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Total zinc quantification by inductively coupled plasma-mass spectrometry and its speciation by size exclusion chromatography–inductively coupled plasma-mass spectrometry in human milk and commercial formulas: Importance in infant nutrition

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ABSTRACT

This paper summarises results of zinc content and its speciation in human milk from mothers of preterm and full-term infants at different stages of lactation and from synthetic formula milks. Human milk samples (colostrum, 7th, 14th, and 28th day after delivery) from Spanish and Brazilian mothers of preterm and full-term infants (and also formula milks) were collected. After adequate treatment of the sample, total Zn was determined, while speciation analysis of the Zn was accomplished by size exclusion chromatography coupled online with the ICP-MS.

It is observed that total zinc content in human milk decreases continuously during the first month of lactation, both for preterm and full term gestations. All infant formulas analysed for total Zn were within the currently legislated levels. For Zn speciation analysis, there were no differences between preterm and full term human milk samples. Moreover Zn species elute mainly associated with immunoglobulins and citrate in human milk whey. Interestingly the speciation in formula milk whey turned out to be completely different as the observed Zn^{2+} was bound almost exclusively to low molecular weight ligands (citrate) and only comparatively very low amounts of the metal appeared to be associated with higher mass biomolecules (e.g. proteins).

1. Introduction

During the first months of life, milk constitutes the unique source of nutrients and, therefore, plays an essential role in terms of body growth and development. Breastfeeding ensures the best possible health as well as the best developmental and psychosocial outcomes for the baby [1]. Human milk provides an adequate intake of macronutrients (proteins, lipids, carbohydrates) and micronutrients (minerals, vitamins, enzymes) and can be used as a nutritional reference to develop formulas with a composition similar to that of human milk. From a nutritional point of view, there is a growing interest in the determination of micronutrients in human and formula milks because of its decisive role in human health.

Among the so-called minerals in milk, zinc is an essential trace element required for the metabolic activity of more than 400 of the body's proteins which is considered essential for cell division and the synthesis of DNA and proteins [2]. Zinc has an important role in cell-mediated immune functions and also as anti-inflammatory and antioxidant agent [3]. Therefore, zinc deficiency may be associated with a large variety of clinical disorders [4]. Increased susceptibility to infections, such as pneumonia and diarrhoea is an important consequence of zinc deficiency, especially in children from developing countries [5]. A relatively recent meta-analysis showed that zinc supplementation reduced significantly the frequency and severity of diarrhoea and respiratory illnesses and the duration of diarrhoeal morbidity in children under 5 years of age [6]. Zinc deficiency in full term infants is rare but may be frequent in

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preterm babies resulting from inadequate dietary intake, prolonged intravenous feeding, immaturity of gastrointestinal absorption systems, increased renal excretion or a combination of these factors [7–10]. Although human breast milk usually ensures an adequate supply of zinc for the infant, zinc concentrations may be insufficient especially in preterm babies, so that zinc supplementation or zinc-fortified formulas may be necessary [11].

In human milk Zn is known to be bound to high molecular mass proteins (HMM) and also to low molecular mass (LMM) ligands. Currently, it is well known that the bioavailability, biological activity, and toxicity of trace elements from food strongly depend on their chemical form (species) [12]. Thus, actual zinc absorption by formula fed children could be different from that of breast fed children. This fact explains the present necessity to carry out studies based on speciation analysis in milk samples, rather than only the more commonly used total determinations [12,13].

Inductively coupled plasma mass spectrometry is a powerful analytical technique for rapid multielemental analysis of biological samples, including milk, and has been shown to be a valuable method to assess the concentration of elements of nutritional interest (e.g. Fe, Cu, Zn, Se, I, Cr, Co) in both human milk and infant formulas [14–18]. Hyphenated techniques based on coupling on line size exclusion chromatography (SEC) with the ICP-MS are now established as the most realistic and potent analytical tools available for elemental speciation analysis [19–22]. According to different authors, zinc concentration in maternal milk may change with postpartum time, from colostrum to mature milk [23,24], and also depending on the mother's pregnancy period [25]. Thus, the aim of this study is twofold: (*a*) to investigate the zinc content and its speciation in human milk from mothers of preterm and full-term infants at different stages of lactation, and (*b*) to assess differences in zinc content and speciation between human milk of the mothers of preterm infants and from preterm commercial infant formulas. A first preliminary comparison between European (Spain) and South American (Brasil) situations on this essential element is also aimed at.

