Journal of Developmental Origins of Health and Disease

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Association of serum and erythrocyte zinc levels with breastfeeding and complementary feeding in preterm and term infants

Talita Rodrigues Azevedo-Silva[1], Anna Caroline Pereira Vivi[1], Fernando Luiz Affonso Fonseca[2], Cibele Wolf Lebrão[3], Maria Wany Louzada Strufaldi[4], Roseli Oselka Saccardo Sarni[5] and Fabíola Isabel Suano-Souza[6]

[1]Department of Pediatrics, Escola Paulista de Medicina – Universidade Federal de São Paulo, São Paulo, Brazil; [2]Clinical Analysis, Department of Pathology of Centro Universitário Faculdade de Medicina do ABC, Universidade Federal de São Paulo – Campus Diadema, Diadema, São Paulo, Brazil; [3]Neonatal Unit, Hospital Municipal Universitário de São Bernardo do Campo – HMSBC. São Bernardo do Campo, São Paulo, Brazil; [4]Department of Pediatrics, Escola Paulista de Medicina – Universidade Federal de São Paulo, Brazil; [5]Department of Pediatrics, Centro Universitário Faculdade de Medicina do ABC; Allergy, Immunology and Clinical Rheumatology, Escola Paulista de Medicina – Universidade Federal de São Paulo, São Paulo, Brazil and [6]Escola Paulista de Medicina – Universidade Federal de São Paulo; Department of Pediatrics, Centro Universitário Faculdade de Medicina do ABC, Santo André, São Paulo, Brazil

Original Article

Cite this article: Azevedo-Silva TR, Vivi ACP, Fonseca FLA, Lebrão CW, Strufaldi MWL, Sarni ROS, and Suano-Souza FI. (2023) Association of serum and erythrocyte zinc levels with breastfeeding and complementary feeding in preterm and term infants. *Journal of Developmental Origins of Health and Disease* **14**: 53–60. doi: 10.1017/S2040174422000447

Received: 23 April 2022
Revised: 16 June 2022
Accepted: 6 July 2022
First published online: 29 July 2022

Keywords:
Zinc; preterm infant; food intake; infants; breastfeeding

Address for correspondence:
Fabíola Isabel Suano DE Souza, St. Botucatu, 598 Vila Clementino. São Paulo (SP), CEP 04023-062, Brazil.
Email: suano.souza@unifesp.br

Abstract

Zinc is an important nutrient involved in cell division, physical growth, and immune system function. Most studies evaluating the nutritional status related to zinc and prematurity were conducted with hospitalized preterm infants. These studies show controversial results regarding the prevalence of deficiency, clinical implications, and the effect of zinc supplementation on mortality, infectious diseases, and growth in these groups. This study aimed to compare serum and erythrocyte zinc levels in a group of preterm and full-term infants after 9 months of age, and related the zinc levels to dietary intake and anthropometric indicators in both groups. This cross-sectional study compared 43 preterm infants (24 to 33 weeks) aged 9–24 months to 47 full-term healthy infants. Outcome measures: anthropometric indicators and dietary intake. Blood sample for serum and erythrocyte zinc levels (ICP-MS, Inductively Coupled Plasma Mass Spectrometry). There was no difference between the groups regarding the mean of serum and erythrocyte zinc. Variables associated with higher serum zinc levels were breastfeeding at evaluation ($\beta = 20.11$ μg/dL, 95% CI 9.62–30.60, $p < 0.001$) and the later introduction of solid foods ($\beta = 6.6$ μg/dL, 95% CI 5.3–11.4, $p < 0.001$). Breastfeeding was also associated with higher erythrocyte zinc levels. The zinc levels were adequate in both groups, there was no association with anthropometric indicators or dietary intake and were slightly influenced by breastfeeding and time of solid food introduction.

