

PEDIATRICS®

The Effect of Zinc Supplementation on Linear Growth, Body Composition, and Growth Factors in Preterm Infants

N. Marta Díaz-Gómez, Eduardo Doménech, Flora Barroso, Silvia Castells, Carmen Cortabarría and Alejandro Jiménez

Pediatrics 2003;111;1002-1009

DOI: 10.1542/peds.111.5.1002

This information is current as of October 19, 2004

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://www.pediatrics.org/cgi/content/full/111/5/1002>

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2004 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



The Effect of Zinc Supplementation on Linear Growth, Body Composition, and Growth Factors in Preterm Infants

N. Marta Díaz-Gómez, PhD, MD*‡; Eduardo Doménech, PhD, MD§¶; Flora Barroso, PhD, MD§; Silvia Castells, PhD‡; Carmen Cortabarría, MD§¶; and Alejandro Jiménez, PhD*

ABSTRACT. *Objective.* The aim of our study was to evaluate the effect of zinc supplementation on linear growth, body composition, and growth factors in premature infants.

Design. Thirty-six preterm infants (gestational age: 32.0 ± 2.1 weeks, birth weight: 1704 ± 364 g) participated in a longitudinal double-blind, randomized clinical trial. They were randomly allocated either to the supplemental (S) group fed with a standard term formula supplemented with zinc (final content 10 mg/L) and a small quantity of copper (final content 0.6 mg/L), or to the placebo group fed with the same formula without supplementation (final content of zinc: 5 mg/L and copper: 0.4 mg/L), from 36 weeks postconceptional age until 6 months corrected postnatal age. At each evaluation, anthropometric variables and bioelectrical impedance were measured, a 3-day dietary record was collected, and a blood sample was taken. We analyzed serum levels of total alkaline phosphatase, skeletal alkaline phosphatase (sALP), insulin growth factor (IGF)-I, IGF binding protein-3, IGF binding protein-1, zinc and copper, and the concentrations of zinc in erythrocytes.

Results. The S group had significantly higher zinc levels in serum and erythrocytes and lower serum copper levels with respect to the placebo group. We found that the S group had a greater linear growth (from baseline to 3 months corrected age: Δ score deviation standard length: $1.32 \pm .8$ vs $.38 \pm .8$). The increase in total body water and in serum levels of sALP was also significantly higher in the S group (total body water: 3 months; corrected age: $3.8 \pm .5$ vs $3.5 \pm .4$ kg, 6 months; corrected age: $4.5 \pm .5$ vs $4.2 \pm .4$ kg; sALP: 3 months; corrected age: 140.2 ± 28.7 vs 118.7 ± 18.8 $\mu\text{g/L}$).

Conclusions. Zinc supplementation has a positive effect on linear growth in premature infants. *Pediatrics* 2003;111:1002–1009; zinc, insulin-like growth factors, preterm infants, growth, body composition.

ABBREVIATIONS. GH, growth hormone; IGF, insulin growth factor; BP, binding protein; BI, bioelectrical impedance; S, supplemental; P, placebo; sALP, skeletal alkaline phosphatase; BW, birth weight; GA, gestational age; PCA, postconceptional age; CA, corrected postnatal age; TBW, total body water; BIA, bioelectrical impedance analysis; HSD, honest significance difference; SDS, score deviation standard; EZn, erythrocyte mean zinc.

From the *Research Unit, University Hospital of the Canaries, La Laguna, Spain; †Nursing School, University of La Laguna; §Faculty of Medicine, University of La Laguna, La Laguna, Spain; and ¶Department of Paediatrics, University Hospital of the Canaries, Tenerife, Spain.

Received for publication May 2, 2002; accepted Sep 23, 2002.

Address correspondence to N. Marta Díaz Gómez, MD, PhD, Avda, Trinidad, 48-bajo, 38204 La Laguna, Tenerife, Canary Islands, Spain. E-mail: nmdiaz@ull.es

PEDIATRICS (ISSN 0031 4005). Copyright © 2003 by the American Academy of Pediatrics.

