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Original Article

Breast milk zinc transfer and early post-natal growth among urban South Indian term infants using measures of breast milk volume and breast milk zinc concentrations

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Abstract

Zinc (Zn) deficiency in infancy and early childhood is of public health concern in developing countries. This study aimed to longitudinally assess Zn intake of urban South Indian term infants in the first 6 months of life using measures of breast milk (BM) volume and BM Zn concentrations and, additionally, to study the effect of BM Zn intake on infant length and weight gain. BM intake by the deuterium dilution technique, BM Zn concentration at months 1, 3 and 6, as well as serum Zn level at months 3 and 6 were assessed in 50 mother–infant pairs. BM intake significantly declined from 627 mL day^{-1} at month 1 to 608 mL day^{-1} at month 6 ($P < 0.01$). BM Zn concentration and intake significantly declined from month 1 to month 6 ($P < 0.001$ for both). Mean infant serum Zn level at months 3 and 6 were 93.0 ± 27.1 and 99.6 ± 30.1 μg dL^{-1}, respectively. Infant BM Zn intake at months 1 and 3 was not associated with the weight and length gain between 1–3 and 3–6 months, respectively. Zn intake from BM, maternal BM Zn content and serum Zn levels were not significantly different between small-for-gestational age and appropriate-for-gestational age infants. Therefore, among urban south Indian term infants less than 6 months of age, BM Zn intakes were low, owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range. Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

Keywords: infant, breast milk zinc content, breast milk zinc intake, serum zinc, length gain and weight gain.

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Introduction

Zinc (Zn) deficiency in infancy and early childhood is an important cause of stunting, increases infectious disease morbidity and mortality due to diarrhoea and pneumonia, and therefore is of public health concern especially in developing countries (Hambidge *et al*. 1997; Hambidge & Krebs 2003). Zn deficiency is responsible for approximately 4% of the worldwide morbidity and mortality burdens of young children (Black *et al*. 2008).

For term infants with normal birthweight, Zn requirements are generally assumed to be met by exclusive breastfeeding due to high bioavailability of Zn in human milk (Krebs *et al*. 1996). However, at 5–6 months of age, infants may become marginally Zn deficient due to the physiological decline in breast milk (BM) Zn concentrations, making the infants vulnerable to sub-optimal Zn intakes and thereby impaired growth (Walravens *et al*. 1992; Krebs 1999). In addition, a decreased intake of Zn from BM due to lower volumes of BM intake, attributed to poor breastfeeding practices, may predispose infants to an elevated risk of Zn deficiency even before 6 months. Equally, an infant born small-for-gestational age (SGA) may be at increased risk for Zn deficiency in comparison to infants born appropriate-for-gestational age (AGA) as they may either have increased requirements for Zn during the first few months of life which could limit their potential for catch up growth during these months (Castillo-Duran *et al*. 1995), or they may have lower neonatal reserves of Zn (Hambidge & Krebs 2003) and/or have impaired Zn absorption or increased endogenous losses (Krebs *et al*. 2006). As SGA infants form a large proportion of low-birthweight infants in developing countries (Hambidge & Krebs 2003), it would also be of interest to assess intakes and levels of Zn among these infants as well. In general, there is a paucity of data on Zn intakes among breastfed infants less than 6 months of age in India based on measured volumes of BM intake and BM Zn concentrations.

We therefore assessed the longitudinal Zn intake of urban South Indian term infants in the first 6 months of life by measuring actual volumes of BM consumed using a non-invasive and accurate 'dose to mother' deuterium dilution method and measuring the maternal BM Zn concentrations. In addition, we assessed if Zn intake from BM was associated with the infant's length and weight gain.

Methods

Study setting

The present study was a prospective observational study of infants from birth to 6 months of age in St. John's Medical College Hospital, Bangalore, India, which is a tertiary care urban hospital drawing patients of diverse socio-economic status, from urban slums to high-income residential areas. The Institutional Ethical Review Board at St. John's Medical College Hospital approved all study procedures and a signed informed consent was obtained from each study subject at enrolment.

Study population

Pregnant women aged 18–40 years, who planned to exclusively breastfeed their infants until 6 months of age, and who were in the last trimester of pregnancy, were invited to participate in the study. Women with multiple pregnancies and any illness were excluded. Of 65 women who consented to be part of the study, 58 were recruited into the study. During the study, 8 mother–infant pairs were lost to follow-up such that 50 mothers and their infants completed the study successfully and were followed up at months 1, 3 and 6.

