	<10	<10
L	12.6	<10
M	12.7	<10
N	<10	<10
O	<10	<10
P	<10	<10
Q	12.5	12.1
R	10.6	12.8

RESULTS IN HUMAN MILK

In Table 3, the data ranges, the (arithmetic) mean (±SD) values and the median of Ce concentrations in human milk and blood samples of the lactating German and Spanish mothers are shown. The average value for each German mother was obtained from two or more specimens prepared/digested from the same sample and triplicate measurements of each digest. From these data the mean of the individual sample was formed. Individual means were the basis for calculating the (arithmetic) mean (SD) of the German sample collective. Single values from Spanish samples were obtained (owing to low sample volume) and used to form the mean of the Spanish collective. The range of the Ce concentration in the human milk samples was between the limit of determination at 5 and 65 ng/L. In the preliminary study of 2007, the median value of the German milk samples was 13 ng/L, which is practically identical to the median of the milk from German mothers in 2008/9 at 12.6 ng/L. The Ce concentration in the Spanish human breast milk (from one digestion and triplicate measurements of this digest) showed similar values with both, median and mean close to German samples. All values from both collectives were in the same range, and there was no statistically significant difference between the data of the German and Spanish samples.

Table 3 Concentrations of Ce in breast milk and blood plasma/serum of German and Spanish lactating mothers.

Milk	Numbers of samples (*n*)	Range (ng/L)	Mean (±SD) (ng/L)	Median (ng/L)	*n* < LOQ*
Munich 2007	11	11–25.8	15.4 (±5.9)	13	4
Munich 2008/9	51	5.04–65.8	15.7 (±5.8)	12.6	3
Madrid 2009	26	5.03–45.7	13.9 (±9.2)	10.6	3
Plasma/serum	Numbers of samples (*n*)	Range (ng/L)	Mean (±SD) (ng/L)	Median (ng/L)	*n* < LOQ**
Munich 2007	not determined	–	–	–	–
Munich 2008/9	31	<10–12.7	<10	<10	29
Madrid 2009	26	21.6–70.3	38.8 (±11.4)	40.4	0

*LOQ of milk 2007: 10 ng/L, 2008/9: 5 ng/L.
**LOQ of blood plasma/serum 2008/9: 10 ng/L.

There is still only sparse information on concentration of Ce in breast milk in literature. Two further publications dealing with Ce determination in breast milk [5,16] are known to the authors aside from our recently published paper [17]. These two other working groups partly applied acid-cleaned breast milk pumps for milk collection and ICP-MS for measurements. Friel et al. [16] found the Ce concentration in human milk "extremely low", overall reporting Ce concentrations below their LOD of 5 µg/L. This LOD is three orders of magnitude higher than the one obtained in the present study. In the work of Wappelhorst et al. [5], the results of Ce content in human milk are partly inconsistent: On the one hand, these authors report a Ce concentration range of 60–240 ng/L, with a median value of 100 ng/L, but later in the text one sample is assigned to 1.36 µg/L (approximately 5 times above the "maximum" of range) whilst in their Fig. 4 other samples are assigned to ca. 0.000 075 µg/L, being about a factor 1000 below the given "minimum" value of range. The results presented in our work were below the range indicated by Wappelhorst et al. in their table [5]; our median is even eight times lower than the median found by them [5]. Their higher Ce values could be explained by the multitude of preanalytical steps, including freeze-drying, pooling of digests, and open evaporation, thereby increasing the risk of contamination. No information is given about blank determinations in their complicated procedure. In our study, the work schedule of the samples comprised only a pressure digestion step and—after adding Ir as an internal standard—the resulting solution was directly given to ICP-MS for measurements of Ce. In breast milk, trace element concentrations can considerably vary during the course of lactation [19]. Therefore, we investigated Ce values from mothers who donated milk several times during their lactation period. However, no clear course could be deduced from the samples of these few individuals as they showed either slight increases or slight decreases over their lactation period. When plotting Ce values from different mothers vs. respective (different) lactation states, this indifferent picture was confirmed. Values are scattered around the mean of 15 ng/L along the whole lactation period; the linear equation showed nearly a parallel to the *x*-axis with a minimal declination close to zero (–0.0284). Overall, it is shown in Fig. 1 that the Ce concentration seems to be fairly constant.