Introduction

Hidden hunger is a type of malnutrition associated with micronutrients deficiency. The World Health Organization (WHO) reports that more than two billion people worldwide have 'hidden hunger', and the principal micronutrients related to this condition are iron, zinc, vitamin A, iodine, and folic acid.[1,2] Zinc is a vital micronutrient involved in cell division, physical growth, and immune system function. This micronutrient is involved in more than 300 metalloenzymes, influences more than two thousand transcription factors, and participates in the regulation and expression of hundreds of genes.[3,4] Mild zinc deficiency can be associated with impaired immune response and cell replication, increasing the susceptibility to infectious and impairing children's growth. The moderate and severe deficiency is called acrodermatitis enteropathica, whose manifestations are irritability, alopecia, diarrhea, and stunting.[3-5]

A meta-analysis that used zinc serum levels (<65 μg/dL), stunting prevalence, and dietary zinc intake inadequacy as markers of zinc deficiency found an alarming deficiency percentage (>20% of the population under five years old) in 23 of the 25 countries assessed.[5] Zinc deficiency is a public health problem in low- and middle-income countries.[3] The zinc serum levels alone are not a good marker of mild zinc deficiency.[4] Zinc supplementation in infants younger than 6 months old, and from 6 to 12 months old was related to a slight improvement in linear growth and lower prevalence of the diarrheal disease, respectively.[6,7]

Preterm newborns are at risk for zinc deficiency due to lower reserves, accelerated postnatal growth, immature gastrointestinal tract, diseases developed during hospitalization, and lower dietary intake of this micronutrient in postnatal follow-up.[8,9] During hospitalization, preterm newborns receive zinc through parenteral and enteral nutrition. Breast milk is the principal source for enteral nutrition in preterm newborns. The use of human milk in preterm infant is associated with a reduced risk of necrotizing enterocolitis, sepsis, and better indicators of long-term neuropsychomotor development.[10] However, the amount of zinc in breast milk varies considerably and decreases with lactation time, which may be insufficient to meet the needs of preterm newborns who may require human milk fortification.[11]

This observation was well documented in several studies, that is, there is a physiological drop in breast milk zinc concentrations with progressing lactation that occurs regardless of maternal zinc status, diet, and supplementation.[12] Foods which are source of zinc such as beef, eggs, and fish should be included in the complementary feeding from the sixth month of life. Plant-based foods such as pulses and grain products are also a good source of zinc, but they contain phytates, which decrease its bioavailability. Previous studies show that the introduction of food sources of animal origin occurs later, between 8 and 12 months, which increases the risk of zinc deficiency at this stage.[13] Specifically, concerning infants born prematurely, a group that is even more vulnerable to zinc deficiency, there are few studies available that assess in more detail the composition of the complementary feeding and the repercussions in short- and long-term outcomes in this population.

Few studies have evaluated the zinc-related nutritional status in moderately and extremely preterm infants after hospital discharge.[14-20] This study aimed to compare serum and erythrocyte zinc levels in a group of preterm and full-term infants after 9 months of age and related the zinc levels to dietary intake and anthropometric indicators in both groups.

Method

Study design

A cross-sectional study was carried out from 2018 to 2019 with 43 preterm infants (preterm group, gestational age from 24 to 33 weeks), with chronological age 9 to 24 months, at the follow-up clinic of the Hospital Municipal Universitário de São Bernardo do Campo, São Paulo, Brazil. The comparison group consisted of 47 healthy full-term infants (term group), adequate for gestational age and weighing more than 2,500 grams, of the same age, in follow-up at the Primary Health Care of the same city.

The Hospital adopts the Kangaroo Method and is a Baby-Friendly Hospital. About 14% of births are preterm, and the breastfeeding rate for preterm infants at hospital discharge is 82%. Preterm newborns with gestational age <34 weeks or birth weight <1,500 grams are followed at the outpatient clinic up to 6 years of age by a multidisciplinary team. The follow-up happens in parallel to that performed by the Primary Care Health. For all preterm newborns were prescribed daily supplementation of vitamin D (400 IU) and iron (2–4 mg/kg of body weight) after hospital discharge up to 2 years of age. None of the infants received zinc supplementation.

Infants with severe malformations (heart and central nervous system defects), genetic syndromes, cerebral palsy, oxygen-dependent children at evaluation, those who did not feed exclusively orally, who had intolerances and food allergies, who were unable to provide telephone contact or missed at the day of data collected, and whose family refused to participate were excluded from the sample (Fig 1).