Socio-demographic and anthropometric information

Information on age, education, parity, occupation, income and obstetric history was obtained by interview from the study subjects. A digital balance (Soehnle, Murrhardt, Germany) was used to record the weights of all mothers to the nearest 100 g, and height was measured to the nearest 0.1 cm using a locally constructed stadiometer. Maternal body mass index (BMI) was calculated as weight in kilograms by the square of height in meters (kg m^{-2}). The nude weight of the infant was measured to the nearest 10 g using a portable paediatric weighing scale (Salters, Tonbridge, Kent, UK), and length was measured using a locally constructed infantometer to the nearest 0.1 cm within 48 h of birth. Only term infants (gestational age >37 weeks) were considered. The newborn was designated SGA if the birthweight was

Key messages

- Among urban south Indian term infants less than 6 months of age, BM Zn intakes were low; owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range.
- BM Zn intake was not related to infant weight gain and length gain.
- Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

<10th percentile for the gestational age at birth compared with the reference population and AGA if the birthweight was >10th percentile for the gestational age at birth (World Health Organization 1995). Infant weight and length were also recorded at months 1, 3 and 6. Infant weight-for-length, length-for-age and weight-for-age were calculated and presented as deviations in z-scores [standard deviation (SD)] from the World Health Organization (WHO) reference population mean value for age and gender (World Health Organization 2006). All anthropometric measurements were done by a trained research assistant in duplicate and the mean was recorded.

Dietary data

A pre-tested interviewer-administered 24-h recall was used to assess the intake of nutrients in mothers at 1, 3 and 6 months post-partum. While administering the questionnaire, standard measures were placed before the mother to quantify the portion size of each food item. The nutrient values were obtained from the nutrient database developed (Bharathi *et al*. 2008) and intake of Zn was thereby calculated. A 24-h dietary recall from the mother, of the infant's solid or liquid intake (if any) during the previous day of the interview, was also captured at months 1, 3 and 6. Mothers were asked to quantify the amount of the formula used (usually in powder form) to prepare the feed using standard measures and the nutrient composition (Zn content) of that particular formula was recorded. In addition, questions were asked about leftovers and care was taken to record the amount consumed after excluding the leftovers. In the case of biscuits, the number consumed was noted and the nutrient content was calculated using the nutrient information available on the pack. In the case of homemade porridges and other breakfast foods, the nutrient database previously described was used to compute intakes of Zn.

Information on infant morbidity

Infant morbidity symptom data (for diarrhoea, dysentery, fever, cough and cold) was collected by maternal recall at months 1, 3 and 6. The percentage of infants with any particular illness was calculated.

BM and non-breast milk (NBM) water intake

BM intake and NBM water intake was measured using the 'dose-to-the mother' deuterium-oxide turnover technique at the infant age of 1, 3 and 6 months (IAEA 2010). A baseline saliva sample of 2 mL from the mother and infant was collected on day 0, after which the mother received an oral dose of 30 g ^2H_2O, measured to the nearest 0.01 g. Saliva samples were then collected using adsorbent sorbettes (Salimetrics, Suffolk, UK) from the mother and the infant over a period of 14 days. The samples were then centrifuged at 3500 rpm and stored at –20°C for subsequent analysis of their ^2H enrichment using Fourier transformed infrared spectrophotometry (IAEA 2010). Intake of BM and water from NBM sources [an indicator of complementary foods (CFs) introduced] was calculated by fitting the isotopic enrichment data to a mathematical model for water turnover in the mother–baby pair and the transfer of water from mother to the baby, based on assumptions as described earlier (IAEA 2010).

BM Zn and serum Zn analysis

Women were asked to provide three mid-feeding BM samples (5 mL), one each in the morning, afternoon and evening. Milk samples were collected in acid-washed plastic bottles to avoid Zn contamination, by hand expression after the nipple and areola were cleaned with deionised water and dried. The samples were centrifuged and stored immediately at –20°C in tightly sealed containers until analysis. BM Zn was determined using flame atomic absorption spectrophotometry (Ice 3500, Thermo, Cambridge, UK) (Rajalakshmi & Srikantia 1980). Briefly, BM samples were centrifuged to remove solid particles, and supernatant was diluted with 0.2% nitric acid solution. The Zn in the sample was emulsified with a drop of 1% triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), aspirated into an acetylene flame and measured at 213.9 nm. Zn standards (Merck, Darmstadt, Germany) ranging from 0.2 to 1.0 ppm of zinc, prepared in 0.2% nitric acid solution, were used to create calibration curves. The intra- and inter-day assay coefficients of variability (CVs) were 1.6% and

