

# Nutritional Zinc Balance in Extremely Low-Birth-Weight Infants

\*Andrea Loui, †Andrea Raab, \*Michael Obladen, and †Peter Brätter

\*Department of Neonatology, Charité Virchow-Hospital, Humboldt University Berlin; and the †Department of Molecular Trace Element Research, Hahn-Meitner-Institute Berlin, Berlin, Germany

## ABSTRACT

**Background:** Zinc is important for metabolism, cell growth, immunity, and defense against oxygen radicals. Extremely low-birth-weight (< 1000 g) infants have higher nutritional needs, but information on zinc is scarce. The authors performed nutritional balances in 10 infants with birth weights of 500 to 999 g and who were fed with fortified human milk.

**Methods:** The authors collected infant feces, urine, and blood and human milk samples during 72 hours at 7 and 12 weeks of age. Zinc concentration was measured by inductively coupled plasma–mass spectrophotometry, atomic emission spectrophotometry, and instrumental neutron activation analysis.

**Results:** Mean (SD) intake via human milk was 379 ( $\pm$  373)  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  during both balances. Urinary excretion was

high at 7 weeks of age, decreased to half at 12 weeks, and was negatively correlated ( $P < 0.01$ ) with weight gain. Mean absorption was slightly positive at 7 weeks of age but zero or negative in most infants at 12 weeks of age. Retention was negative in all infants at both observation periods, except in one infant during the second balance. Clinical zinc deficiency developed in one infant at 12 weeks of age.

**Conclusions:** Zinc balances in extremely low-birth-weight infants are highly variable and usually negative. Controlled trials are needed to assess need for and benefits and risks of zinc supplementation. *JPGN* 32:438–442, 2001. **Key Words:** Prematurity—Extremely low-birth-weight infants—Breast-feeding—Zinc deficiency—Human milk fortifier. © 2001 Lippincott Williams & Wilkins, Inc.

Most preterm infants with birth weights less than 1000 g (extremely low-birth-weight [ELBW] infants) now are surviving in industrialized countries; therefore, their quality of life becomes very important. Adequate nutrition influences their neurodevelopmental outcome.

Zinc is a cofactor for approximately 300 metalloenzymes and hormones. It is essential for growth. Zinc deficiency impairs unspecific immune response, T-cell function, and cytokine response in mononuclear cells. It is the second most important deficiency in infants after iron and has been described in preterm infants predominantly fed with breast milk (1–3). Recently we observed a 640-g infant with severe zinc deficiency and infants of less than 1500 g with serum zinc concentrations less than 0.5 mg/L, the limit assumed to be necessary for normal development (4). Risk factors for zinc deficiency are low fetal stores, immaturity of the digestive system, low content in breast milk, and rapid growth. Intake recommendations for ELBW infants are derived from studies in older infants (5–7) and adults, or from consensus of nutritional committees (8–11). We are aware of two studies

that included infants less than 1000 g in nutritional zinc balances (12,13).

We assumed that the nutritional needs of rapidly growing ELBW infants for zinc are higher than for preterm infants of an older gestational age. Intake, absorption, and retention of zinc were studied in infants with birth weights of 500 to 999 g.

## METHODS

The study was carried out in the Department of Neonatology, Charité Virchow-Hospital Berlin, from January to September 1998. The local ethics committee approved the protocol and informed consent was obtained from the parents. Zinc was measured at the Department of Molecular Trace Element Research, Hahn-Meitner-Institute Berlin.

### Patients and Nutritional Protocol

Nineteen ELBW infants were admitted during the study period. Four died in the first weeks of life, three received parenteral nutrition within 7 days before the balance, and two parents gave no consent. Ten infants subsequently born between 25 and 27 weeks of gestation, weighing 730 to 995 g at birth comprised the study group, which consisted of six boys males, four girls, and two sets of triplets (Table 1).

The balances were performed during stable growth and in the absence of infection, necrotizing enterocolitis, other catabolic

Received April 26, 2000; Accepted December 15, 2000.

Address correspondence and reprint requests to Dr. Andrea Loui, Department of Neonatology, Charité Virchow-Hospital, Humboldt University Berlin, Augustenburger Platz 1, 13353 Berlin, Germany (e-mail andrea.loui@charite.de).