The Research Ethics Committee of Universidade Federal de São Paulo approved the study (N° 2.937.127), and all methods were performed following the Declaration of Helsinki. The children's legal guardians signed the informed consent form after the interview and explanation by the researchers regarding the study's steps and procedures.

Collected data

General information

Information on the socioeconomic status, household income, mother's education level, and maternal health during pregnancy were collected. Data collected from medical records were weight, length, head circumference, gestational age, and Apgar score at birth. The gestational age was calculated according to the date of the last menstruation. If this information was not available, we used the first-trimester ultrasound and, finally, the clinical evaluation of the newborn (*New Ballard Score*).[21] Fenton's reference[22] was adopted to classify the newborns into small (SGA), adequate (AGA), and large (LGA) for gestational age when the birth weight for gestational age was below the 10th percentile, from the 10th to the 90th, and above the 90th, respectively.

Anthropometry

At the time of evaluation, anthropometric measurements were performed by an experienced dietician at clinical evaluation. Weight was measured on a digital scale graduated in grams, length with a measuring board graduated in millimeters, and head and arm circumference with an inextensible measuring tape.[23] The infants were unclothed and without diapers during all procedures.

The anthropometric measures were used to calculate the indicators z-scores of body mass index (BMIZ), length/age (LAZ), and head circumference/age (HCAZ) through the WHO Anthro v.3.2.2. The cutoff points employed to classify anthropometric indicators were proposed by the World Health Organization.[24] The corrected age of 40 weeks was used to calculate anthropometric indicators for preterm infants.

Dietary intake

Three 24-hour recalls were collected over a 2-week period by a trained nutritionist. Three, two, and one 24-hour recalls were available in 24 (26.7%), 16 (17.7%), and 45 (50%) infants included in the study. Preterm infants had lower number of 24-hour recall responses when compared to the control group (two or three responses: preterm group 32.6% *vs.* term group 57.4%, $p = 0.021$).

Dietary recalls were analyzed using DietWin® program, which uses the food composition tables proposed by the United States Department of Agriculture[25] and the Brazilian Food Composition Table.[26] Consumption of milk and infant formula was excluded from the calculation of main meals (solid foods). The frequency of breast feedings per day (times in 24 hours) was recorded. The main meals were defined as solid foods consumed at lunch and dinner per the traditional Brazilian eating habits, in general, rice, beans, meats, poultry, fish, and vegetables.[27] The dietary intake of energy, protein, zinc, and iron in main meals, infant formula, and cow´s milk was showed separately between preterm and full-term groups, stratified into breastfed and non-breastfed infants because was not possible to measure the volume of breast milk consumed in breastfed children.

Additional dietary data collected included the age of onset and sequence of introduction of complementary foods, breastfeeding practices and duration, and use of infant formula and whole cow's milk.