2.0%, respectively. Zinc intake of the infant day^{-1} was calculated as the product of BM intake day^{-1} in mL day^{-1} and BM Zn concentration mL^{-1} of BM. A 2 mL of fasting blood sample from the mother and 1 mL of non-fasting blood sample from the infant was drawn by venipuncture and collected into trace metal-free vacuette tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 3 and 6 months post-partum. Once the blood samples were obtained, they were immediately stored in cool boxes and transferred to the laboratory for further processing. The median time between the blood draw and the spinning and the separation of serum from the clot was 3 h. Blood samples for Zn analysis were centrifuged at 3000 rpm for 10 min, and the serum was kept frozen at –80°C until analysis. The serum Zn concentration was measured using flame absorption spectrophotometer (Ice 3500, Thermo) (Smith *et al*. 1979). We were able to obtain infant blood samples only for 31 and 32 infants at 3 and 6 months, respectively. The intra- and inter-day assay CVs were 1.4% and 1.4%, respectively. An infant was considered to be Zn deficient if the serum Zn level was below 65 μg dL^{-1}, while the mother was considered to be Zn deficient if the serum Zn level was below 70 μg dL^{-1} (Executive Summary 2007).

Statistical analysis

Statistical analyses were carried out with SPSS (version 16.0, SPSS, Chicago, IL, USA). Data are expressed as numbers and percentages, mean ± SD for normally distributed data and median (25th, 75th percentile) for non-normal data. Infant BM intake and NBM water intake were compared between months 1, 3 and 6 using repeated measures analysis of variance (RMANOVA), and post hoc pairwise comparisons were performed by paired *t*-test using Bonferroni adjustment. As the BM Zn concentrations of the morning, afternoon and evening BM samples did not differ significantly from each other at any of the time points, the mean BM Zn for each month was used in further analysis. The BM Zn concentrations and BM Zn intakes were compared between months 1, 3 and 6 using Friedman test and pairwise comparisons were performed by Wilcoxon rank test using Bonferroni adjustment. The weight and the length gain from months 1 to 3 and 3 to 6 were calculated as the difference in the weights and lengths between these time points. Separate linear regressions of weight and length gain on BM Zn intakes were performed. Potential confounders such as NBM water intake, infant age, gender, and infant weight and length at birth and month 3 were adjusted for in the model. Independent *t*-test was used to examine whether BM intake was significantly different between SGA and AGA infants, while Mann–Whitney *U*-test was used to study whether maternal BM Zn content, BM Zn intakes and serum Zn (mothers and infants) were significantly different between the SGA and AGA groups. Two-sided *P*-value <0.05 was considered statistically significant.

Results

Socio-demographic and anthropometric characteristics of infants and their mothers at birth/delivery

Socio-demographic and anthropometric characteristics of infants and their mothers at birth/delivery are detailed in Table 1. The study mothers were on average 23.0 ± 2.9 years old, and approximately 76% of them were primiparous. The median family income was 9000 Indian Rupees, indicating that the mothers were not from a low socio-economic status. Nearly half of the infants born in the study had birthweight less than the 10th percentile for the gestational age at birth.

Infant and maternal dietary intake, maternal BM Zn content and serum Zn

Maternal and infant dietary intake, serum Zn and anthropometric measurements are presented in Table 2. BM intake significantly declined from 627 to 608 mL day^{-1} and NBM water intake increased from 83 to 389 mL day^{-1} from month 1 to month 6 ($P < 0.01$ for both). The BM Zn concentrations declined significantly from month 1 to month 6 ($P < 0.001$), and so did the Zn intakes from BM ($P < 0.001$). The mothers reported having introduced

Table 1. Socio-demographic and anthropometric characteristics of study infants and their mothers at birth/delivery

Maternal antenatal characteristics (last trimester of pregnancy) ($n = 50$)	Mean ± SD or n (%)
Age (years)	23.0 ± 2.9
Total family income (INR)*	9000 (5000, 15 000)
Parity	
Primiparous	38 (76)
Multiparous	12 (24)
Weight(kg)	60.4 ± 12.1
Height (cm)	154 ± 5.9
Body mass index	25.4 ± 5.1
Type of delivery	
Spontaneous vaginal delivery	34 (68)
Caesarean section	16 (32)
Infant characteristics at birth ($n = 50$)	
Gestational age at birth by ultrasonography (weeks)	39.1 ± 0.9
Birth outcome	
Small-for-gestational age	24 (48)
Appropriate-for-gestational age	26 (52)
Gender	
Male	29 (58)
Female	21 (42)
Birthweight (kg)	2.7 ± 0.5
Birth length (cm)†	49.6 ± 1.7
Weight-for-age z-score	−1.21 ± 0.93 ($n = 48$)
Weight-for-length z-score	−2.00 ± 1.43 ($n = 47$)
Length-for-age z-score	0.04 ± 0.95 ($n = 47$)

INR, Indian Rupees; SD, standard deviation. *Median (25th, 75th percentile). †$n = 48$.