TABLE 1. Infants clinical and nutritional characteristics

At birth		Balance period 1			Balance period 2			During entire study				
Infant No	Sex	Birthweight (g)	Gestational Age (wk)	Age at study (d)	Weight at study (g)	AP (U/L)	Age at study (d)	Weight at study (g)	AP (U/L)	Weight gain (g/kg/d)	Head growth (mm/wk)	Longitudinal Growth (mm/wk)
1	m	995	27 + 0	43	1585	568	64	2025	561	11.9	7.1	8.1
2	f	845	26 + 6	45	1465	494	68	1950	805	11.9	7.1	5.5
3	m	780	25 + 3	50	1640	648	76	2060	490	12.5	7.7	8.6
4	f	779	26 + 0	48	1315	612	82	1870	407	10.5	6.8	6.8
5	f	808	26 + 0	48	1290	545	82	1855	279	9.8	5.4	6.9
6	m	850	26 + 0	62	1460	425	97	2525	162	11.7	5.7	6.5
7	m	945	26 + 0	62	1315	763	91	2080	558	10.5	6.3	6.7
8	f	885	26 + 0	62	1445	448	91	2280	284	10.0	5.0	4.8
9	m	835	25 + 0	52	1270	477	77	1705	420	9.4	6.7	7.8
10	m	730	25 + 4	49	1185	1320	71	1615	784	11.3	5.9	7.2
mean		845	25.9	52	1397	630	80	1997	475	11.0	6.4	6.9
SD		76	0.6	7	138	250	10	253	200	1.0	0.8	1.1

AP, alkaline phosphatase activity in serum

disease, cholestasis, and disturbance of liver function. Nine infants received transfusions (mean cumulated volume, 65.7 ± 31.6 ml/kg). Three infants were treated with dexamethasone before the first balance. Enteral caffeine was given to seven infants during the first balance and to one during both periods. Diuretics were given to one infant during the first balance and to four infants at both periods. Infants received parenteral nutrition that contained trace element solution (Peditrace; Pharmacia & Upjohn GmbH, Erlangen, Germany) during the first weeks. Infants were fed with mother's or donor milk fortified with 5% FM 85 (Nestlé, München, Germany). Fortification was started at 20 ± 7 days and complete enteral nutrition was achieved at 27 ± 9 days (mean ± SD). All infants were enterally supplemented with iron, calcium, and phosphorus. Body weight was measured daily using an electronic precision scale (S 10-2720; Soehnle, Murrhardt, Germany), body length was measured once a week using a metallic tape inside the incubator and with a precision measuring device outside the incubator (Schäfer Kunststofftechnik, Germany), and frontooccipital head circumference was measured once a week using a tape measure. Mean weight gain was 11.0 ± 1.0 g · kg<sup>-1</sup> · d<sup>-1</sup>; birth weight doubled within 65 ± 8 days.

### Sample Collection

Nutritional balance studies were performed for 72 hours at 7 and 12 weeks of age. We used a mass balance and no isotopic tracer method. Recommendations of Cooke et al. (14) for sample collection without contamination were adopted. Collection vials, bottles, tubes, and urine bags were precleaned and free of zinc contamination (15). Feces were collected with preweighed urine bags attached to the perianal area (Hollister Overseas LTD., Ballina, Ireland), and urine with bags were tubed to a preweighed collection bottle (Urinocol premature, B Braun S.A., Paris, France). Bottles were changed every 6 hours. Vial and bag weight was assessed using a precision scale (Basic plus 221-S OCE, Sartorius AG, Göttingen, Germany). To account for losses, the infant was placed on a preweighed absorbent diaper. Blood was drawn once during each balance study and a pooled sample of human milk, used for nutrition at 1 day, was collected. All samples were frozen at -20°C until analysis.