Preterm birth is often associated with nutritional compromise and impaired growth performance. It is believed that the relation between nutrition and growth is mediated by changes in the hormone and growth factors axis.^{1,2} In humans, nutrient restriction causes impaired signaling of the growth hormone (GH) receptor, producing GH resistance and reducing the synthesis of liver insulin growth factor (IGF)-I and its binding protein (BP) IGFBP-3. On the other hand, the reduction of glucose availability causes a decrease in insulin secretion which increments IGFBP-1 levels, thus reducing IGF-I bioavailability.³ These changes in the GH axis associated with malnutrition could be explained in part by zinc deficiencies.⁴

Zinc deficiency during infancy has a negative effect on the endocrine system, leading to growth failure among other clinical manifestations. Zinc is a key component of the cell architecture and function in the organism. It is required for the production of over 200 enzymes including phosphatases, metalloproteinases, oxidoreductases, and transferases which are involved in protein synthesis, nucleic acid metabolism, and immune functions. In addition, it is a structural component of various proteins, hormones, and nucleotides.⁵ Zinc also plays an important role in gene transcription. Numerous transcription factors contain a functional domain named "zinc-finger", composed of ~30 amino acids around a zinc ion. Zinc-finger containing transcription factors play many roles in protein-DNA or protein-RNA interactions.⁶ Zinc is one of the most prevalent trace elements in the brain. Accordingly, there is evidence that zinc may be essential for brain function as well as for growth in the fetus and child.⁷ However, it remains unclear how zinc deficiency relates to any of these changes at the biochemical level. Studies of zinc-deficient animals have demonstrated that one of the mechanisms involved in the slowing down of growth is the low concentration of circulating IGF-I. It has been shown that serum IGF-I decreases during zinc depletion and increases again as soon as zinc is supplied in the diet.⁸ Despite this, growth failure is not reversed by maintaining IGF-I levels through exogenous administration, suggesting that the defect occurs in hormone signaling.⁹

Of greater practical concern is the fact that a high intake of zinc reduced copper absorption. For this reason, it has been recommended that the zinc-to-copper molar ratio in infant formulas should not

exceed 20.¹⁰ The main biological role of copper is as an electron transfer intermediate in redox reactions. There are numerous enzymes with important redox activity which contain copper as a cofactor for catalytic activity. These include cytochrome-c oxidase, ceruloplasmin, amine oxidases, and superoxide dismutases. Clinical manifestations of copper deficiency in the premature infant have been well documented and include anemia, neutropenia, skeletal abnormalities, and possibly, disorders of the central nervous system function.¹¹

Preterm infants have a high risk of zinc, copper, and other micronutrient deficiencies and are frequently growth-retarded. There are multiple contributing factors which explain this. As a consequence of shorter gestation and the immaturity of the gastrointestinal tract, these infants have lower body stores. Premature infants also have a high nutrient demand because of rapid postnatal growth and an increased risk of intercurrent diseases, which means that the intake of nutrients may be inadequate during the first months of life. In an attempt to improve the growth of premature infants, various controlled nutritional intervention studies have been conducted. These studies have shown that zinc supplementation has a positive influence on linear growth,^{12,13} motor development,¹³ and weight gain, and a lower prevalence of diarrhea.¹⁴ To our knowledge, neither these studies conducted in premature infants nor other clinical trials of zinc supplementation at different ages (fetal life, childhood, and adolescence) have investigated the effect of zinc on body composition, estimated by bioelectrical impedance (BI) analysis and on IGFs. For this reason, we decided to address this particular field of research.

METHODS

We designed a longitudinal double-blind, randomized clinical trial to detect differences in growth between supplemental (S) and placebo (P) groups (ie, a higher growth rate in the S group). Thus, we tested the effect of zinc supplementation on linear growth, body composition, serum levels of skeletal alkaline phosphatase (sALP), and growth factors in premature infants during the first 6 months of life.

Participants

Our study was conducted in a third level neonatal unit at the University Hospital of the Canaries which serves a small island population and has a relatively low number of newborn admissions (~30 per annum under 1500 g birth weight [BW]). From these, we selected 37 preterm infants before discharge home. The criteria for inclusion were: 1) gestational age (GA) below 37 weeks; 2) BW between 1000 and 2500 g; 3) BW appropriate for GA (BW between the 10th and the 90th percentile for gestational age); 4) the infants were in a stable clinical condition, without any evidence of disease likely to influence growth and neurodevelopment; and 5) had been formula-fed during the hospital stay. GA, in weeks, was estimated from maternal history and according to the Dubowitz test, applied by a trained pediatrician.

The study was approved by the Ethics Committee of the University Hospital of the Canaries, and written informed consent to participate in the study was obtained from the parents of all the children. Parents knew they could withdraw their child from the study at any time. No attempts were made to influence the mothers in their choice of infants feeding.