CFs only by 6 months; however, data from the deuterium method indicated that NBM water was introduced among 44% of the infants at month 1, 77% at month 3 and 85% at month 6, leaving only 14.2% of the infants being exclusively breastfed by 6 months of age. The commonly introduced foods by 6 months of age were fortified commercial cereal formula, cow's milk, biscuits, mixed grain porridges and traditional Indian breakfast foods such as steamed rice and lentil cakes. The contribution of Zn from CF among the infants whose mothers reported having started CF ($n = 16$) at 6 months was 0.90 (0.45, 1.12) mg day^{-1}.

The maternal energy and Zn intakes were much lower than the recommended dietary allowance for lactating women throughout the post-partum period of 6 months; however, the mean maternal serum Zn levels were above the cut-off levels for Zn deficiency. The proportion of Zn deficient mothers was 0.32 and 0.16 at months 3 and 6, respectively. In addition, the maternal BM Zn concentrations were not low, nor were they correlated with maternal dietary Zn intake and Zn status. However, the Zn intakes of the infants calculated as the product of the BM volume consumed in mL day^{-1} and the BM Zn concentration mL^{-1} of BM appeared to be low. Nevertheless, the mean infant serum Zn levels appeared to be in the normal range, with a very small proportion of the infants being deficient in Zn. We observed no significant correlations between infant Zn intake and serum Zn levels. The BM Zn intakes as well as the serum Zn concentrations were not significantly different between male and female infants (data not shown). The serum Zn concentrations were also not significantly different among infants who received Zn from CF in addition to BM Zn (data not shown).

Infant BM Zn intake and serum Zn in relation to growth

The bodyweights of the infants were significantly lower ($P < 0.05$) in comparison to WHO standards throughout the early post-natal period of 6 months. Table 3 represents the infant BM Zn intakes in relation to weight and length gain. The mean weight gain of infants was 1.88 ± 0.59 and 1.47 ± 0.59 kg, translating to a daily weight gain of 31.3 and 15.2 g day^{-1} during the 1- to 3-month and 3- to 6-month periods, respectively. The mean length gain among infants was 7.1 ± 2.4 and 5.7 ± 1.8 cm in the 1- to 3-month and 3- to 6-month periods, respectively. The weight and length gain were not significantly different between boys and girls, except for the weight gain from 1 to 3 months ($P = 0.01$) which was higher in boys. The weight and length gain were also not significantly different among infants who received Zn from CF in addition to BM Zn (data not shown).

The weight and length gain from 1 to 3 and 3 to 6 months were not associated with the BM Zn intakes at months 1 and 3, respectively, when examined in separate linear regression analyses. The regression

Table 2. Infant and maternal dietary intake, maternal breast milk zinc content and serum zinc

Parameters*	Month 1	Month 3	Month 6
Infant age (days)	30 ± 2 (n = 50)	94 ± 13 (n = 48)	182 ± 17 (n = 50)
Infant dietary intake			
Infant BM intake (mL day^{-1})	627 ± 170 (n = 50)	744 ± 183 (n = 48)	608 ± 235† (n = 49)
Infant NBM water intake (mL day^{-1})	83 ± 115 (n = 50)	160 ± 147 (n = 48)	389 ± 455‡ (n = 49)
BM zinc content (mg L^{-1})§	2.50 (2.03, 3.26) (n = 50)	1.37 (0.89, 1.79) (n = 47)	1.17 (0.80, 1.60) (n = 50)
Zinc intake from BM (mg day^{-1})§	1.60 (1.13, 2.19) (n = 50)	0.88 (0.67, 1.36) (n = 47)	0.68 (0.51, 1.13) (n = 49)
Zinc intake from BM (mg kg^{-1} day^{-1})§	0.43 (0.32, 0.53) (n = 50)	0.15 (0.11, 0.22) (n = 47)	0.10 (0.06, 0.16) (n = 49)
Zinc intake from reported CFs (mg day^{-1})¶	–	–	0.90 (0.45, 1.12) (n = 16)
Maternal dietary intake			
Energy intake/kg bodyweight (kcal kg^{-1} day^{-1})	32.7 ± 12.8 (n = 50)	34.6 ± 13.1 (n = 48)	32.3 ± 13.8 (n = 48)
Zinc intake (mg day^{-1})	7.6 ± 2.9 (n = 50)	7.7 ± 2.3 (n = 48)	7.8 ± 2.8 (n = 50)
Infant and maternal zinc levels			
Maternal serum zinc (μg dL^{-1})	–	83.5 ± 26.5 (n = 40)	92.2 ± 25.1 (n = 43)
Infant serum zinc (μg dL^{-1})	–	93.0 ± 27.1 (n = 31)	99.6 ± 30.1 (n = 32)
Proportion of zinc deficient mothers**	–	0.32 (0.18, 0.46) (n = 40)	0.16 (0.05, 0.27) (n = 43)
Proportion of zinc deficient infants**	–	0.16 (0.03, 0.29) (n = 31)	0.09 (–0.01, 0.19) (n = 32)
Infant anthropometry			
Bodyweight (kg)	3.8 ± 0.6 (n = 50)	5.6 ± 0.8 (n = 48)	7.2 ± 1.0 (n = 50)
Body length (cm)	53.2 ± 2.4 (n = 49)	60.8 ± 2.8 (n = 47)	65.8 ± 2.7 (n = 50)
Weight-for-age z-score	–1.21 ± 1.27 (n = 50)	–1.07 ± 1.14 (n = 48)	–0.80 ± 1.30 (n = 50)
Weight-for-length z-score	–0.62 ± 1.38 (n = 49)	–0.39 ± 1.32 (n = 47)	–0.71 ± 1.26 (n = 50)
Length-for-age z-score	–1.03 ± 1.81 (n = 48)	–1.00 ± 1.65 (n = 47)	–0.34 ± 1.48 (n = 49)

