See corresponding editorial on page 1373.

Zinc supplementation reduces morbidity and mortality in very-low-birth-weight preterm neonates: a hospital-based randomized, placebo-controlled trial in an industrialized country[1–3]

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ABSTRACT

Background: Zinc plays a pivotal role in the pathogenesis of many diseases and in body growth. Preterm neonates have high zinc requirements.

Objective: The objective of the study was to investigate the efficacy of zinc supplementation in reducing morbidity and mortality in preterm neonates and to promote growth.

Design: This was a prospective, double-blind, randomized controlled study of very-low-birth-weight preterm neonates randomly allocated on the seventh day of life to receive (zinc group) or not receive (control group) oral zinc supplementation. Total prescribed zinc intake ranged from 9.7 to 10.7 mg/d in the zinc group and from 1.3 to 1.4 mg/d in the placebo control group. The main endpoint was the rate of neonates with ≥1 of the following morbidities: late-onset sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, periventricular leucomalacia, and retinopathy of prematurity. Secondary outcomes were mortality and body growth.

Results: We enrolled 97 neonates in the zinc group and 96 in the control group. Morbidities were significantly lower in the zinc group (26.8% compared with 41.7%; $P = 0.030$). The occurrence of necrotizing enterocolitis was significantly higher in the control group (6.3% compared with 0%; $P = 0.014$). Mortality risk was higher in the placebo control group (RR: 2.37; 95% CI: 1.08, 5.18; $P = 0.006$). Daily weight gain was similar in the zinc (18.2 ± 5.6 g · kg^{-1} · d^{-1}) and control (17.0 ± 8.7 g · kg^{-1} · d^{-1}) groups ($P = 0.478$).

Conclusion: Oral zinc supplementation given at high doses reduces morbidities and mortality in preterm neonates. This trial was registered in the Australian New Zealand Clinical Trial Register as ACTRN12612000823875. *Am J Clin Nutr* 2013;98:1468–74.

INTRODUCTION

Zinc is a structural component of hormones, nucleotides, and proteins. It is required for the production of a wide variety of enzymes involved in essential metabolic patterns (ie, protein synthesis, nucleic acid metabolism, immune function, and organogenesis) (1–3). Zinc is crucial for the development of the brain, respiratory and intestinal tracts in pre- and postnatal life (1, 4–11).

Preterm neonates have lower zinc reserves than term infants because ~60% of fetal zinc is acquired during the third trimester of pregnancy (3). Preterm infants are also less efficient at absorbing and retaining zinc for growth (3). Adequate absorption may be compromised by limited intake, immature digestive and absorptive processes; excessive endogenous losses occur secondary to either poorly regulated secretion or interference with reabsorption (3). Thus, premature neonates have relatively high dietary zinc requirements (12–14). However, studies on zinc homeostasis in neonates have produced somewhat conflicting results regarding zinc requirements and optimal daily intakes (1–3, 14). There is a wealth of evidence that zinc, at different doses, has a positive influence on morbidity, mortality, and growth in infants and children (15–17). Recent trials have suggested that high doses of zinc as co-treatment against infections early in the life (18, 19). In this trial we aimed to investigate the efficacy of high zinc doses to reduce morbidity and mortality and promote growth in preterm neonates.

SUBJECTS AND METHODS

Study design and period

A double-blind, single-center, randomized, placebo-controlled trial was conducted between January 2009 and May 2012. The study design was approved by the ethics committee of our Center (#0901). Written informed consent was obtained from the parents of the infants enrolled in the study.

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[2] No honorarium, grant, or other form of payment was given to anyone to produce the article.

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Received November 14, 2012. Accepted for publication August 22, 2013.

First published online September 11, 2013; doi: 10.3945/ajcn.112.054478.

Participants

All very-low-birth-weight (VLBW)[4] neonates (birth weight: 401–1500 g or a gestational age of 24 to 32 wk), consecutively observed in a neonatal intensive care unit, were considered eligible for the study. Exclusion criteria were as follows: congenital or maternal infections, immunodeficiency, malformations, syndromes, genetic defects, evidence of infection, and necrotizing enterocolitis (NEC) diagnosed before enrollment, critically ill conditions (defined as a blood pH <6.8 or hypoxia with persistent bradycardia for ≥ 1 h), and hospitalization for <1 wk.