Interventions

The infants were evaluated at 36 weeks postconceptional age (PCA); baseline, and at 3 and 6 months corrected postnatal age

(CA). At each evaluation, anthropometric variables and BI were measured, a 3-day dietary record was collected, and a blood sample taken. Baseline measurements were conducted while the infants were still being fed a premature formula as per nursery protocol. Supplemented and placebo study formula was started after the first blood sampling. All infants received the formula from 36 weeks PCA until 6 months CA.

Infants in the S group (32.2 ± 2.3 weeks GA, 1717 ± 401 g BW) were fed with a standard term infant formula supplemented with zinc sulfate (final content 10 mg/L), whereas the P group (31.9 ± 1.9 weeks GA, 1692 ± 333 g BW) received the same formula without supplementation (final content of zinc: 5 mg/L). To avoid inhibition of copper absorption by zinc, we added an extra amount of copper sulfate to the zinc-supplemented formula to a final content of .6 mg/L. The other components of the formula were kept the same (Table 1). Zinc and copper supplements were added to the formula during manufacturing.

An iron supplement (1 mg/kg, as sulfate) was given once daily, 20 minutes before food, to all infants during the study period. Solid foods were introduced after 4 months of age.

Outcomes

Primary outcome measure was growth (length, weight, cephalic perimeter). Secondary outcome measures were: total body water (TBW) estimated by BI, brachial perimeter, skinfolds, analytical variables (serum levels of copper, zinc, total alkaline phosphatase, sALP, IGF-I, IGFBP-3, and IGFBP-1; blood hemoglobin levels; and levels of zinc in erythrocytes), and daily intake of copper and zinc, controlling for the following confounding variables: BW, GA, sex, 1- and 5-minute scores in the Apgar test, target height, daily intake of energy and proteins, milk volume intake at each evaluation, and percentage of energy from solid food at 6 months CA.

Dietary Assessment

Food records were completed by nursing staff at 36 weeks PCA (baseline) and by the parents at each follow-up visit, using household measures. Dietary records at baseline and 3 months CA only consisted of the daily volume of formula milk intake. Solid food was included in all cases after the 3 months evaluation. All records were checked for accuracy and consistency by a nutritionist. We used a 3-day dietary record, validated according to Goldberg et al's method.¹⁵ Mean daily intake of energy, proteins, zinc, and copper was calculated using food composition tables.^{16,17} For infant formula and industrially prepared infant food, information regarding nutrient content was obtained from the manufacturers.

Anthropometry

The anthropometric measurements were always taken by the same person. Nude weight was recorded using an electronic balance with an accuracy of ± 5 g (Soehnle, West Germany). Crown-heel length was measured in the supine position to the nearest .1 cm using a Holtain infantometer (Holtain Limited, Dyfed, United Kingdom). Occipitofrontal head circumference was determined as the largest measurement taken across the occiput and forehead

TABLE 1. Nutrient Composition of the Supplemented and Standard Study Formulas (per 100 mL)

	Standard Formula	Supplemented Formula
Energy (kcal)	67	67
Protein (g)	1.5	1.5
Fats (g)	3.4	3.4
Carbohydrate (g)	7.7	7.7
Sodium (mg)	16	16
Potassium (mg)	66	66
Calcium (mg)	42	42
Phosphorus (mg)	21	21
Chloride (mg)	44	44
Iron (mg)	.8	.8
Zinc (mg)	.5	1.0
Copper (μ g)	40	60

Formula zinc and copper concentrations were confirmed by chemical analysis in our laboratory.

using a metal tape. Mid-upper arm circumference was recorded on the right limb, as the mean of 2 successive measurements and rounded to the nearest .1 cm. Skinfold measurements were taken using a Holtain skinfold caliper (Holtain Limited) from the triceps, biceps, subscapular, and suprailiac sites. Skinfold was recorded as the mean of 2 successive measurements and rounded to the nearest .1 mm.

Bioelectrical Impedance

BI measurements were recorded using a tetrapolar electrode configuration at a fixed 50 KHz frequency (BI analysis [BIA]/Maltron BF 905; SanoCare Human Systems, Essex, United Kingdom). Infants were placed in a supine position with legs extended and slightly apart. The arms were placed with the forearms parallel to, but separate from, the body, with the hands comfortably extended, palm down. Measurements were made using electrodes cut to 1-cm diameter. The electrodes were placed in the standard position. The current electrodes were positioned distal to the voltage electrodes, at a center to center distance of 3 cm. The impedance index ($\text{length}^2/\text{impedance}$) was calculated. We applied the predictive equation of Kushner et al¹⁸ to estimate TBW ($\text{TBW} = .591 \text{ length}^2/\text{impedance} + .065 \text{ weight} + .04$).