### Trace Element and Data Analysis

Zinc concentration in serum, red blood cells (RBCs), human milk, feces, urine, supplements, and drugs was measured for quality assessment by means of three different methods: instrumental neutron activation analysis, inductively coupled plasma (ICP)-mass spectrophotometry (MS) and ICP atomic emission spectrophotometry (ICP-AES). Serum, RBCs, human milk, and urine were analyzed without sample pretreatment. Feces were digested with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> before analysis. All reagents used were of suprapure or ultrapure grade. Zinc concentration in urine and feces was determined without pooling the samples. Quality was controlled by using certified reference materials. The within-day and day-to-day variation coefficients for ICP-MS and ICP-AES were less than 5% (n = 7). The recovery of zinc in serum, urine, RBCs, and milk was 98 ± 4% (n = 7) and in feces was 96 ± 6% (n = 7), measured with ICP-MS and ICP-AES. The results obtained from instrumental neutron activation analysis, ICP-MS, and ICP-AES were compared by applying the maximum likelihood fitting of a functional relationship (16). We found the methods to be free of systematic errors. Therefore, a mean value derived from these methods was used to calculate the balance data. Intake was estimated by measuring zinc concentration in milk, supplements, and drugs. Absorption was calculated from intake minus excretion in feces and retention from intake minus excretion in feces and urine.

The Spearman rank correlation coefficient R<sub>s</sub> (software SPSS, PC+, Chicago, IL) and 95% confidence interval (software StatXact-4, version 4.0.1, copyright 1989-1999, Cytel Software Corporation) were used to estimate the relation between measurements and clinical data; significance was assumed for P < 0.05.

### RESULTS

Mean zinc concentration in milk was similar in both periods but varied individually (data not shown). The mean intake via breast milk was 373 ± 290 μg · kg<sup>-1</sup> · d<sup>-1</sup> during the first and 353 ± 563 μg · kg<sup>-1</sup> · d<sup>-1</sup> during the second period (Table 2). Urinary zinc excretion was high at 7 weeks of age, decreased to half at 12 weeks of age, and correlated negatively with weight gain (P < 0.001, r = -0.74, 95% confidence interval [CI] = -0.92, -0.56).

TABLE 2. Zinc balance data and blood concentrations for each infant

Infant No	Intake $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	Excretion		Absorption $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	Retention $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	Retention [% intake]	Serum mg/L	RBC mg/L
		Fecal $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	Urinary $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$					
Balance Period 1								
1	160	242	205	-51	-287	-179	1,44	1,71
2	140	396	184	-183	-440	-315	0,61	2,02
3	321	204	90	36	27	8	1,20	2,22
4	409	139	310	66	-40	-25	1,26	4,19
5	398	113	1046	72	-761	-331	0,57	4,69
6	417	88	491	79	-161	-39	0,45	2,99
7	433	594	608	-37	-768	-177	1,07	4,65
8	395	176	648	56	-429	-109	1,10	5,56
9	844	942	235	-12	-333	-39	0,53	6,09
10	239	113	137	53	-10	-4	1,11	4,69
median	375,6	300,7	395,2	7,8	-320,3	-121	0,93	3,88
SD	187,0	260,0	285,6	77,5	272,7	119	0,34	1,46
Balance Period 2								
1	219	210	130	9	-120	-55	0,32	1,86
2	2040	426	98	1614	1515	74	0,44	3,24
3	217	191	146	25	-121	-56	0,44	2,98
4	258	234	596	24	-572	-222	0,40	2,24
5	260	604	216	-344	-561	-216	0,42	3,51
6	419	1055	176	-636	-813	-194	0,49	3,07
7	100	118	79	-18	-97	-98	0,58	3,23
8	90	301	435	-211	-646	-717	0,47	2,46
9	174	600	150	-426	-576	-330	0,74	6,62
10	131	68	102	63	-39	-56	0,53	3,67
mean	390,7	380,8	212,9	9,9	-202,9	-187	0,48	3,29
SD	557,1	285,5	160,0	579,4	630,5	208	0,11	1,24

RBC, red blood cells

Urinary zinc excretion and cumulated doses of diuretics or caffeine did not correlate.

Zinc absorption varied within a wide range, especially during the second period. Mean absorption was positive at 7 weeks of age, but was mostly zero or negative at 12 weeks of age. Only infant 2, with a high zinc concentration in human milk, had a significant positive absorption at this age. Absorption correlated positively with intake ( $P < 0.05$ ,  $r = 0.46$ , 95% CI = 0.07, 0.85).

Retention was found to be negative in all infants at both ages except in infant 2 during the second period. Alkaline phosphatase (AP) activity correlated positively with absorption ( $P < 0.05$ ,  $r = 0.48$ , 95% CI = 0.11, 0.84), retention ( $P < 0.01$ ,  $r = 0.48$ , 95% CI = 0.34, 0.89), and linear growth ( $P < 0.05$ ,  $r = 0.46$ , 95% CI = 0.16, 0.76) (Table 1).