Intervention, randomization, and blinding

On the seventh day of life, the neonates enrolled were randomly allocated to 2 study groups: group 1 (zinc group), who received a commercially available multivitamin product in drops containing zinc (Previtamin 0; DUPI), and group 2 (placebo control group), who received a similar multivitamin preparation without zinc (Previtamin; DUPI). The composition of the multivitamin products used in this study is reported in **Table 1**. Daily doses of zinc and vitamins were prescribed irrespective of body weight. The 2 multivitamin preparations were provided in identical bottles without indication of group identity or content. Each bottle was labeled with a unique serial number according to the randomization list; this information was not available to the investigators until the data had been obtained, entered in the database, and analyzed by a blinded statistician. After randomization, nurses blinded to the study aims administered the assigned preparation as drops, once daily, 2 h after feeding. The supplement was given again to subjects who vomited within 15 min after the administration. All episodes of vomiting and other adverse events were reported on the clinical chart. Vomiting episodes within 15 min from administration/number of doses administered per patient were recorded. Compliance was measured by the rate of number of doses administered/number of doses prescribed, as documented by nurses in the clinical chart. The supplement assigned was discontinued at discharge or at 42 wk postconceptual age.

Outcome measures

The main endpoint was the rate of neonates presenting, during the hospitalization period, at least one of the following morbidities: late-onset sepsis, NEC, bronchopulmonary dysplasia (BPD), periventricular leucomalacia (PVL), and retinopathy of prematurity (ROP). Secondary outcomes were mortality and growth.

Data collection

Data regarding gestational age, weight at birth, mode of delivery, multiple birth, sex, Apgar score, Critical Risk Index for Babies score (20), presence of respiratory distress syndrome, patent ductus arteriosus (PDA), mode and duration of mechanical ventilation, start of enteral feeding, time to reach full enteral feeding, breast milk use, presence and duration of central vascular access, time-to-discharge, occurrence of late-onset sepsis, NEC, BPD, PVL, intraventricular hemorrhage, and ROP were collected by researchers not in charge of the clinical management of the participants. Diagnosis of late-onset sepsis, NEC, BPD, PVL, and ROP was performed according to standardized criteria (21–25) by physicians unaware of the study design and aims. Discharge was decided by the same physicians with the use of criteria indicated by the American Academy of Pediatrics (26). The patients' study courses are reported in **Figure 1**.

TABLE 1
Composition of the multivitamin preparations used in the study[1]

	Group 1 (zinc)	Group 2 (control)
Energy (kcal)	0.05	0.12
Vitamin A (μg)	480	750
Vitamin C (mg)	60	60
Vitamin E (mg)	6	6
Folic acid (μg)	120	100
Vitamin D_3 (IU)	800	800
Vitamin B-12 (μg)	0.6	0.6
Zinc (mg)	9	0

[1] Data refer to a daily dose of vitamins and zinc sulfate provided with commercially available products used in the trial. Doses reported were maintained for the entire duration of the study and were not modified when the neonates gained weight.

Feeding protocol and vitamin supplementation

Enteral feeding was started on the first day of life at 10 mL $\cdot$ $kg^{-1} \cdot d^{-1}$ divided into 8 feeds by using preterm formula in all stable infants, as previously described (27). When available, maternal milk was administered. Human milk fortification was added when full enteral feeding was achieved. Aspirate residual from an orogastric tube and abdominal aspect were checked before each feeding. The total amount of gastric residue was calculated daily. In the absence of signs of feeding intolerance in the previous 24 h, enteral nutrition was increased daily by 10–20 mL/kg. Enteral nutrition was discontinued in the case of erythematic abdominal wall, absence of bowel sounds, blood in the stools, or bile or blood in aspirates associated with a radiologic marker of NEC-Bell stage $\geq$II (22). Parenteral nutrition was maintained through a central vascular access in all infants to ensure adequate intake of fluids, electrolytes, and nutrients until full enteral feeding was reached (28). Neonates receiving total parenteral nutrition received intravenously 1 mg Zn/d, starting from day 7 of life. Vitamins and zinc prescriptions during the study period are reported in **Table 2** (29). Iron was supplemented orally according to recommendations (30).

Statistics

To demonstrate a 20% reduction in morbidity in subjects receiving zinc with 85% power and a type 1 error = 0.05 (2-tailed test), a minimum sample size of 100 patients for each group was required. The Kolmogorov-Smirnov test was used to determine whether variables were normally distributed. For continuous variables, groups were compared by using the test of equality of means, the Mann-Whitney U test, and the Kruskal-Wallis test. The chi-square test and Fisher's exact test were used for categorical variables. For 2 related dichotomous variables, the McNemar test was used to detect differences between study

[4] Abbreviations used: BPD, bronchopulmonary dysplasia; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; PVL, periventricular leucomalacia; RCT, randomized controlled trial; ROP, retinopathy of prematurity; VLBW, very low birth weight.