Biochemical Analyses

Blood samples were obtained by means of an antecubital vein puncture, between 9:00 and 9:15 AM, at least 3 hours after the last bottle feed. Approximately 3 mL of blood was immediately transferred to a vacutainer and 2 mL was transferred to an ethylenediaminetetraacetic acid tube.

Hemoglobin concentrations were assayed within half an hour of blood extraction, using the cyanmethemoglobin method. One milliliter of ethylenediaminetetraacetic acid blood was used to determine the erythrocyte zinc concentrations.

The erythrocytes were washed with saline and dried at 90°C for 18 hours in a drying oven, weighed, and then ashed at 500°C for 18 hours in a muffle furnace. The ashed erythrocytes were diluted with deionized water and 0.1 N hydrochloric acid to measure the concentration of zinc.

After the serum was separated, total alkaline phosphatase levels were measured by the colorimeter method. The remaining serum was stored in disposable polystyrene tubes at -40°C until use.

Serum zinc, copper, and the concentration of zinc in ashed erythrocytes were measured by flame atomic absorption spectrophotometry in a Perkin-Elmer 2380 (Perkin-Elmer Corp, Wellesley, MA). To avoid contamination of the samples with the minerals under study, disposable plastic material was used where possible. All remaining material was washed in nitric acid 1:1 and distilled water and then dried in an oven at 150°C. We used deionized water for the dilution of the samples, the standards, and the controls. The coefficient of variation of the measurements was always <5%.

IGF-I was measured by radioimmunoassay after separation of the IGFs from the serum-BPs by acid-ethanol extraction, avoiding interference with the technique (Nichols Institute Diagnostics, San Juan Capistrano). We also analyzed serum levels of IGFBP-3, by immunoradiometric assay (Nichols Institute Diagnostics), IGF-BP-1 (by immunoenzymometric assay; Medix Biochemica OY Ab, Kauniainen, Finland), and sALP (immunoradiometric assay; Beckman Coulter, Fullerton, CA). All samples were assayed in duplicate. For the IGF-I assay, intra and interassay coefficient of variation was 3.4% and 8.6%, respectively. These values were 4.6% and 6.7% for the IGFBP-3 assay, 3.5% and 7.2% for IGFBP-1, and 4.4% and 7.6% for skeletal phosphatase alkaline, respectively.

Sample Size

Sample sizes were calculated for an assumed 5% difference in linear growth, a standard deviation of 7.5, a power of 80%, and a 1 side level of significance of .05.

Randomization

Those infants who complied with all the inclusion criteria were selected at 34 to 35 weeks PCA. Once we received informed parental consent in writing, the infants were assigned to 1 of the 2 study groups using randomization tables generated with Statistica version 5.0 (Statsoft, Tulsa, OK).

The formula was manufactured for us by a company in the food industry (Nestle Espana SA) was identical in appearance and flavor, and was packaged in identical containers. To maintain the blindness of the study, only the person who provided the appropriate milk formula (identified by a sticker with the random letter: A or B) to the parents knew to which group the random letters corresponded and this person took no further part in the study procedures. Participants were enrolled and assigned to a random letter by one of the investigators (C.C.) who was not aware which group the letters represented. Assignment to the zinc or P group was not known either to the families or to the other investigators.

Statistical Analyses

Statistical analysis was performed by using the Statistical Package for Social Science version 10.0.1 (SPSS, Chicago, IL). Means were compared by using the analysis of variance test for repeated measurements. Initial length was controlled by using covariance analysis. For simple comparison between 2 means we used the Student *t* test. Simple linear correlations are expressed with *r*-Pearson's coefficient. *P* < .05 was taken as the limit of significance. All of the results for continuous variables are expressed as mean ± standard deviation.

The *z* scores for weight-for-age, length-for-age, and head perimeter-for-age were calculated. Combined intra- and extra-uterine Largo growth curves¹⁹ were used as the reference pattern until 40 weeks, PCA and Hernandez growth curves²⁰ after that (at 3 and 6 months CA evaluations).