One infant (7 weeks of age) and seven infants (12 weeks of age) had serum zinc concentrations less than 0.5 mg/L. Red blood cell zinc content was less than in adults (Table 2) and correlated with the cumulated transfusion volume ( $P < 0.001$ ,  $r = 0.74$ , 95% CI = 0.50, 0.99).

Infant 6 was 86 days old when severe dermatitis developed in the genital region. Serum zinc concentration was 0.17 mg/L and AP activity was 162 IU/L. He was treated enterally with zinc orotate ( $137 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 13 days), resulting in healing of the skin lesions. The

second balance (14 weeks) remained negative despite zinc supplementation.

## DISCUSSION

We found nutritional zinc balances in ELBW infants highly variable, but usually negative. Retention was negative in nearly all infants at both periods. Because most zinc is acquired during the last trimester of pregnancy, ELBW infants have low stores. Their growth during the first weeks, however, is rapid and it is difficult to meet the zinc requirements. Nutritional balance studies are one method to estimate retention of nutrients. To obtain valid information about ELBW infants, we did not change the nutritional regime. Infants treated with dexamethasone, diuretics, or caffeine were not excluded.

We found low mean zinc concentrations in human milk of 2.48 mg/L (range, 0.92–7.0 mg/L) at 7 and 2.42 mg/L (range 0.79–13.4 mg/L) at 12 weeks of age, respectively, which is similar to the range of 1.96 to 2.94 mg/L during the first 4 weeks of lactation and of 0.98 to 1.64 mg/L at 3 months reported by others (17–21). Zinc intake in this population was low but within the reported range of 177 to 1825  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (5,6). Higashi et al. (12) found a negative balance in infants younger than 36 weeks of gestation, with a lower zinc intake than the

current study population. Schanler et al. (13) reported a mean zinc intake of  $1903 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , the net retention was more than the intrauterine accretion rate. Zinc intake in the infants in this study obviously was too low. Only the donor milk for patient 2 was rich in zinc during the second balance. A sample contamination could be excluded by measuring various milk samples. Protein fortification of human milk increases zinc bioavailability (22). Only two of the infants in the study population reached the recommended protein intake of  $3.0 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 7 weeks of age, despite fortification with 5% FM 85. All infants had serum protein concentrations less than 5.1 g/L.

Zinc homeostasis is maintained by changes in excretion mainly via feces. The zinc excretion in this study via feces was nearly constant at 7 and 12 weeks of age, as reported by others (5,12). We found absorption to be positively correlated with zinc intake. As seen from our results, the regulatory system could not compensate for the low milk concentration of zinc by increasing the absorption rate or by maturation between 7 and 12 weeks of age.

A relevant question is, whether enteral iron influences zinc absorption because both metals may compete for the same absorption pathway or carrier protein. Conflicting reports (24) have been published. Some authors reported a negative effect of iron on zinc absorption (25,26); other studies showed no effect (27). Iron intake and zinc absorption and retention did not correlate in our study.

We found urinary zinc excretion at 7 weeks of age to be 10-fold higher than in other studies (6,7,12), decreasing markedly until 12 weeks of age. Maturing renal reabsorption may cause this. Sievers et al. (28) and Higashi et al. (12) reported decreasing urinary excretion with age. Extremely low-birth-weight infants are often treated with diuretics and methylxanthines. Sievers et al. (28) reported augmented urinary zinc excretion in infants treated with caffeine. However, we found no correlation between zinc excretion and diuretics or caffeine.

Zinc retention changed with age. For the intrauterine retention rate of 249 to  $458 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (11,29), a minimal enteral intake of  $785 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  was suggested by Higashi et al. (12). For a weight gain of more than  $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , a zinc intake of  $1504 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  was proposed by Wastney et al. (30). The low zinc and protein intake in our study may have prevented a positive zinc balance and impaired growth. Zinc absorption and retention correlated ( $P < 0.01$ ,  $r = 0.65$ , 95% CI = 0.25, 1.0) (Table 2), which confirmed the results of Ehrenkranz et al. (6). Urinary zinc excretion and weight gain correlated negatively.