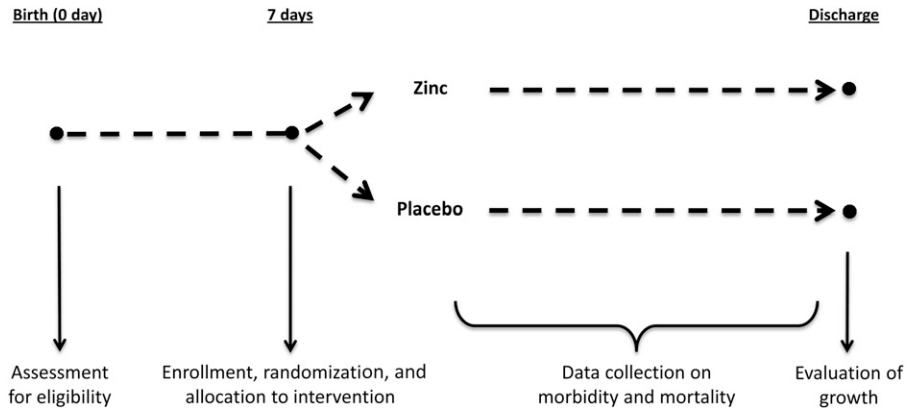

FIGURE 1. Study design.

groups. We performed a multivariate analysis using binary logistic regression analysis to evaluate whether morbidity was influenced by gestational age, modality of delivery, multiple birth, body weight, sex, Apgar score at 5 min, or study group assignment. The level of significance for all statistical tests was 2-sided ($P < 0.05$). Statistical analysis was performed, per intention-to-treat, by a statistician blinded to patient group assignment, by using SPSS version 16.0 for Windows (SPSS Inc).

RESULTS

A total of 203 VLBW neonates were considered eligible for the study. Ten subjects were excluded because of malformations ($n = 4$), early-onset sepsis ($n = 3$), and corioamnionitis ($n = 3$). Eight of these neonates died. Five subjects were lost to follow-up because they required surgery for PDA and ROP. A total of 188 VLBW neonates completed the study protocol (**Figure 2**). The main clinical and demographic characteristics of the study population are reported in **Table 3**.

No difference was observed in the rate of neonates with sepsis, BPD, PVL, or ROP between groups (**Table 4**), but the risk of developing at least one of the abovementioned morbidities was significantly reduced (OR: 0.513; 95% CI: 0.280, 0.939; $P = 0.030$) in subjects receiving zinc supplementation (Table 4). NEC was observed only in neonates in the control group (Table 4). The rate of breast milk use was not significantly different between neonates with (50%) or without (15%) NEC ($P = 0.166$).

Mortality risk after enrollment was higher in neonates who received placebo than in those who received zinc supplementation at a high dose (17 of 96 compared with 5 of 97; RR: 2.37; 95% CI: 1.08, 5.18; $P = 0.006$). No significant differences were observed when the causes of death in the 2 groups were compared (sepsis: 9 of 17 compared with 3 of 5; intraventricular hemorrhage: 4 of 17 compared with 1 of 5; PDA: 3 of 17 compared with 1 of 5; NEC: 1 of 17 compared with 0 of 5). The duration of hospitalization was similar in the 2 groups (group 1: 59 ± 31 d; group 2: 49 ± 35 d; $P = 0.181$). Body weight at discharge was higher in the zinc group than in the placebo group (2208 ± 501 g compared with 1889 ± 639 g; $P = 0.001$). Daily weight gain was similar in the zinc (18.2 ± 5.6 g · kg^{-1} · d^{-1}) and control (17.0 ± 8.7 g · kg^{-1} · d^{-1}; $P = 0.478$) groups.

Binary logistic regression analysis showed that morbidity was influenced by male sex (OR: 2.291; 95% CI: 1.147, 4.577; $P = 0.019$), Apgar score at 5 min (OR: 0.545; 95% CI: 0.370,

TABLE 2
Daily doses of vitamins and zinc in the 2 study populations[1]

	When receiving total parenteral nutrition		When full enteral feeding was reached[2] (enteral nutrition >110 kcal/kg)			
			Preterm-formula fed		Fortified breast-milk fed	
	Group 1 (zinc)	Group 2 (control)	Group 1 (zinc)	Group 2 (control)	Group 1 (zinc)	Group 2 (control)
Vitamin A (μg)	780	1050	573	843	833	1104
Vitamin C (mg)	75	75	75	75	79	73
Vitamin E (mg)	6.8	6.8	7	7	9	9
Folic acid (μg)	146	126	160	140	161	141
Vitamin D_3 (IU)	800	800	1000	1000	1000	1000
Vitamin B-12 (μg)	0.8	0.8	0.8	0.8	0.9	0.9
Iron (mg)[3]	0	0	1.7	1.7	0.04	0.04
Zinc (mg)	10	1	10.3	1.3	10.4	1.4

[1] Doses were calculated including the amounts in parenteral nutrition or enteral nutrition formula and oral multivitamin supplements.