BM, breast milk; CFs, complementary foods; NBM, non-breast milk. *Parameters are mean ± standard deviation/median (25th, 75th percentile). †BM intake at month 3 is significantly different from that of month 1 ($P < 0.05$) and BM intake at month 6 is significantly different from that of month 3 ($P < 0.05$) by Bonferroni adjusted pair wise comparisons in repeated measures analysis of variance (RMANOVA). ‡NBM water intake at month 6 is significantly different from that of month 1 ($P < 0.05$) and month 3 ($P < 0.05$) by Bonferroni adjusted pairwise comparisons in RMANOVA. §BM Zn content and BM Zn intake significantly different between months 1, 3 and 6 by Friedman test, $P < 0.001$. All pair wise comparisons were significant by Wilcoxon rank test, Bonferroni adjusted P-value <0.001. ¶The median Zn intake from CFs has been reported only for those infants whose mothers reported having given CFs. **Values are expressed as proportion (95% confidence interval: lower bound, upper bound).

for 1–3 months was adjusted for infant NBM water intake at month 1, infant age at month 1, gender and birthweight/length. The regression for 3–6 months was adjusted for infant NBM water intake at month 3, infant age at month 3, gender and weight/length at month 3. Serum Zn at months 3 and 6 was not associated with weight or length gain in the months preceding those time points.

Table 3. Infant zinc intakes in relation to post-natal growth[1]

Months 1–3

Weight gain†	B	95% CI	*P*-value	Length gain†	B	95% CI	*P*-value
BM Zn intake at month 1	–0.03	–0.25, 0.19	0.785	BM Zn intake at month 1	0.34	–0.83, 1.53	0.555
NBM water intake at month 1‡	–0.76	–2.16, 0.63	0.278	NBM water intake at month 1‡	–6.34	–13.5, 0.84	0.082
Infant age at month 1	–0.74	–1.54, 0.06	0.069	Infant age at month 1	4.80	0.70, 8.90	0.023
Infant gender	–0.48	–0.82, –0.15	0.005	Infant gender	–0.08	–1.84, 1.67	0.925
Infant birthweight	0.07	–0.27, 0.42	0.665	Infant birth length	–0.20	–0.72, 0.32	0.437

Months 3–6

Weight gain†	B	95% CI	*P*-value	Length gain†	B	95% CI	*P*-value
BM Zn intake at month 3	–0.13	–0.41, 0.15	0.362	BM Zn intake at month 3	0.62	–0.41, 1.66	0.231
NBM water intake at month 3‡	0.56	–0.67, 1.80	0.363	NBM water intake at month 3‡	–0.74	–5.46, 3.96	0.750
Infant age at month 3	0.06	–0.30, 0.44	0.714	Infant age at month 3	–2.16	–3.65, –0.67	0.006
Infant gender	–0.00	–0.38, 0.38	0.984	Infant gender	–0.88	–2.21, 0.44	0.186
Infant weight at month 3	0.05	–0.17, 0.29	0.609	Infant length at month 3	–0.25	–0.51, 0.01	0.061

BM, breast milk, CI, confidence interval; NBM, non-breast milk. *Multiple linear regression with weight and length gain from month 1 to month 3 and from month 3 to month 6 as dependent variables and BM Zn intakes at months 1 and 3, NBM water intakes at months 1 and 3, infant age at months 1 and 3, infant gender, birthweight and length and infant weight and length at months 1 and 3 as independent variables. †Weight gain and length gain from month 1 to month 3 and from month 3 to month 6 are calculated as the difference in weight and length, respectively, during these time points. ‡NBM water intake at months 1 and 3 is calculated using 'dose-to-mother' deuterium dilution method.