The technique used in our study allows measurement of net zinc absorption across the intestinal tract, but gives no information on the endogenous zinc excretion via desquamation, loss by pancreatic and intestinal endogenous secretions. Therefore, we tended to underestimate zinc bioavailability. For direct measurements of endog-

enous losses, intravenous injections of stable isotopes are needed (23,30–32).

Serum zinc concentration is easily available and widely used to detect zinc deficiency. The values in the current study were less than those found by Altigani et al. (33). Because zinc is mainly bound to albumin, mild hypoproteinemia may have altered serum zinc concentrations in these patients. Redistribution between serum and tissues in acute inflammation additionally may reduce zinc concentrations in serum. None of the infants had increased C-reactive proteins at the time of balance studies.

Data about zinc in the RBCs of preterm infants are few. Red blood cell zinc concentration exceeds that of serum, changes slowly in response to deficiency, and may be a useful long-term indicator of zinc status. No changes in RBC zinc concentrations from 7 to 12 weeks of age and no correlations between RBC concentrations and intake, absorption, or retention were found in our study. Red blood cell concentration in the case of zinc deficiency was within the normal range reported by others (34). The duration of relevant deficiency has been too short to reduce zinc concentrations in RBCs. The cumulated transfusion volume and RBC zinc concentration correlated because of high zinc content in adult RBCs.

Alkaline phosphatase is a zinc-dependent enzyme important for bone metabolism, and often is increased in osteopenia praematurorum. The positive correlation of serum AP with zinc absorption and retention shows the central role of zinc in AP activity. In zinc deficiency, AP activity may be low despite bone mineral depletion. Lucas et al. (35) described a correlation between serum AP and growth in breast-fed preterm infants.

In the patient with zinc deficiency, we found a prompt healing of the skin lesions after zinc supplementation. Therefore, we presumed that supplemented zinc was used and incorporated in metabolic processes. For further supplementation, we must proof the best chemical form of zinc. In most studies, zinc sulfate was used. We preferred zinc orotate because this compound releases zinc more slowly and functions as a store.

Our data confirm that with usual European breast milk fortifiers many ELBW infants are malnourished with respect to protein and zinc. Nutritional needs are higher for this group. Fortifiers specifically designed for ELBW infants are needed. To assess benefits and risks of zinc supplementation, controlled trials in ELBW infants are necessary. We must investigate interactions with other nutrients, minerals, and trace elements. The goal remains to improve growth and neurodevelopmental outcome.

## REFERENCES

1. Heinen F, Matern D, Pringsheim W, et al. Zinc deficiency in an exclusively breast fed preterm infant. *Eur J Pediatr* 1995;154: 71–5.
2. Paupe A, Lenclen R, Andre MC, et al. Zinc deficiency in a premature breast-fed infant. *Arch Pediatr* 1996; 3:507–8.