RESULTS

After allocation, the parents of 1 patient refused to continue in the trial and the infant was replaced by another to maintain a comparable number of cases in both groups (Fig 1). The rest of the participants in each group received the intended treatment, completed the study protocol, and were analyzed for the primary outcome. The only protocol deviation occurred on those occasions when we were unable to obtain sufficient blood to complete all the analyses. Missing values were not substituted (see Table 4).

The infants included in the study were recruited between June 1998 and December 1999. Follow-up evaluations were carried out at 36 weeks PCA, 3 months and 6 months CA.

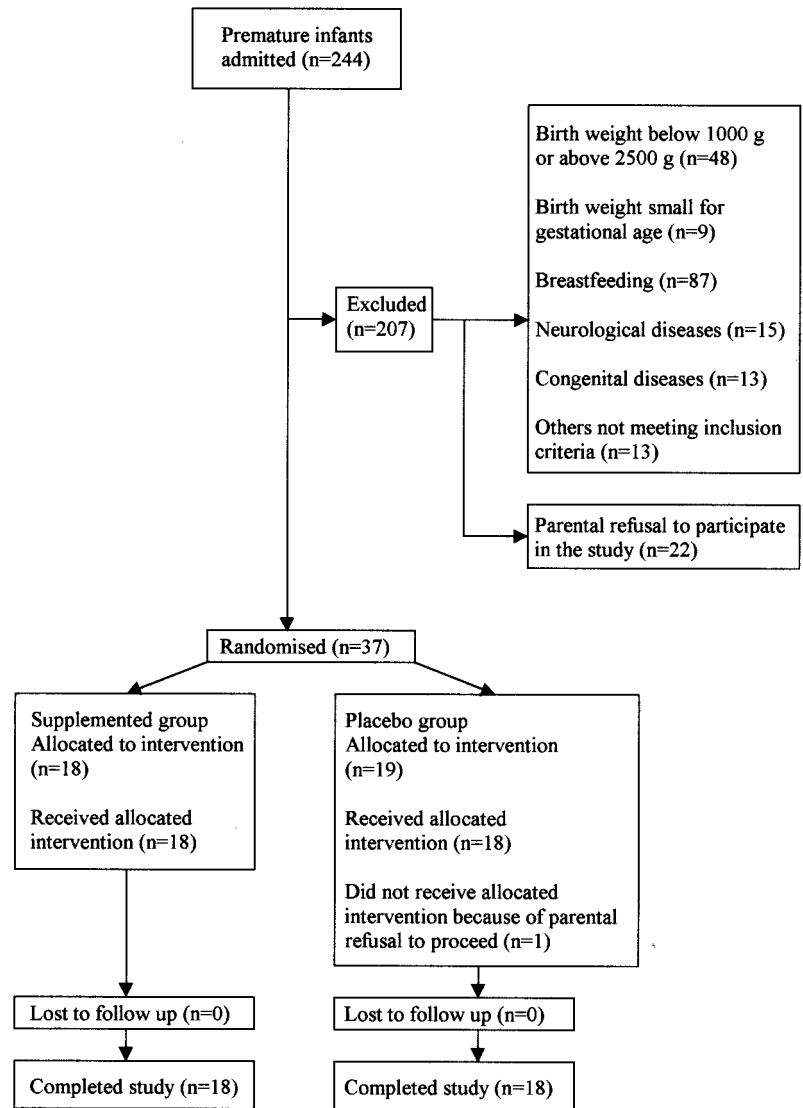
Baseline Data

On admission, the S and P groups did not differ in the variables studied (see Tables 3 and 4 and Fig 2). Infants of both groups were comparable for Apgar score (at 5 minutes: 7.9 ± 2.0 vs 7.8 ± 1.3) and for male:female ratio (12:6 vs 8:10). The target height was similar in both groups (S group: males 174.7 ± 4.9 cm, females 161.8 ± 6.4 cm; P group: males 175.4 ± 4.7 cm, female 160.0 ± 4.3 cm).

Nutritional Intake

Calculated energy and protein intakes were not higher in the S group than in the P group at any time during the study. At 3 months CA, mean zinc intake in the S group was about twice that of the P group. The introduction of complementary foods after this age caused a decrease in milk intake in both groups at 6 months CA. There was no statistical difference among the groups in the percentage of energy intake from milk and from solid food at 6 months CA (Table 2). Both study formulas were well-tolerated and no infants showed adverse reactions or side effects.