[2] Data are expressed according to the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition, where 100 kcal corresponds to a minimum energy intake of 110 kcal/kg body wt (29).

[3] Doses reported in the table refer to the content of iron in parenteral and enteral nutrition; iron was further supplemented orally at 2–5 mg · kg^{-1} · d^{-1} starting from 4 wk of life in all neonates according to recommendations (30).

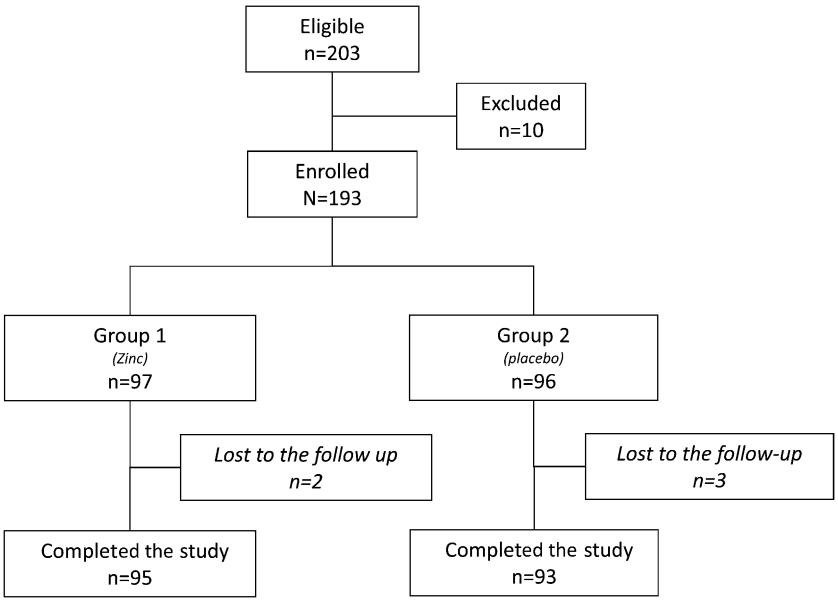

FIGURE 2. Flow of neonates through the study. Mean (±SD) age at discharge: group 1, 59 ± 31 d; group 2, 49 ± 35 d.

0.803; $P = 0.002$), and assignment to the zinc group (OR: 0.500; 95% CI: 0.249, 0.998; $P = 0.048$). Occurrence of vomiting after treatment was similar in the zinc (2.4 ± 4.0%) and the control (2.0 ± 3.7%) groups ($P = 0.396$). No adverse events associated with the administration of multivitamin products containing zinc or not were observed. Compliance was similar between groups (all of the doses prescribed were administered).

DISCUSSION

Our findings indicate a significant reduction in morbidity and mortality associated with the use of high-dose zinc supplements in the diet of VLBW neonates. These results agree with those of previous studies that support an advantage of zinc supplementation early in life. Twelve randomized controlled trials (RCTs) were conducted in subjects aged <28 d of life (13, 15, 19, 31–39). Seven studies had a double-blind design, and most of these trials enrolled slightly preterm or small-for-gestational-age neonates (13, 15, 19, 32, 34, 37, 38). Three studies, including infants with a birth weight <1500 g (31, 36, 39), investigated the effect of zinc on body growth and neurodevelopment. Although positive, the conclusions of these studies were limited by the small number of observations. The 2 largest trials reported a reduced morbidity and mortality associated with zinc use early in life (15, 38). None of these trials enrolled VLBW preterm neonates. A systematic review (16) on the effect of neonatal zinc supplementation on preventing morbidity and mortality evaluated 3 trials including low-birth-weight infants receiving zinc within the first month of

TABLE 3
Main demographic and clinical characteristics of the study population[1]