Wappelhorst et al. [5] presents a figure showing a fast decrease from ca. 150 to 100 ng/L within 0–20 days and a slower decrease down to ca. 70 ng/L between 20–75 days. However, as mentioned above, their results were partly confusing and about 8–10 times higher than our values.

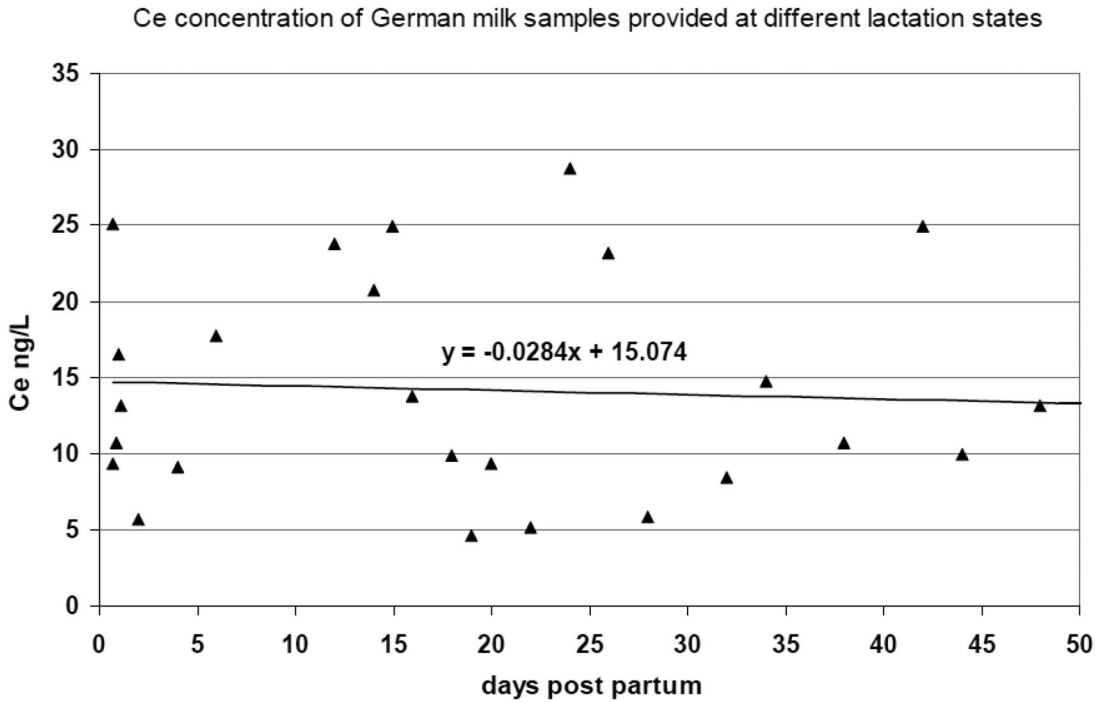

Fig. 1 Ce concentrations of milk samples collected from mothers of Munich region. Mothers from Munich donated milk at different lactation states, some of them even on several occasions. There is no clear trend visible along the lactation state. The linear regression equation shows an insignificant declination close to zero (–0.0284), also showing that the Ce concentration is fairly constant.

RESULTS IN PLASMA OR SERUM

Ce concentrations in the German human plasma samples were below the quantification limit of 10 ng/L throughout, except for two samples which had 11.8 and 12.7 ng/L. However, the serum samples of the Spanish mothers showed Ce values ranging between 21.6 to 70.3 ng/L. These higher Ce serum concentrations of the Spanish mothers could be explained by a higher daily Ce intake. Ce is immediately taken up at high rates from inhalative exposure and is transported via blood to other organs, as demonstrated in an animal experiment from Shiao-Shan et al. [20]. A Ce source in the ambient air of traffic-rich cities like Madrid could be diesel vehicles and the widespread use of catalysts in automobiles, since Ce is employed as a diesel additive and as a promoter in catalytic converters [1]. Thus, a higher inhalative Ce intake could be argued owing to the much higher traffic volume in Madrid, compared to Munich. An analogous conclusion was drawn for Pt and Pd, as both noble metals are catalyst components, too, and both are elevated in road dust of streets with higher traffic volume [21]. Environmental studies from Morelli et al. and Gomez et al. confirmed traffic to be the source of Pt, Rh, and Ce, especially in populated urban areas [22,23]. Moreover, Gomez et al. [24] found lower Pt (Rh) content in airborne particles in Munich compared to Madrid. Thus, it could be concluded that the higher Ce content in serum of the Spanish mothers may be caused by a higher inhalative incorporation of Ce in Madrid owing to a higher traffic volume.