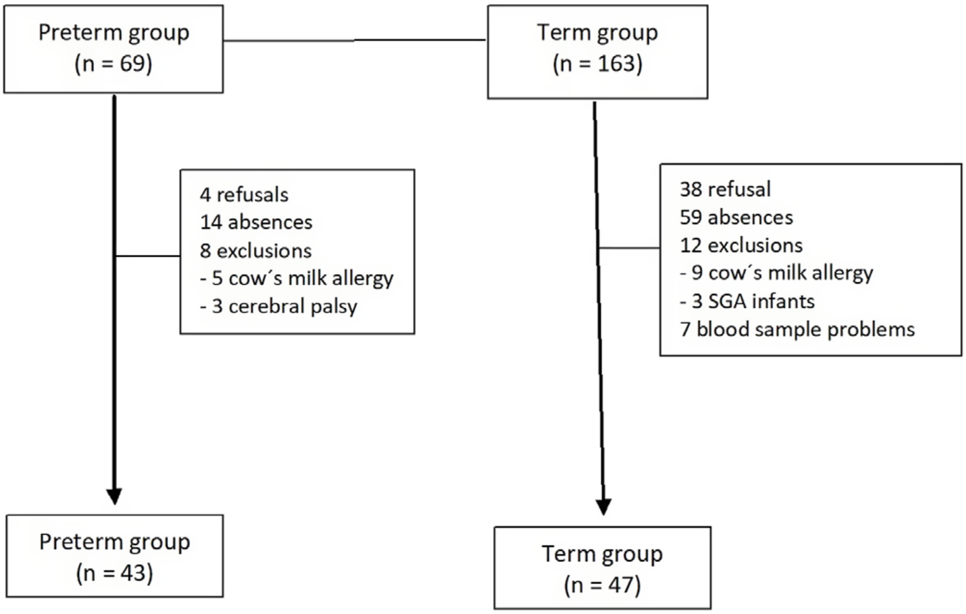

Fig. 1. Sample inclusion flowchart.

Laboratory tests

The blood samples (8 mL) were collected with 3 hours of fasting in the morning. The blood sample was divided into tubes for collecting metals (Trace EDTA BD Ref. 368381), dry tube, and EDTA tube. The material was immediately transported to the Clinical Analysis Laboratory, where the sample preparation, laboratory analysis, and storage were performed. Samples that were not analyzed immediately were stored in a freezer at −80 °C.

The blood count was performed with the multi-parameter automated hematology analyzer (Cell-Dyn Ruby) using the Multi-Angle Polarized Scatter Separation technology and laser flow cytometry. Anemia was defined by the level of hemoglobin (Hb) below 11 g/dL.[28] The ultrasensitive C-reactive protein (us-CRP) was measured [human CRP (C-reactive protein) ELISA Kit, FineTest® Wuhan Fine Biotech].

Serum and erythrocyte zinc levels were determined by the inductively coupled plasma mass spectrometry method (ICP-MS). Red cell lysis was performed with phosphate buffer. The reference values adopted for serum and erythrocyte zinc were 65 µg/dL[4] and 40 µg/g hemoglobin (µg/gHb),[29] respectively.

Statistical analysis

The analyses were performed in the statistical package SPSS 25.0 (IBM®). Categorical variables were presented as absolute numbers and percentages and compared with the Chi-square test. The distribution of continuous variables was assessed using the Shapiro–Wilk test, histograms, and Kurtosis values. The variables with normal distribution were presented as mean±standard deviation and compared by the Student's *t*-test for independent variables. Variables with non-normal distribution were presented as medians and interquartile ranges (p25–p75) and compared with the Mann–Whitney test.

The linear regression method was employed for the multivariate analysis. The variables were included in the model after the analysis of multicollinearity and interpretation of "Tolerance" and "Variance inflation factor" (VIF) values. The serum and erythrocyte zinc were used as dependent variables. The independent variables without collinearity, which showed a statistically significant difference between the preterm and full-term groups, and the ones with clinical relevance were included in this analysis. A significance level of 5% was adopted in all analyses.

Employing α-bidirectional = 0.05 and β = 0.20 allowed the included sample (45 infants per group) to detect a difference of 10 µg/dL of serum zinc between the groups (standardized magnitude of the effect of 0.6). For this calculation, we used data from the paper published by Cho et al., 2019,[17] which found a mean and standard deviation in serum zinc levels of 81.4 ± 18.7 µg/dL in a group of preterm infants.

Results

Table 1 shows the general characteristics of population studied. In the preterm group, 24 (55.8%) of the infants were male, the mean birth weight, gestational age, and corrected age were 1,245 ± 381.7 grams, 29.9 ± 2.3 weeks, and 14.3 ± 6.4 months, respectively. There was no difference between the groups in the socioeconomic variables studied, such as household income and maternal education.

The duration of total breastfeeding was lower [7.0 months (3.0; 9.2) *vs.* 11.3 months (6.0; 16.4); $p = 0.003$] and the age of introduction of solid foods was earlier [7.0 months (6.3; 8.0) *vs.* 6.0 months (5.0; 6.0), $p < 0.001$] in infants of preterm group compared to the full-term group, using chronological age (Table 1).