The final goal is of great nutritional importance: to obtain as much information as possible about adequate zinc speciated content in infant formulas, as compared to natural maternal milk. This goal is pursued here using a powerful hyphenation of a chromatographic separation of the Zn-biomolecules followed on line by a final ICP-MS detection.

2. Experimental

2.1. Subjects and sampling

Spanish human breast milk samples from 15 mothers of preterm infants (25th–36th gestational week) and 16 mothers from full-term infants (>36th gestational week) were obtained from the Departments of Neonatology and Obstetrics & Gynecology, respectively, of the 'Hospital Central de Asturias', Oviedo (Spain) during the first month of lactation (colostrum, 7th, 14th, and 28th day after delivery). Also, data from one Brazilian mother (colostrum, 7th, 14th and 21st day after delivery) was investigated. Women with underlying chronic diseases and those who had received zinc supplementation during pregnancy were excluded. Each participant gave written informed consent for participating in this study which was approved by the institutional review board.

Mother's milk was sampled by manual expression into sterile zinc-free plastic containers. Samples were collected in the morning ≥1 h after the previous breastfeeding. The nipple area of the breast was washed with soap and water and dried with a tissue before sampling. Containers were labelled with the lactation day and the mother's gestational week. All samples were stored in a freezer until they were picked up and, once in the laboratory, were kept frozen (−20 °C) until analysis.

Preterm ($n = 8$) and full-term ($n = 8$) commercial Spanish formula milks studied were also supplied by the Service of Neonatology of the hospital. Brazilian full-term ($n = 3$) commercial formula milks were purchased in local drugstores. Formula milks were reconstituted with ultrapure water as suggested by the manufacturer (a water content of 87.3% [w/w] as expected for human milk).

2.2. Instrumentation and chemicals

For Spanish measurements, a quadrupole ICP-MS Agilent model 7500ce (Agilent Technologies, Tokyo, Japan) was used. A flow of 4 mL min^{-1} of H_2 was used in order to pressurise the octapole cell for Zn determinations. The sample introduction system consisted of a Meinhard nebuliser with double-pass glass spray chamber cooled down to 2 °C. Plasma operating conditions and acquisition parameters are presented in Table 1.

Sample digestions were carried out with a Milestone microwave oven model Ethos-1 (Microwave Laboratory Systems, Sorisole, Italy).

The chromatographic system consisted of a Shimadzu Model LC-10 A HPLC pump (Kyoto, Japan) and a Rheodyne Model 7125 (Cotati, CA, USA) injection valve fitted with a 100 μL loop. Chromatographic separations were carried out on a Superdex 200 HR 130/310 size exclusion column (10 mm × 310 mm) (Pharmacia, Amersham, Upsala, Sweden). Chromatographic operating conditions are given in Table 1

A Waters Model M484 (Waters corporation, MA, USA) UV–vis absorption detector was employed for molecular detection. Retention times and peak heights were obtained using a Philips Model PU 4815 (Cambridge, UK) computing integrator.

A Heraeus Model BiofugeStratos refrigerated ultracentrifuge was used in order to obtain milk whey from whole milk samples.

For Brazilian measurements, a quadrupole ICP-MS Thermo Scientific model iCAP Qc (Thermo Scientific, Bremen, Germany)

Table 1
Instrumental parameters for ICP-MS and HPLC.

Parameter	ICP-MS	
	Agilent 7500ce	Thermo iCAP Qc
RF power (W)	1500	1550
Carrier gas flow rate (L min^{-1})	1.12	1.0
H_2 flow rate (L min^{-1})	4	4.0
$Q_{colision\ cell}$ (V)	−13	−21
$Q_{quadrupole}$ (V)	−11.5	−18
Measured isotopes	^{64,66,67,68}Zn and ^{69,71}Ga	^{64,66,67,68,70}Zn and ^{69,71}Ga
Parameter	Chromatographic system	
	Shimadzu LC-10	Amersham ÄKTA Explorer
Column	Superdex™ 200 10/300 GL	Superdex™ 200 10/300 GL
Mobile phase	Tris–HCl 0.1 mol L^{-1}, pH = 7	Tris–HCl 0.01 mol L^{-1}, pH = 7
Injection volume (μL)	100	50
Eluent flow-rate (mL min^{-1})	0.5	0.5
UV detection (nm)	280	280
Separation range (kDa)	600–10	600 – 10
Calibration equation[a]	t_R = 88.83 – 13.81 log(MW)	t_R = 42.16 – 9.90 log(MW)

[a] t_R is the retention time (in min) and MW is the protein molecular weight (in kDa).