3. Stapleton KM, O'Loughlin E, Relic JP. Transient zinc deficiency in a breast-premature infant. *Australas J Dermatol* 1995; 36:157-9.
4. Obladen M, Loui A, Kampmann W, et al. Zinc deficiency in rapidly growing infants. *Acta Paediatr* 1998;87:685-91.
5. Mendelson RA, Bryan MH, Anderson GH. Trace mineral balances in preterm infants their own mother's milk. *J Pediatr Gastroenterol Nutr* 1983;2:256-61.
6. Ehrenkranz RA, Gettner PA, Nelli MC, et al. Zinc and copper nutritional studies in very low birth weight infants: comparison of stable isotopic extrinsic tag and chemical balance methods. *Pediatr Res* 1989;26:298-307.
7. Wauben I, Gibson R, Atkinson S. Premature infants fed mother's milk to 6 months corrected age demonstrate adequate growth and zinc status in the first year. *Early Hum Dev* 1999;54:181-94.
8. Canadian Society of Pediatrics, Nutrition Committee. Nutrient needs and feeding of premature infants. *Can Med Assoc J* 1995; 152:1765-85.
9. ESPGAN Committee on Nutrition of the preterm infant (Bremer HJ, Brooke OG, Orzalesi M, et al). Nutrition and feeding of preterm infants. *Acta Paediatr Scand* 1987;78(suppl 336):2-14.
10. American Academy of Pediatrics, Committee on Nutrition. Nutritional needs for low birth weight infants. *Pediatrics* 1985;75: 976-86.
11. Reifen RM, Zlotkin S. Microminerals. In: Tsang RC, Lucas A, Uauy R, et al., eds. *Nutritional needs of the preterm infant: scientific basis and practical guidelines*. Baltimore; Williams & Wilkins, 1993;195-207.
12. Higashi A, Ikeda T, Iribe K, et al. Zinc balance in preterm infants given the minimal dietary zinc requirement. *J Pediatr* 1988;112: 262-6.
13. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics* 1999;103:1150-7.
14. Cooke RJ, Perrin F, Moore J, et al. Methodology of nutrient balance studies in the preterm infant. *J Pediatr Gastroenterol Nutr* 1988;7:434-40.
15. Cornelis R, Heinzow B, Herber RFM, et al. Sample collection guidelines for trace elements in blood and urine. *J Trace Elements Med Biol* 1996;10:103-27.
16. Thompson M, Brown DW, Ellison S, et al. Handling false negatives, false positives and reporting limits in analytical proficiency tests. *Analyst* 1997;122:495-7.
17. Krebs NF, Reidinger CJ, Hartley S, et al. Zinc supplementation during lactation: effects on maternal status and milk zinc concentrations. *Am J Clin Nutr* 1995;61:1030-6.
18. Aquilio E, Spagnoli R, Seri S, et al. Trace element content in human milk during lactation of preterm newborns. *Biol Trace Element Res* 1996;51:63-70.
19. Lönnnerdal B. Effects of milk and milk components on calcium, magnesium, and trace element absorption during infancy. *Physiol Rev* 1997;77:644-64.
20. Atkinson SA, Whelan D, Whyte RK, et al. Abnormal zinc content in human milk. *Am J Dis Child* 1989;143:608-11.
21. Bates CJ, Prentice A. Breast milk as a source of vitamins, essential minerals and trace elements. *Pharmacol Ther* 1994;62:193-220.
22. Knudsen E, Sandstrom B, Andersen O. Zinc and manganese bioavailability from human milk and infant formula used for very low birth weight infants, evaluated in a rat pup model. *Biol Trace Elem Res* 1995;49:53-65.
23. Davidsson L. Minerals and trace elements in infant nutrition. *Acta Paediatr Suppl* 1994;395:38-42.
24. Whittaker P. Iron and zinc interactions in humans. *Am J Clin Nutr* 1998;68:442-6S.
25. Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *J Nutr* 1986;116:927-35.
26. Sandström B, Davidsson L, Cederblad A, et al. Oral iron, dietary ligands and zinc absorption. *J Nutr* 1985;115:411-4.
27. Haschke F, Ziegler EE, Edwards BB, et al. Effect of iron fortification on infant formula on trace mineral absorption. *J Pediatr Gastroenterol Nutr* 1986;5:768-73.
28. Sievers E, Oldigs HD, Dörner K, et al. Longitudinal zinc balances in breast-fed and formula-fed infants. *Acta Paediatr* 1992;81:1-6.
29. Shaw ICL. Trace elements in the fetus and young infants: I. Zinc. *Am J Dis Child* 1979;133:1260-6.
30. Wastney ME, Angelus PA, Barnes RM, et al. Zinc absorption, distribution, excretion, and retention by healthy preterm infants. *Pediatr Res* 1999;45:191-6.
31. Aggett PJ. Aspects of neonatal metabolism of trace elements. *Acta Paediatr Suppl* 1994;402:75-82.
32. Lowe NM. Comparison of estimates of zinc absorption in humans by using 4 stable isotopic tracer methods and compartmental analysis. *Am J Clin Nutr* 2000;71:523-9.
33. Altigani M, Murphy JF, Gray OP. Plasma zinc concentration and catch up growth in preterm infants. *Acta Paediatr Scand Suppl* 1989;357:20-33.
34. Hatano S, Aihara K, Nishi Y, et al. Trace elements (copper, zinc, manganese, and selenium) in plasma and erythrocytes in relation to dietary intake during infancy. *J Pediatr Gastroenterol Nutr* 1985; 4:87-92.
35. Lucas A, Brooke OG, Baker BA, et al. High alkaline phosphatase activity and growth in preterm neonates. *Arch Dis Child* 1989;64: 902-9.