At the time of evaluation, the anthropometric indicators showed lower mean values, and we observed a lower percentage of breastfed infants (18.6% *vs.* 53.2%; $p = 0.001$) in the preterm group compared to the full-term group (Table 1).

There was no statistically significant difference between the preterm and full-term group in the serum zinc (94.0 ± 23.4 µg/dL *vs.* 90.3 ± 18.0 µg/dL; $p = 0.450$) and erythrocyte zinc levels (119.4 ± 23.8 µg/gHb *vs.* 112.7 ± 23.1 µg/gHb; $p = 0.307$) (Table 2). There was no difference in the dietary intake of zinc, iron, energy, and protein between the groups (Table 3).

In the linear regression, the variables associated with higher serum zinc concentrations were breastfeeding at evaluation (β = 20.11 µg/dL, 95% CI 9.62–30.60, $p < 0.001$) and time of introduction of solid foods (β = 6.6 µg/dL, 95% CI 5.3–11.4, $p < 0.001$). Breastfeeding was also associated with higher erythrocyte zinc

Table 1. General characteristics of infants evaluated

Variables		Preterm group (*N* = 43)	Term group (*N* = 47)	*P*-value
General				
Gender	Male	24 (55.8%)	20 (42.8%)	0.291*
Gestational age	Weeks	29.9 ± 2.3	39.0 ± 1.2	<0.001§
Birth weight	grams	1245 ± 381.7	3288 ± 459	<0.001§
Delivery type	Cesarean	27 (62.8%)	23 (48.9%)	0.209*
Twin	Yes	4 (9.3%)	0 (0.0%)	0.002*
GA Classification	SGA	8 (18.6%)	0 (0.0%)	<0.001*
Chronological age	Months	16.7 ± 6.3	15.9 ± 4.5	0.493§
Adjusted age	Months	14.3 ± 6.4	15.9 ± 4.5	0.165§
Maternal characteristics				
Maternal age	Years	29.9 ± 7.3	29.4 ± 6.8	0.781§
Primiparous	Yes	12 (38.7%)	17 (37.0%)	0.532*
Supplementation	Iron	23 (53.5%)	29 (61.7%)	0.523*
	Folic acid	22 (51.2%)	18 (87.2%)	<0.001*
Pregestational BMI	Malnutrition	3 (10.3%)	1 (2.4%)	
	Eutrophy	11 (37.9%)	20 (48.8%)	0.505*
	Overweight	9 (31.0%)	12 (29.3%)	
	Obesity	6 (20.7%)	8 (19.5%)	
Current eating habits				
Breastfeeding	Yes	8 (18.6%)	25 (53.2%)	0.001*
Infant formula	Yes	17 (39.5%)	14 (29.8%)	0.379*
Cow milk	Yes	28 (65.5%)	32 (68.1%)	0.825*
Iron supplementation	Yes	28 (65.1%)	20 (42.6%)	0.037*
Iron dosage	mg/kg	1.6 (1.2; 2.3)	1.1 (0.8; 1.3)	0.008‖
Dietary background				
Exclusive BF	Yes	30 (69.8%)	37 (78.7%)	0.346*
Exclusive BF time	Months	3.0 (2.0; 6.0)	4.0 (3.0; 6.0)	0.430‖
Total BF time	Months	7.0 (3.0; 9.2)	11.3 (6.0; 16.4)	0.003‖
Infant formula use	Yes	34 (79.1%)	35 (74.5%)	0.628*
Onset of infant formula use	Months	2.0 (1.0; 3.0)	2.0 (0.0; 5.0)	0.086§
Whole cow´s milk use	Yes	14 (32.6%)	28 (59.6%)	0.012*
Onset of cow´s milk use	Months	12.0 (7.0; 16.5)	8.0 (7.0; 9.0)	0.001‖
Onset of fruits	Months	7.0 (6.2; 8.0)	6.0 (4.0; 6.0)	<0.001‖
Onset vegetables	Months	7.0 (6.3; 8.0)	6.0 (5.0;6.0)	<0.001‖
Onset of meat/poultry	Months	7.0 (7.0; 8.0)	6.0 (6.0;7.0)	<0.001‖
Onset of fish	Months	7.5 (6.0; 9.0)	8.0 (7.0; 11.0)	<0.001‖
Onset eggs	Months	7.0 (6.0; 9.0)	8.0 (6.0; 8.0)	<0.001‖
Family food	Months	11.5 (8.2; 12.0)	10.0 (9.0; 12.0)	0.250‖
Anthropometric indicators				
Body Mass Index	Z-score	−0.42 ± 1.15	0.33 ± 1.22	0.004§
Length age	Z-score	−1.27 ± 1.42	0.30 ± 1.44	<0.001§
Head circumference	Z-score	−0.60 ± 1.30	0.18 ± 1.04	0.003§