Fig 1. Flow diagram of patient randomization protocol.



Anthropometric and BI Measurements

We found a significant interaction between the independent variables (study period and group) in both length and TBW estimated by BI (length in cm $F[2,68] = 5.03, P = .009$; TBW $F[2,62] = 4.59, P =$

.014). Posthoc pairwise comparisons revealed that there were significant differences between groups (S and P) in both variables, with higher values in the S group at 3 and 6 months CA (Tukey honest significance difference [HSD]: length in cm $>1.046, P = .01$;

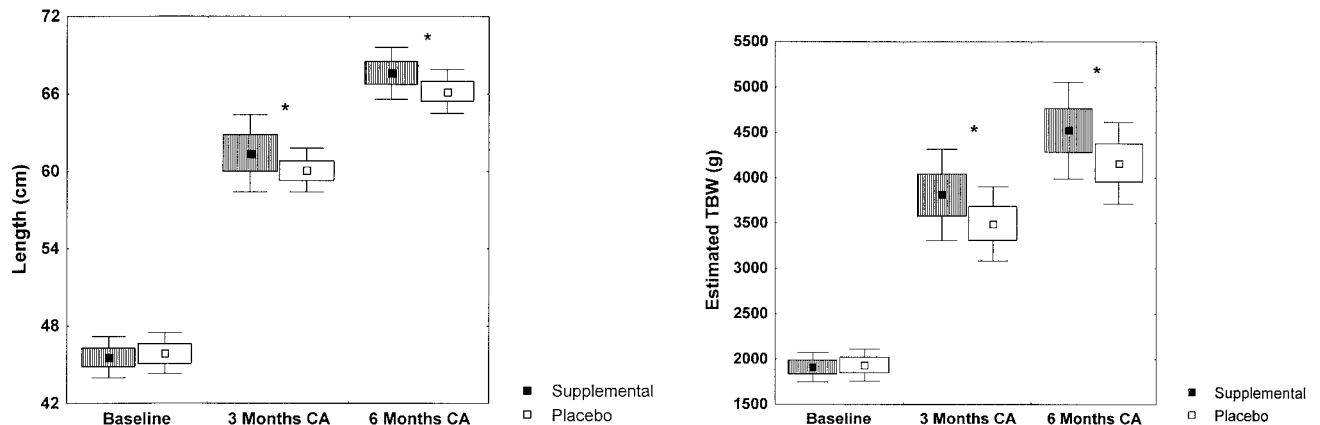


Fig 2. Changes in length and TBW estimated by BIA through the study period in the P and S groups at baseline (36 weeks PCA), 3 and 6 months CA. Closed boxes: S group mean \pm standard error. Open boxes: P group mean \pm standard error. Whisker: Mean \pm standard deviation. *, S versus P: $P = .01$.

TABLE 2. Dietary Intake for Study Infants

Sampling Time		S Group	P Group	P
36 wk PCA (Initial)	Energy (kcal/kg/d)	123 ± 14	129 ± 13	NS
	Protein (g/kg/d)	3.5 ± .4	3.6 ± .4	NS
	Zinc (mg/kg/d)	.88 ± .09	.91 ± .09	NS
	Copper (mg/kg/d)	.11 ± .01	.11 ± .01	NS
	Milk volume (mL/kg/d)	170 ± 23	184 ± 20	NS
3 mo CA	Energy (kcal/kg/d)	109 ± 16	104 ± 22	NS
	Protein (g/kg/d)	2.4 ± .3	2.3 ± .4	NS
	Zinc (mg/kg/d)	1.43 ± .24	.71 ± .11	.0001
	Copper (mg/kg/d)	.09 ± .01	.06 ± .01	.0001
	Milk volume (mL/kg/d)	146 ± 26	130 ± 30	NS
6 mo CA	Energy (kcal/kg/d)	113 ± 22	109 ± 16	NS
	Protein (g/kg/d)	2.5 ± .4	2.5 ± .4	NS
	Zinc (mg/kg/d)	.94 ± .17	.55 ± .09	.0001
	Copper (mg/kg/d)	.09 ± .01	.08 ± .01	.01
	Milk volume (mL/kg/d)	77 ± 19	77 ± 21	NS
	% energy intake from			
	Milk	46 ± 16	47 ± 11	NS
	Cereals	17 ± 9	15 ± 7	NS
	Vegetables	17 ± 5	18 ± 4	NS
	Fruits	16 ± 9	15 ± 9	NS
Meats	2 ± 2	3 ± 2	NS	
Yogurt	2 ± 4	2 ± 4	NS	

NS indicates not significant. Values are mean ± standard deviation.