Interestingly, the Spanish Ce serum concentrations were different depending on the date of sampling. Ce concentrations in serum samples collected in March 2009 were higher than Ce concentrations in samples collected in mid-May 2009 (Fig. 2), whereas all Ce values of breast milk were similar. Differences with respect to sampling time were insignificant. This finding is also expressed in the elevated ratio of Ce concentration of milk vs.s Ce of serum.

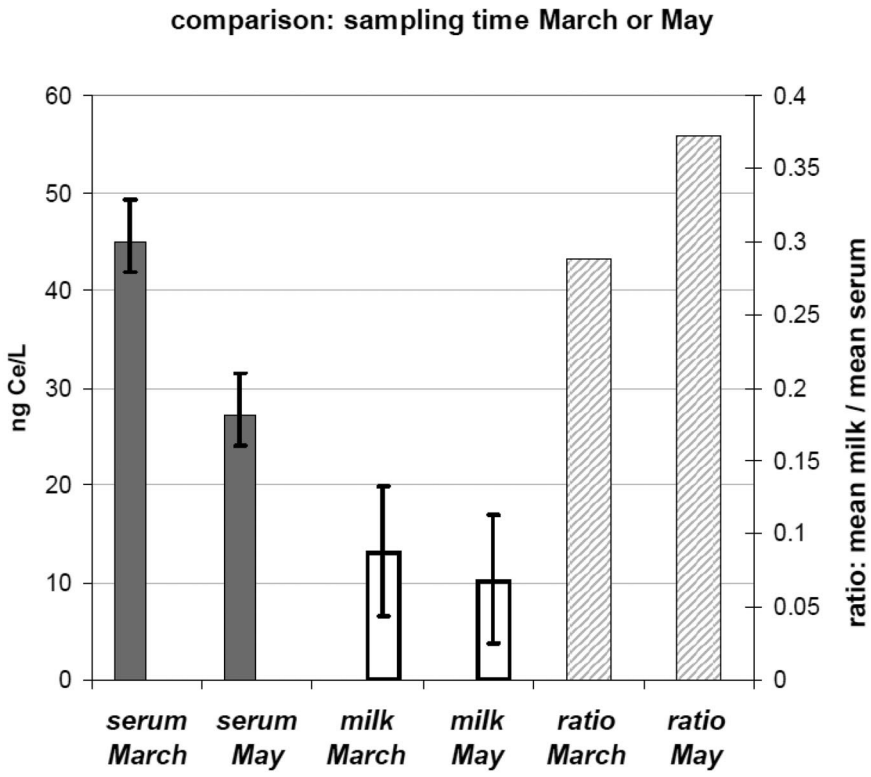

Fig. 2 Mean Ce concentrations in serum and breast milk of mothers from Madrid region. All samples were collected four weeks postpartum, however, during two collection periods, either in March or in May. It becomes clear that Ce concentrations in serum samples collected in March are significantly higher than of those collected in May, whilst Ce milk concentration differs only insignificantly. As a consequence, the ratio (Ce milk/serum) is higher in May.

The varying Ce levels of the Spanish serum samples could be traced to different concentrations of particulate matter owing to changes in climate and heating situation as well as traffic volume in March and in May in Madrid, which was also reported by the study of Artinano et al. [25].

In the recent literature [17] blood plasma values of Ce from "not traceable" to 30 ng/L were found for adult males and females living in northern Germany. Considering these low plasma Ce concentrations (mean <8 ng/L) and our own measurements in the plasma samples from Munich (mean <10 ng/L), together with the analyzed Ce concentrations in all breast milk samples (mean 15 ng/L), no significant enrichment of Ce from plasma/serum to milk could be assumed during lactation, since both Ce concentrations were in the same range. Even with higher Ce values in the Spanish serum samples (mean 38.8 ng/L) an increased transport of Ce from the serum to the breast milk to reach similar values was not indicated. Figure 3 shows the paired milk-serum/plasma values. The linear regression shows a parallel to the *x*-axis, proving that there is no dependence of milk Ce on blood Ce (r^2 practically zero).