The BM Zn intake and serum Zn levels were not significantly different between infants with and without reported minor symptomatic illnesses such as diarrhoea, fever, cough, cold and dysentery at months 1, 3 and 6. Diarrhoea was reported among 6% (3 out of 50), 12.8% (6 out of 47) and 14% (7 out of 50) of the infants at months 1, 3 and 6, respectively. Fever was reported among 4% (2 out of 50), 8.3% (4 out of 48) and 22% (11 out of 50) of the infants at months 1, 3 and 6, respectively. Cough was reported among 4% (2 out of 50), 29% (14 out of 48) and 20% (10 out of 50) of the infants at months 1, 3 and 6, respectively. Cold was reported among 14% (7 out of 50), 33% (16 out of 48) and 42% (21 out of 50) of the infants at months 1, 3 and 6, respectively. None of the infants had dysentery during the study period (data not shown).

Comparison between SGA and AGA infants

A comparison of the BM intakes and intakes of Zn through BM, maternal BM Zn content, maternal and infant serum Zn levels between SGA and AGA infants is presented in Table 4. None of these parameters were significantly different between the two groups. However, the weight-for-age z-scores were significantly lower at birth and months 1, 3 and 6 in SGA infants in comparison to AGA infants ($P < 0.001$ at birth and months 1 and 3, $P = 0.005$ at month 6). Similarly, the SGA infants had lower weight-for-length z-scores at birth ($P < 0.001$) and lower length-for-age z-scores at months 1 and 3 ($P = 0.034$ and $P = 0.011$, respectively). However, intakes of BM Zn or serum Zn were not related to any of these standardised anthropometric indices in either SGA or AGA infants (data not shown).

Discussion

In a prospective observational study to assess longitudinal concentrations of maternal BM Zn and Zn intakes among their infants at 1, 3 and 6 months of age from urban South India, we observed that BM Zn intakes were low, owing to low volumes of BM intake,

Table 4. Infant Zn intakes and serum Zn levels, maternal BM zinc content and infant anthropometric measures in term SGA and AGA infants

	Birth/Delivery	Month 1	Month 3	Month 6
Infant BM intake (mL day^{-1})				
SGA	–	620 (500, 757) ($n = 24$)	755 (575, 780) ($n = 22$)	500 (390, 750) ($n = 23$)
AGA	–	665 (507, 755) ($n = 26$)	775 (672, 940) ($n = 26$)	660 (440, 841) ($n = 26$)
P-value*	–	0.690	0.153	0.288
BM zinc content (mg L^{-1})				
SGA	–	2.42 (2.08, 3.23) ($n = 24$)	1.37 (0.84, 1.99) ($n = 21$)	1.24 (0.73. 1.57) ($n = 24$)
AGA	–	2.54 (1.83, 3.30) ($n = 26$)	1.35 (0.95, 1.74) ($n = 26$)	1.06 (0.85, 1.63) ($n = 26$)
P–value**	–	0.900	0.856	0.627
Zn intake from BM (mg day^{-1})				
SGA	–	1.48 (1.06, 2.33) ($n = 24$)	0.72 (0.56, 1.57) ($n = 22$)	0.62 (0.54, 1.00) ($n = 24$)
AGA	–	1.67 (1.26, 2.19) ($n = 26$)	0.93 (0.73, 1.33) ($n = 26$)	0.61 (0.43, 1.20) ($n = 26$)
P-value**	–	0.634	0.286	0.946
Maternal serum zinc (μg dL^{-1})				
SGA	–		82.8 (64.5, 106.3) ($n = 18$)	86.2 (72.2, 103.9) ($n = 22$)
AGA	–		76.9 (55.7, 101.5) ($n = 22$)	87.2 (73.0, 117.2) ($n = 21$)
P-value**	–	–	0.348	0.752
Infant serum zinc(μg dL^{-1})				
SGA	–		100.0 (72.6, 114.2) ($n = 11$)	98.3 (82.6, 135.8) ($n = 20$)
AGA	–		90.0 (67.5, 106.5) ($n = 20$)	89.3 (77.3, 109.3) ($n = 12$)
P-value**	–	–	0.433	0.276
Weight-for-age *z*-score				
SGA	–1.94 ± 0.74	–1.84 ± 1.24	–1.68 ± 1.11	–1.33 ± 1.38
AGA	–0.55 ± 0.49	–0.62 ± 1.01	–0.55 ± 0.89	–0.32 ± 1.02
P-value*	0.000	0.000	0.000	0.005
Weight-for-length *z*-score				
SGA	–2.82 ± 1.10	–1.37 ± 1.96	–1.33 ± 1.81	–0.72 ± 1.69
AGA	–1.27 ± 1.29	–0.71 ± 1.65	–0.73 ± 1.50	–0.00 ± 1.19
P-value*	0.000	0.214	0.229	0.162
Length-for-age *z*-score				
SGA	–0.21 ± 0.69	–1.04 ± 1.26	–0.92 ± 1.39	–0.85 ± 1.18
AGA	0.27 ± 1.13	–0.21 ± 1.39	0.03 ± 1.11	–0.42 ± 1.04
P-value*	0.082	0.034	0.011	0.179