(*Continued*)

Table 1. (*Continued*)

Variables		Preterm group (*N* = 43)	Term group (*N* = 47)	*P*-value
Length Z-score	Short stature < −2 SD	11 (25.6%)	1 (2.1%)	<0.001*
BMI Z-score	Thinness/malnutrition < −2 SD	5 (11.6%)	0 (0.0%)	
	Normal −2 to + 1 SD	38 (88.4%)	44 (93.6%)	0.016*
	Overweight/Obesity > +1 SD	0 (0.0%)	3 (6.4%)	

GA, gestational age; SGA, small for gestational age; BF, Breastfeeding; BMI, Body Mass Index; SD, standard deviation.
*Significance level of the Chi-square.
[§] Student's *t*-test.
[‖] Mann–Whitney tests.

Table 2. Comparison of laboratory variables between preterm and full-term group

Variables		Preterm group (*N* = 43)	Term group (*N* = 47)	*P*-value
C-reactive protein (*n* = 79)	mg/L	1.0 (0.2;2.0)	1.0 (1.0;2.6)	0.372[§]
Hemoglobin (*n* = 75)	g/dL	12.1 ± 1.3	12.0 ± 0.8	0.747*
Mean corpuscular volume	mcm^3	76.0 ± 6.6	74.7 ± 4.2	0.315*
Serum zinc (*n* = 78)	µg/dL	94.0 ± 23.4	90.3 ± 18.0	0.450*
Erythrocyte zinc (*n* = 70)	µg/dL	1413.6 ± 219.3	1352.1 ± 267.0	0.358*
Erythrocyte zinc (*n* = 70)	µg/gHb	119.4 ± 23.8	112.7 ± 23.1	0.307*
Inadequate values				
Hemoglobin	<11 g/dL	4 (13.3%)	4 (9.1%)	0.707[‖]
Serum zinc	<65 µg/dL	2 (6.3%)	2 (4.4%)	0.554[‖]
Erythrocyte zinc	<40 ug/Hb	0 (0.0%)	0 (0.0%)	–

*Significance level of the Student's *t*-test.
[§] Mann–Whitney.
[‖] Chi-Square tests.

levels at evaluation (β = 18.8 µg/dL, 95% CI 3.7–33.8, $p = 0.015$) (Table 4).

Discussion

Serum zinc deficiency occurred in less than 5% of infants born prematurely and was not associated with thinness/malnutrition and short stature. There are no well-defined cutoffs for serum and erythrocyte zinc levels in term and preterm infants, and these measurements by themselves could not be sensitive enough to act as a biological marker for zinc deficiency, especially for subclinical zinc deficiency. A study with 27,801 individuals aged between 6 months and 74 years showed that values below 65 µg/dL (2.5th percentile) of serum zinc could be low for children under ten years of age.[29] However, the authors did not propose specific cutoffs for infants, nor did they relate these inappropriate values to relevant clinical outcomes.

Preterm newborns have lower serum zinc levels than term infants in the first months of life, and in this group, zinc supplementation during hospitalization is associated with reduced mortality, improved weight gain, and linear growth up to two years of age.[14,15,29] Studies that evaluated serum zinc levels and the effects of supplementation in clinical outcomes such as growth[7,18,19] and development[15,18] in preterm infants after hospital discharge show divergent results.[30-32] These publications included moderate or late preterm infants with younger age and lower breastfeeding rates than our study.