Since milk and plasma/serum concentrations are in the same low range, and milk Ce concentration is fairly constant independently of higher serum Ce levels, it is concluded that there is no significant transfer rate, specifically none which would be increased under environmental pollution. Rossipal et al. [26] reported that for Cu, Se, and Co a regulatory effect of the mammary gland on the transfer of these trace elements from blood to milk was assumed. In a similar manner, we suggested that Ce transport into human milk might be regulated so that Ce content in milk is independent of the Ce content in blood. Thus, concerning a possible radiation exposure from radioactive Ce isotopes to newborns and

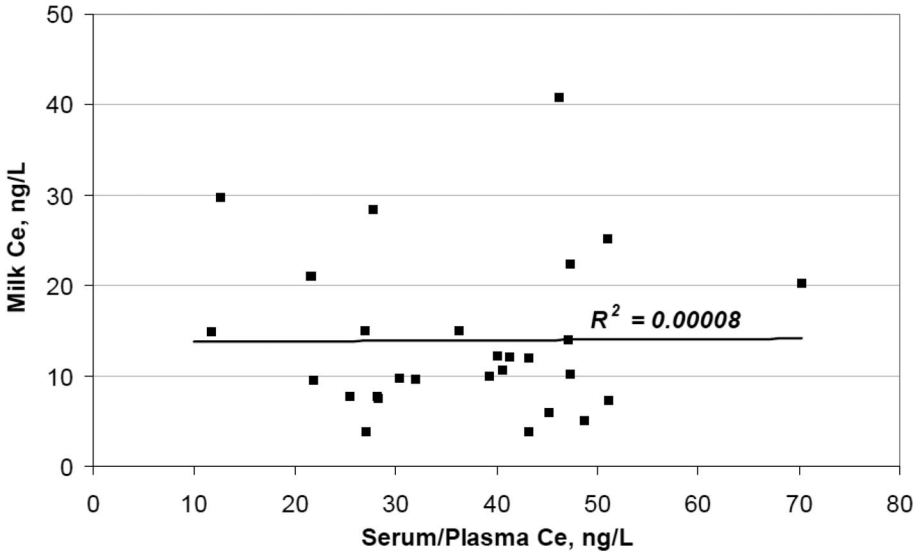

Fig. 3 Concentrations of Ce in human milk vs. serum of all Spanish mothers. The samples are paired samples, i.e., milk and blood samples from each mother were taken at the same time. No positive relation is apparent. Ce concentration in milk is independent from Ce concentration in serum as also demonstrated by a regression coefficient being practically zero.

infants through breast-feeding, the results of our study could not confirm an increased level of Ce in human breast milk compared to plasma values.

CONCLUSIONS

The present study successfully established a QC method for determination of low Ce concentrations in biological fluids.

Instrumental and vessel blanks were low at ca. 0.3 ng/L, blank digestions showed low values at ca. 0.5 ng/L. The serial precision was satisfying with 2.1–5.4 % at 1 ng/L, the day-to day precision was sufficient at 7.9 % at 1 ng/L and the recovery in spiked milk samples was close to 100 %. LOD and LOQ were low at 2 and 5 ng/L (milk) or 3 and 10 ng/L (plasma, serum), respectively.

Our study substantially contributed to defining reference values of Ce in human breast milk collected from German and Spanish breast-feeding mothers at various stages of lactation from 5 days to 51 weeks postpartum. The arithmetic mean values of the Ce concentrations in human milk varied between the quantification limit of 5 ng/L up to 65 ng/L; and the median value amounted to about 13 ng/L. The data were about a factor of eight lower than the values found in another study. Ce concentrations in the German plasma samples were mostly under the detection limit, whereas the Spanish samples showed Ce values between 21.6 and 70.3 ng/L. These higher data could be explained by an enhanced intake of Ce in humans in Madrid, compared to Munich, possibly caused by increased Ce concentrations in particulate matter emissions owing to a higher traffic volume. Our results showed that varying Ce values in plasma/serum depend on the environmental condition, but no increased level of Ce in human breast milk compared to plasma/serum values was confirmed. Ce transport and amount in human milk seem to be regulated and independent of the Ce content in plasma. Ce content in plasma could be an indicator for environmental Ce, which is not valid for breast milk.

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