AGA, appropriate-for-gestational age; BM, breast milk; SGA, small-for-gestational age. *Two-sided *P*-values using independent *t*-test; **two-sided *P*-values using Mann–Whitney *U*-test.

despite BM Zn concentrations being in the normal range. Despite the lower maternal dietary Zn intake, the lactating women in this study were able to maintain higher levels of BM Zn concentration at months 1, 3 and 6 in comparison to the age-specific BM Zn concentrations generated from the combined analysis of 33 studies that investigated the BM Zn content of non-supplemented mothers of healthy term infants (Brown *et al*. 2009). This may be brought forth by a physiological adaptive response during lactation, such as increased absorption, reduced excretion from the kidney and the intestine, and mobilisation and re-equilibration from maternal Zn pools (Krebs 1998). A study among Chinese lactating women at 2 months post-partum has demonstrated that they were able to secrete 2 mg Zn day^{-1} into their BM, in the presence of marginal Zn intakes of 7.6 mg Zn day^{-1} (Sian *et al.* 2002). The Zn concentrations in the BM of mothers in our cohort declined from 1 to 6 months post-partum; however, this physiological pattern of decline in BM Zn concentrations as lactation progresses has been previously reported from other studies as well (Krebs *et al.* 1995; Krebs 1999). It is possible that as maternal zinc pools decline, a progressive decline in BM zinc may be seen. The decline in BM Zn concentrations of the mothers in our cohort could not be compensated by an increase in BM intake by the infant, as over the period of 6 months there was a decline in the weight-specific BM intake of the infant and a proportional increase in NBM water intake. The BM intake of our infants was lower than that reported by others using test weighing method (Coulibaly *et al.* 2004; Islam *et al.* 2008) and 'dose–to-mother' deuterium dilution method (Galpin *et al.* 2007; Moore *et al.* 2007).

The Zn intakes of infants from BM reported in our study (calculated as the product of the BM volume consumed in mL day^{-1} and the BM Zn concentration mL^{-1} of BM) were lower at months 1, 3 and 6 in comparison to calculated Zn intakes based on measured BM Zn concentrations and an assumed BM intake volume of 780 mL day^{-1} (FNB & IOM 2001), as well as the measured Zn intake of term, AGA infants born to mothers in the United States (Krebs *et al.* 1994). However, they were in close agreement with the simulated mean daily Zn transfer in BM to exclusively breastfed infants at months 1, 3 and 6 (Brown *et al.* 2009). With the assumptions of urinary and sweat Zn losses to be 20 $\mu g\ kg^{-1}\ day^{-1}$, endogenous fecal Zn losses to be 50 $\mu g\ kg^{-1}\ day^{-1}$ and Zn required for new tissue accretion to be 20 $\mu g\ g^{-1}$ weight gain or 30 $\mu g\ g^{-1}$ lean tissue gain (Butte *et al.* 2002), the total requirement of Zn for infants (boys and girls) would be 0.94, 0.86 and 0.79 mg day^{-1} at 1, 3 and 6 months, respectively. Assuming a mean fractional absorption of 0.55 for BM Zn (Krebs 1999), in the present study, the amount of Zn available for absorption from BM at 1, 3 and 6 months would only amount to 0.88, 0.48 and 0.37 mg day^{-1}, respectively, indicating increasing gap between the measured and recommended dietary intake of Zn.