	Group 1 (zinc; $n = 97$)	Group 2 (control; $n = 96$)	P
Body weight at birth (g)	1114 (1056, 1172)[2]	1033 (969, 1097)	0.066
Gestational age (wk)	28 (28, 29)	28 (27, 29)	0.720
Cesarean delivery [n (%)]	92 (94.8)	87 (90.6)	0.258
Twins [n (%)]	23 (23.7)	18 (18.8)	0.399
Male [n (%)]	37 (38.1)	43 (44.8)	0.349
Apgar score at 1 min	5.3 (4.93, 5.74)	5.3 (4.98, 5.67)	0.969
Apgar score at 5 min	7.5 (7.28, 7.67)	7.5 (7.31, 7.69)	0.880
Critical Risk Index for Babies	2.1 ± 1.3[3]	2.2 ± 1.7	0.114
Respiratory distress syndrome [n (%)]	92 (94.8)	93 (96.9)	0.721
Invasive mechanical ventilation [n (%)]	46 (47.4)	56 (58.3)	0.129
Patent ductus arteriosus requiring pharmacotherapy [n (%)]	35 (36.1)	34 (35.4)	0.923
Intraventricular hemorrhage, grade ≥3 [n (%)]	8 (8.2)	11 (11.5)	0.454
Start of enteral nutrition (h)	3 (1.6, 4.0)	2 (1.8, 2.2)	0.176
Time to reach full enteral feeding (d)	17 (15.3, 19.8)	14 (11.6, 16.7)	0.440
Breast milk/total enteral nutrition at enrollment (%)	45.5 (39.2, 51.8)	46.9 (40.2, 53.5)	0.760
Breast milk/total enteral nutrition at discharge (%)	30.8 (24.9, 36.6)	27.5 (20.8, 34.3)	0.470

[1] For continuous variables, groups were compared by using the test of equality of means, the Mann-Whitney U test, and the Kruskal-Wallis test. The chi-square test and Fisher's exact test were used for categorical variables. For 2 related dichotomous variables, the McNemar test was used to detect differences between study groups.

[2] Mean; 95% CI in parentheses (all such values).

[3] Mean ± SD (all such values).

TABLE 4
Neonatal morbidities observed in the 2 study groups[1]

	Group 1 (zinc; n = 97)	Group 2 (control; n = 96)	P
Late-onset sepsis [n (%)]	16 (16.5)	12 (12.5)	0.431
Necrotizing enterocolitis [n (%)][2]	0 (0)	6 (6.3)	0.014
Bronchopulmonary dysplasia [n (%)][3]	10 (10.3)	15 (15.6)	0.272
Periventricular leucomalacia [n (%)]	3 (3.1)	9 (9.4)	0.071
Retinopathy of prematurity[4]	0 (0.0)	3 (3.17)	0.121
Patients with ≥1 of the conditions listed above [n (%)]	26 (26.8)	40 (41.7)	0.030

[1] For continuous variables, groups were compared by using the test of equality of means, the Mann-Whitney U test, and the Kruskal-Wallis test. The chi-square test and Fisher's exact test were used for categorical variables. For 2 related dichotomous variables, the McNemar test was used to detect differences between study groups.

[2] Necrotizing enterocolitis Bell stage >2 (22).

[3] Oxygen therapy at 36 wk of postconceptional age (23).

[4] Stage >2 (25).

life for ≥14 d (32, 34, 38). They concluded that the available data were too limited to evaluate the efficacy of zinc on long-term morbidity and mortality and that the 3 trials analyzed were not adequately powered to evaluate these outcomes. In addition, all of the trials were conducted in developing countries, and 2 were from India, which limited the generalization of findings to industrialized countries. Finally, the predictors for heterogeneity were not investigated because of the small number of trials, and information on exclusive or predominant breastfeeding status was available only at the time of initiation of the study.

The dietary recommendations for preterm neonates suggest 1.1–2 mg · kg Zn^{-1} · d^{-1} (29). We used higher zinc doses; the use of zinc doses above the dietary guidance level is an emerging research area in pediatric nutrition (10, 16). Many studies suggest that 10–20 mg Zn/d is able to limit the risk of infections in children (40–42). Total zinc intake in the zinc group ranged from 9.7 to 10.7 mg/d. High doses in preterm neonates seem justified because of the lower stores at birth, the higher requirements for growth, and potentially other stress factors present during prematurity (16). In a large trial, Sazawal et al (15) showed a reduction in mortality in the first 9 mo of life in an RCT that enrolled full-term small-for-gestational-age infants who received oral zinc supplementation at high doses (5 mg/d). A dose of 10 mg/d was adopted in other studies involving neonates early in life to prevent bacterial infections (18, 19). Our study was the first trial to use high doses of zinc in VLBW neonates. Zinc requirements are higher in the first days of life than subsequently. We administered zinc irrespective of body weight to provide higher dose in early life. The intakes of zinc in the control group are at the low end of the recommended range (29). Our results suggest that the recommended intakes of zinc meet the metabolic requirement of preterm neonates but may be insufficient to affect morbidity, and, finally, that only high doses of zinc may exert a real pharmacologic effect. No side effects were observed in our study, as previously shown by others (1–3). Further studies should be carried out to establish the optimal dose of zinc in this particular population.