Serum and erythrocyte zinc levels were adequate in boths groups, and breastfed infants at evaluation in this study had higher zinc levels. This finding is similar to Waunen et al., 1999,[18] that found higher zinc concentrations in the hair of breastfed preterm and full-term infants at 6- and 12-month corrected age. However, these results differ from other studies, which found lower zinc concentrations in breastfed infants than those receiving infant formulas.[33,34] The zinc content in human milk varies considerably and decreases with lactation time.[34,35] Despite this progressive reduction and lower content, the bioavailability of zinc in human milk is better than in infant formula (60% *vs.* 24%).[36,37] A recent pilot study showed that a mother's zinc intake (diet and supplementation) was positively associated with zinc content in breast milk.[38]

In addition to the better bioavailability of zinc in human milk, two other factors may explain the association of breastfeeding with better blood concentrations of zinc in this study. Breastfed children usually have adequate time of introduction, quality, and variety of

Table 3. Dietary intake in preterm and full-term infants stratified by breastfed or not breastfeeding

	Preterm group ($n = 43$)		Term group ($n = 47$)	
Variables	Breastfed ($n = 8$)	Not breastfed ($n = 33$)	Breastfed ($n = 25$)	Not breastfed ($n = 21$)
Main meals				
Energy (kcal/kg)	35.4 (19.7; 50.6)	26.9 (21.9; 36.6)	18.5 (11.5; 27.6)	23.0 (17.0; 35.7)
Protein (g/kg)	2.4 (1.3; 3.0)	1.8 (1.3; 2.8)	1.4 (0.7; 2.0)	1.2 (1.7; 2.7)
Iron (mg)	3.4 (1.8; 4.7)	5.9 (1.93; 11.6)	3.0 (1.9; 4.6)	3.2 (1.9; 7.9)
Zinc (mg)	2.0 (1.5; 4.2)	1.8 (1.2; 3.1)	1.8 (1.0; 2.9)	2.3 (1.1; 2.8)
Infant formula				
Energy (kcal/kg)	62.3 (52.8; 71.9)	51.3 (44.8; 57.9)	53.3 (43.3; 71.9)	50.1 (43.8; 59.6)
Protein(g/kg)	2.4 (1.8; 3.0)	1.7 (1.3; 1.9)	1.4 (1.4; 2.2)	1.5 (1.4; 2.1)
Iron (mg)	10.1 (9.8; 10.4)	6.4 (4.2; 7.5)	5.4 (5.4; 9.4)	8.1 (6.6; 9.4)
Zinc (mg)	7.1 (6.3; 8.9)	4.0 (3.3; 5.3)	5.4 (4.6; 6.6)	5.3 (4.9; 6.2)
Cow´s milk				
Energy (kcal/kg)	12.9 (12.2; 16.7)	36.3 (29.7; 44.4)	20.2 (6.6; 25.6)	40.0 (23.7; 52.7)
Protein (g/kg)	0.6 (0.5; 0.8)	1.5 (1.04; 1.8)	0.8 (0.3; 1.3)	1.7 (1.2; 2.5)
Iron (mg)	0.2 (0.2; 0.6)	0.6 (0.4; 7.5)	0.3 (0.1; 0.5)	0.7 (0.5; 0.8)
Zinc (mg)	0.8 (0.6; 1.6)	2.3 (1.6; 4.8)	1.2 (0.4; 2.1)	2.7 (2.0; 3.1)

Significance level of the Mann–Whitney test between preterm group *vs.* term group ($p > 0.05$).