However, despite the low intakes of BM Zn, the serum Zn levels of the infants appeared to be in the normal range. It may be that the requirements of Zn are partially offset by mobilisation of hepatic Zn bound to metallothionein in the first months of life (Zlotkin & Cherian 1988). It may also be that diarrhoeal diseases were not a major cause of morbidity among our children and, therefore, increased fecal Zn losses may not be an important factor in determining Zn requirements among these infants. In addition to these, we know from the deuterium data that infants were receiving water other than BM (either as water alone or as part of food) as early as month 1, which the mothers were not reporting. The reasons for this need further exploration using qualitative data. It could be possible that these CFs were contributing to the total intake of Zn among infants and thereby resulting in normal serum Zn concentrations. Equally, one may argue that there could have been some leaching of Zn from the red blood cells into the serum between the time of blood draw and the processing of the sample, which may have led to an increase in Zn levels. In addition, serum Zn measurements may not reflect individual Zn status (Golden 1989) and the use of an exchangeable zinc pool may have been a more robust marker of Zn status.

Infants born to mothers in this study were shorter and thinner at birth and throughout the post-natal period of 6 months compared with the reference WHO population; however, the growth velocity appeared adequate. BM Zn intakes at months 1 and 3 were not associated with the change in weight or length during the period of 1–3 or 3–6 months. To support a daily weight gain of 31 g day^{-1} (months 1–3) and 15.5 g day^{-1} (months 3–6) observed among our infants, the estimated Zn retention would be approximately 0.62 and 0.30 mg day^{-1}, respectively. The observed Zn intakes might have been adequate to support this gain in weight in the present study; equally, these intakes may not have been enough to enable catch up growth to the WHO standards, at any point in time during the 6-month study period. In addition, we could speculate effects of low Zn intakes among these children on body composition, as 10 mg Zn supplements day^{-1} among peri urban Guatemalan children aged 81.5 ± 7.0 months has shown a greater increase in median triceps skin-fold *z*-score and a smaller deficit in median mid-arm circumference *z*-score compared with the placebo group (Cavan *et al*. 1993).

Equally noteworthy is the observation that 48% of the infants born to mothers in our cohort were SGA. Prematurely born SGA infants from United States have shown to have smaller exchangeable Zn pool size at birth in comparison to AGA infants, indicating a compromised Zn status at birth (Krebs *et al*. 2006). Large-scale supplementation studies of Zn among breastfed SGA infants in developing countries have demonstrated significant improvements in growth and reduced morbidity and mortality (Sazawal *et al*. 2001). However, such an effect has not been demonstrated among AGA infants in whom the Zn content of the BM is considered adequate to meet their needs for Zn during the first few months of life (Krebs 1999). Post-natal requirements for Zn may be higher in SGA infants, or they may be consuming lower volumes of BM and thereby lower amounts of Zn, or alternatively their mother may have lower concentrations of Zn in their BM. We explored these possibilities among mother–infant pairs in our cohort; however, results from our study did not demonstrate any significant differences in either intakes of BM Zn, maternal BM Zn concentrations or Zn levels between SGA and AGA infants. Results from a similar study in Bangladesh demonstrate that BM Zn concentrations and total BM Zn transfer were not significantly different between mothers of SGA and AGA infants when assessed at 1, 3 and 6 months post-partum (Islam & Brown 2010). It is very likely that the aetiology of growth retardation is multifactorial and affected by deficits of energy or several other limiting nutrients such as protein, iron and vitamin A, D or C, apart from low Zn intakes.

To our knowledge, this study is the first of its kind from India using the accurate and objective deuterium dilution technique to assess BM Zn intake among infants less than 6 months of age. This study demonstrated that among urban south Indian term infants less than 6 months of age, BM Zn intakes were low, owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range. BM Zn intakes were not related to weight gain and length gain among these infants. However, this study is limited by its small sample size and therefore may have low power to detect potentially clinically significant effects in the multiple linear regression models. The effect of low Zn intakes on weight and length gain in stunted or low weight population merits further evaluation using well-designed intervention studies with greater sample size. Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

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Conflicts of interest

Dr. Anura V Kurpad is a member of the Kraft Health and Wellness Advisory Council. His honoraria go entirely to charity. None of the other authors have any personal or financial conflict of interest.

Contributions

TMS collected, analysed and interpreted the data, and wrote the first draft of the manuscript; TT provided statistical guidance in data analysis and contributed to the writing of the manuscript; PT guided the analyses of breast milk zinc and serum zinc, and contributed to the writing of the manuscript; SB was involved in the conception, design and execution of the study; SMV contributed to the writing of the manuscript; and AVK conceived and designed the study, interpreted the results and contributed to the writing of the manuscript. All co-authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual content.

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