The morbidities analyzed in this study are heterogeneous and have a multifactorial pathogenesis. Zinc plays an important role at multiple levels and may interfere with the pathogenesis of many neurologic, respiratory, and intestinal complications typically affecting preterm neonates (4–11). This aspect may explain how a single nutrient may affect the occurrence of different diseases. In addition, zinc participates in the response to environmental injury determined by oxidative stress and free radical–mediated diseases (1–3). This is probably the route by which zinc interferes with the development of many complications involving the brain and respiratory and intestinal tracts by exerting a protective effect (3–8). The most evident beneficial effect was observed against NEC. This finding could be attributable to the numerous positive activities of zinc at the intestinal level. These include regulation of intestinal permeability, epithelial cell growth and differentiation, and immune response (9, 22). The mechanisms of action of zinc were not investigated in this trial. Further studies are necessary to better define the metabolic effects elicited by high zinc doses at different tissue levels and specifically during NEC development.

The administration of zinc early in life, when the organism is in rapid development, has a key role in promoting growth and differentiation (4–11). Zinc is essential for insulin-like growth factor I and growth hormone receptor gene expression and is crucial to ensure normal grow in early life (43). We found an improvement in weight gain at discharge in infants receiving high zinc supplementation. Several RCTs analyzed the effect of zinc on growth in the first months of life (13, 31, 32, 36, 37, 39), but no conclusive results were obtained. Diet supplementation with zinc may limit extrauterine growth retardation (2). The potential benefit of higher zinc intake on growth was suggested by a cross-sectional study showing a direct correlation between daily weight gain and intestinal zinc absorption with a daily intake of ~3 mg (44). However, these authors suggested that additional studies should be performed to assess the potential benefit of higher zinc intakes. Our results support the potential utility of zinc supplementation, not only to limit the most common morbidities of prematurity, but also to drive the optimal development of body growth and functions.

Our findings, although encouraging, should nevertheless be interpreted with consideration of some limitations. Because we evaluated a specific population of VLBW neonates, the conclusions may not be necessarily extended to subjects with different gestational ages or body weights. Although we observed interesting results on the occurrence of NEC, this study was not powered to demonstrate the efficacy of zinc in preventing this condition. We also observed differences in the use of breast milk when comparing neonates with or without NEC. Although not statistically significant, this difference confirms the protective

role of breast milk on the risk of NEC (45). Serum concentrations of zinc or other vitamins were not measured to avoid adjunctive procedures and blood collections in preterm infants at high risk of iatrogenic anemia and to minimize handling (46, 47). The high dose of zinc used during this trial was established on the basis of previous evidence (18, 19, 40–42), whenever the zinc in the placebo group was administered at the low end of the recommended range. Further studies are probably advocated to establish the optimal dose of zinc in this particular population. We used 2 multivitamin formulations with slight differences in some nutrients other than zinc (eg, folic acid and vitamin A). To the best of our knowledge there is no evidence that postnatal folic acid supplementation influences the occurrence of morbidities and survival in VLBW neonates. Previous RCTs on parenteral vitamin A supplementation (48) were unable to demonstrate a reduction in the mortality rate of preterm infants. One RCT evaluated the efficacy of vitamin A given orally in neonates (49). In this study, vitamin A supplementation failed to reduce mortality and morbidity (49). Finally, to the best of our knowledge this was the first time a dose of ~10 mg Zn/d was administered to preterm neonates. Further studies should be performed to evaluate the potential efficacy of different doses of oral zinc supplementation.

In conclusion, this was the first study to investigate the efficacy of zinc supplementation in reducing morbidity and mortality in VLBW preterm neonates during hospitalization. A high zinc intake could be considered a safe and useful nutritional intervention in early life. Further studies should be performed to shed light on the mechanisms underlying the effects observed.

The authors' responsibilities were as follows—GT, AP, and RBC: designed the research; GT and AP: performed the data monitoring and statistical analysis; FM, SC, MGC, and AS: cared for the patients and collected the data; MDC and EB: contributed to the study design and revised the final version of the manuscript; and GT and RBC: wrote the first draft of the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript. The authors had no conflict of interest to declare.