Table 4. Multivariate analysis assessing factors associated with the levels of serum and erythrocyte zinc in infants

Dependent variable	Predictors	β	95% confidence interval		*P*-value
Serum zinc (μg/dL)	Age (months)	0.11	−0.95	1.18	0.833
($n = 70$)	Gender (male)	0.47	−8.61	9.56	0.917
	Group (preterm)	6.20	−5.19	17.60	0.280
	Zinc intake (mg)	0.31	−0.74	1.37	0.559
	BMI Z-score	1.31	−3.13	5.76	0.556
	Length Z-score/age	3.16	−0.20	6.53	0.065
	C-reactive protein (mg/L)	−0.88	−2.02	0.25	0.127
	Breastfeeding (yes)	20.11	9.62	30.60	0.000
	Infant formula (yes)	3.26	−10.63	17.17	0.640
	Whole cow´s milk (yes)	−6.86	−20.24	6.50	0.308
	Solid foods onset (months)	6.63	3.72	9.55	0.000
Erythrocyte zinc (ug/gHb)	Age (months)	0.65	−1.096	2.39	0.457
($n = 55$)	Gender (male)	3.86	−10.127	17.86	0.580
	Group (preterm)	14.20	−3.583	31.99	0.115
	Zinc intake (mg)	0.97	−0.493	2.44	0.188
	BMI Z-score	1.78	−4.552	8.11	0.574
	Length Z-score/age	−2.03	−7.260	3.19	0.438
	C-reactive protein (mg/L)	0.92	−0.675	2.51	0.251
	Breastfeeding (yes)	18.83	3.782	33.88	0.015
	Infant formula (yes)	−12.96	−33.367	7.44	0.207
	Whole cow´s milk (yes)	−17.22	−37.637	3.18	0.096
	Solid foods onset (months)	−1.41	−5.619	2.80	0.503

the foods during complementary feeding[39]; and lower frequency of infectious conditions that lead to more significant zinc depletion, such as diarrheal and respiratory diseases.[40]

In this study, the introduction of solid foods in the preterm group agreed with the current recommendations (5–8 months of chronological age). The early introduction of solid foods (before 3 months corrected age or 5 months chronological age), and consumption of foods with low energy density and micronutrients are common problems in the complementary feeding in preterm infants and are associated with nutritional deficiencies.[41-43]

The infant feeding practices observed in this study, characterized by low breastfeeding rates, and early onset of infant formula (at 2 months) are similar to earlier reports.[44,45] In addition, we observed that the energy intake in the infants who received infant formula was almost double that offered in the main meals. This finding is against the WHO recommendation, which suggests that the principal source of energy and nutrients in an infant´s diet at around 12 months old should come from solid foods in the main meals. Formula-fed infants who consume a higher volume and more energy-dense milk in early life lead to faster growth which could potentially program a greater risk of long-term obesity.[46]

The dosage of zinc in biological samples requires care so that there is no contamination and interference from environmental factors in the pre-analytical phase (hemolysis, tube type, sample processing and storage temperature). The methods used for zinc analysis also vary in sensitivity and complexity of the operation. They include atomic absorption spectrometers (AAS, flame or graphite furnace), inductively coupled plasma optical (atomic) emission spectrometers (ICP-OES/ICP-AES), and ICP mass spectrometers (ICP-MS).[47] In our study, all sample collection, storage, and dosing procedures were carefully controlled at all stages, considering these factors, and ensuring the quality of the operation and the results obtained.

Strengths of this study are the preterm group with moderate (<34 weeks) and very low birth weight (<1500 g) newborns, the inclusion of a healthy comparison group of the same city, with socioeconomic and cultural characteristics similar to the preterm group, and the detailed dietary intake of infants. The limitations are the low number of children included, the impossibility of calculating dietary intake in breastfed infants, the lack of other zinc biomarkers such as metallothioneins, high variation in the age of children included (from 9 up to 24 months), and the non-systematic collection of information about diseases developed by the group of preterm infants after hospital discharge.

Our results showed that the zinc levels were adequate in both groups, and there was no association with anthropometric indicators or dietary intake. The zinc levels were slightly influenced by breastfeeding and time of solid food introduction. Nonetheless, more studies using better biological markers on zinc-related nutritional status are required to define more clearly these associations.

Acknowledgments. Multidisciplinary team of Hospital Municipal Universitário de São Bernardo do Campo and the Primary Care.

Financial Support. FAPESP – São Paulo State Research Support Foundation - Number: 2016/09428-1 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES.

Conflit of interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and this study has been approved by the Research Ethics Committee of Universidade Federal de São Paulo under number N° 2.937.127.

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