REFERENCES

1. Zlotkin SH, Atkinson S, Lockitch G. Trace elements in nutrition for premature infants. Clin Perinatol 1995;22:223–40.
2. Voyer M, Davakis M, Antener I, Valleur D. Zinc balances in preterm infants. Biol Neonate 1982;42:87–92.
3. Giles E, Doyle LW. Zinc in extremely low-birth weight or very preterm infants. Neo Rev 2007;8:165–72.
4. Levenson CW, Morris D. Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr 2011;2:96–100.
5. Gu Y, Dee CM, Shen J. Interaction of free radicals, matrix metalloproteinases and caveolin-1 impacts blood-brain barrier permeability. Front Biosci Schol Ed 2011;3:1216–31.
6. Isikay I, Bilginer B, Narin F, Soylemezoglu F, Akalan N. The effect of intracisternal Zn (II) protoporphyrin IX on vasospasm process in the experimental subarachnoid hemorrhage model. Acta Neurochir Suppl 2011;110:33–7.
7. D'Angio CT, Maniscalco WM. Bronchopulmonary dysplasia in preterm infants: pathophysiology and management strategies. Paediatr Drugs 2004;6:303–30.
8. Strange RC, Cotton W, Fryer AA, Jones P, Bell J, Hume R. Lipid peroxidation and expression of copper-zinc and manganese superoxide dismutase in lungs of premature infants with hyaline membrane disease and bronchopulmonary dysplasia. J Lab Clin Med 1990;116:666–73.
9. Buccigrossi V, Giannattasio A, Armellino C, Lo Vecchio A, Caiazzo MA, Guarino A. The functional effects of nutrients on enterocyte proliferation and intestinal ion transport in early infancy. Early Hum Dev 2010;86(suppl 1):55–7.
10. Canani RB, Ruotolo S. The dawning of the zinc era in the treatment of pediatric acute gastroenteritis worldwide? J Pediatr Gastroenterol Nutr 2006;42:253–5.
11. Iwaya H, Kashiwaya M, Shinoki A, Lee JS, Hayashi K, Hara H, Ishizuka S. Marginal zinc deficiency exacerbates experimental colitis induced by dextran sulfate sodium in rats. J Nutr 2011;141:1077–82.
12. Shah MD, Shah SR. Nutrient deficiencies in the premature infant. Pediatr Clin North Am 2009;56:1069–83.
13. Díaz-Gómez NM, Doménech E, Barroso F, Castells S, Cortabarria C, Jiménez A. The effect of zinc supplementation on linear growth, body composition, and growth factors in preterm infants. Pediatrics 2003; 111:1002–9.
14. Zlotkin SH, Cherian MG. Hepatic metallothionein as a source of zinc and cysteine during the first year of life. Pediatr Res 1988;24:326–9.
15. Sazawal S, Black RE, Menon VP, Dinghra P, Caulfield LE, Dhingra U, Bagati A. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial. Pediatrics 2001;108:1280–6.
16. Gulani A, Bhatnagar S, Sachdev HP. Neonatal zinc supplementation for prevention of mortality and morbidity in breastfed low birth weight infants: systematic review of randomized controlled trials. Indian Pediatr 2011;48:111–7.
17. Yakoob MY, Theodoratou E, Jabeen A, Imdad A, Eisele TP, Ferguson J, Jhass A, Rudan I, Campbell H, Black RE, et al. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria. BMC Public Health 2011;11(suppl 3):S23.
18. Basnet S, Shrestha PS, Sharma A, Mathisen M, Prasai R, Bhandari N, Adhikari RK, Sommerfelt H, Valentiner-Branth P, Strand TA. A randomized controlled trial of zinc as adjuvant therapy for severe pneumonia in young children. Pediatrics 2012;129:701–8.
19. Bhatnagar S, Wadhwa N, Aneja S, Lodha R, Kabra SK, Natchu UC, Sommerfelt H, Dutta AK, Chandra J, Rath B, et al. Zinc as adjunct treatment in infants aged between 7 and 120 days with probable serious bacterial infection: a randomized, double-blind, placebo-controlled trial. Lancet 2012;379:2072–8.
20. International Neonatal Network. The CRIB (clinical risk index for babies) score: a tool for assessing inicial neonatal risk and comparing the performance of neonatal intensive care units. Lancet 1993;342:193–8.
21. Terrin G, Passariello A, Manguso F, Salvia G, Rapacciuolo L, Messina F, Raimondi F, Canani RB. Serum calprotectin: an antimicrobial peptide as a new marker for the diagnosis of sepsis in very low birth weight newborns. Clin Dev Immunol 2011; 2011:291085.
22. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364: 255–64.
23. Bancalari E. Changes in the pathogenesis and prevention of chronic lung disease of prematurity. Am J Perinatol 2001;18:1–9.
24. Whitelaw A. Intraventricular haemorrhage and posthaemorrhagic hydrocephalus: pathogenesis, prevention and future interventions. Semin Neonatol 2001;6:135–46.
25. Sylvester CL. Retinopathy of prematurity. Semin Ophthalmol 2008; 23:318–23.
26. American Academy of Pediatrics Committee on Fetus and Newborn. Hospital discharge of the high-risk neonate. Pediatrics 2008;122:1119–26.
27. Terrin G, Passariello A, Canani RB, Manguso F, Paludetto R, Cascioli C. Minimal enteral feeding reduces the risk of sepsis in feed-intolerant very low birth weight newborns. Acta Paediatr 2009;98:31–5.
28. Terrin G, Passariello A. Artificial nutrition in very low birth weight newborns and impact on growth: practical methods to avoid and treat malnutrition in stable and unstable neonates. In: Preedy VR, ed. Handbook of growth and growth monitoring in health and disease. New York, NY: Springer Science+Business Media LCC Press, 2012: 387–420.
29. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, Domellöf M, Embleton ND, Fusch C, Genzel-Boroviczeny O, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 2010; 50:85–91.
30. Rao R, Georgieff MK. Iron therapy for preterm infants. Clin Perinatol 2009;36:27–42.
31. Friel JK, Andrews WL, Matthew JD, Long DR, Cornel AM, Cox M, McKim E, Zerbe GO. Zinc supplementation in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 1993;17:97–104.

32. Castillo-Durán C, Rodríguez A, Venegas G, Alvarez P, Icaza G. Zinc supplementation and growth of infants born small for gestational age. J Pediatr 1995;127:206–11.
33. Lira PI, Ashworth A, Morris SS. Effect of zinc supplementation on the morbidity, immune function, and growth of low-birth-weight, full-term infants in northeast Brazil. Am J Clin Nutr 1998;68:418S–24S.
34. Sur D, Gupta DN, Mondal SK, Ghosh S, Manna B, Rajendran K, Bhattacharya SK. Impact of zinc supplementation on diarrheal morbidity and growth pattern of low birth weight infants in kolkata, India: a randomized, double-blind, placebo-controlled, community-based study. Pediatrics 2003;112:1327–32.
35. Black MM, Sazawal S, Black RE, Khosla S, Kumar J, Menon V. Cognitive and motor development among small-for-gestational-age infants: impact of zinc supplementation, birth weight, and caregiving practices. Pediatrics 2004;113:1297–305.
36. Loui A, Raab A, Wagner M, Weigel H, Grüters-Kieslich A, Brätter P, Obladen M. Nutrition of very low birth weight infants fed human milk with or without supplemental trace elements: a randomized controlled trial. J Pediatr Gastroenterol Nutr 2004;39:346–53.
37. Bueno O, Bueno G, Moreno LA, Nuviala RJ, Pérez-González JM, Bueno M. Zinc supplementation in infants with asymmetric intra uterine growth retardation; effect on growth, nutritional status and leptin secretion. Nutr Hosp 2008;23:212–9.
38. Taneja S, Bhandari N, Rongsen-Chandola T, Mahalanabis D, Fontaine O, Bhan MK. Effect of zinc supplementation on morbidity and growth in hospital-born, low-birth-weight infants. Am J Clin Nutr 2009;90:385–91.
39. Islam MN, Chowdhury MA, Siddika M, Qurishi SB, Bhuiyan MK, Hoque MM, Akhter S. Effect of oral zinc supplementation on the growth of preterm infants. Indian Pediatr 2010;47:845–9.
40. Lazzerini M, Ronfani L. Oral zinc for treating diarrhoea in children. Cochrane Database Syst Rev 2013;1:CD005436.
41. Malik A, Taneja DK, Devasenapathy N, Rajeshwari K. Short-course prophylactic zinc supplementation for diarrhea morbidity in infants of 6 to 11 months. Pediatrics 2013;132:e46–52.
42. Krebs NF. Update on zinc deficiency and excess in clinical pediatric practice. Ann Nutr Metab 2013;62(suppl 1):19–29.
43. MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000;130:1500S–8S.
44. Jalla S, Krebs NF, Rodden D, Hambidge KM. Zinc homeostasis in premature infants does not differ between those fed preterm formula or fortified human milk. Pediatr Res 2004;56:615–20.
45. Lucas A, Cole TG. total enteral nutrition at and neonatal necrotizing enterocolitis. Lancet 1990;336:1519–23.
46. Strauss RG. Anaemia of prematurity: pathophysiology and treatment. Blood Rev 2010;24:221–5.
47. Vandenberg KA. Individualized developmental care for high risk newborns in the NICU: a practice guideline. Early Hum Dev 2007; 83:433–42.
48. Darlow BA, Graham PJ. Vitamin A supplementation to prevent mortality and short- and long-term morbidity in very low birth weight infants. Cochrane Database Syst Rev 2011;10:CD000501.
49. Wardle SP, Hughes A, Chen S, Shaw NJ. Randomised controlled trial of oral vitamin A supplementation in preterm infants to prevent chronic lung disease. Arch Dis Child Fetal Neonatal Ed 2001;84:F9–13.