TBW >.247, $P = .01$; Fig 2). There were no significant differences between groups in the other anthropometric variables (Table 3).

We found that infants in the S group had a greater linear growth than infants in the P group, either expressed in Δ score deviation standard (SDS) length or in centimeters per week, over the first 3 months and also over the complete study period (Δ SDS length: baseline-3 months CA: $1.32 \pm .81$ vs $.38 \pm .84$, $P = .002$; baseline-6 months CA: $1.31 \pm .69$ vs $.34 \pm .83$, $P = .001$; length increase: baseline-3 months CA: $.91 \pm .08$ vs $.81 \pm .07$ cm/week, $P = .001$; baseline-6 months CA: $.73 \pm .05$ vs $.69 \pm .05$ cm/week, $P = .036$). Covariance analysis showed that the effect of the initial length was not significant. Moreover, after controlling by initial length, the differences in the length increase between the S and the control group remained significant.

The increase in weight and cephalic perimeter was also greater in the S group than in the P group, but differences between groups were not significant.

Biochemical Outcome

From baseline to 3 months CA, serum IGF-I and IGFBP-3 levels increased in both groups. In zinc-supplemented infants, the increase in serum IGF-I levels was higher than in the P group, although not statistically significant (Table 4).

The levels of total alkaline phosphatase showed a significant increase through the first months of the study in both groups ($F[2,50] = 7.15$, $P = .002$), being

higher in the S group than in the P group ($F[1,25] = 6.06$, $P = .021$; Table 4).

The concentration of sALP also showed significant differences depending on the study period in both groups ($F[2,46] = 90.71$, $P < .0001$), achieving maximum levels at 3 months CA and then decreasing, without reaching baseline levels at 6 months CA. There was a significant interaction between independent variables ($F[2,46] = 5.99$, $P = .005$). Posthoc pairwise comparisons revealed that there were significant differences between groups at 3 months CA (Tukey HSD >14.29, $P = .05$), with higher values in S group (Table 4).

The serum levels of sALP at 3 months CA were significantly correlated with Δ SDS length, weight, and head perimeter during the study period ($r = .48$, $P = .005$; $r = .44$, $P = .01$ and $r = .46$, $P = .007$, respectively).

We found significant differences between the S and control groups in serum zinc levels ($F[1,31] = 10.50$, $P = .003$), and a significant interaction between the independent variables ($F[2,62] = 7.77$, $P = .001$). Posthoc pairwise comparisons showed that mean values from the P group at 3 and 6 months CA did not present significant changes with respect to the baseline. However, in the S group serum zinc levels did increase (Tukey HSD >22.13, $P = .01$). Accordingly, the S group had significantly higher serum zinc levels than the P group, at 3 and 6 months CA (Tukey HSD >22.13, $P = .01$; Table 4).

The erythrocyte mean zinc (EZn) levels were sig-

TABLE 3. Changes in Weight, Head Perimeter, Arm Perimeter, and Skinfolds Through the Study Period

Groups	Baseline		3 Months CA		6 Months CA	
	S (N = 18)	P (N = 18)	S (N = 18)	P (N = 18)	S (N = 18)	P (N = 18)
Weight (g)	2314 ± 203	2296 ± 143	6333 ± 708	6056 ± 795	7766 ± 679	7393 ± 819
Head perimeter (cm)	33.0 ± 1.3	32.9 ± .7	42.1 ± 1.2	41.5 ± 1.3	44.7 ± 1.3	44.1 ± 1.5
Arm perimeter (cm)	8.5 ± .5	8.5 ± .4	13.7 ± .9	13.2 ± 1.0	14.7 ± .7	14.1 ± .9
Sum 4 skinfolds (mm)	15.7 ± 2.2	17.4 ± 2.8	32.1 ± 5.1	31.0 ± 6.4	29.5 ± 4.2	30.5 ± 6.6

Values are mean ± standard deviation.

TABLE 4. Changes in Analytical Data Through the Study Period

	Baseline	3 Months CA	6 Months CA	
Haemoglobin: (g/dL)				
S	10.3 ± 1.8 (18)	11.9 ± .9 (17)	12.2 ± 1.1 (16)	NS
P	10.1 ± 2.0 (18)	11.7 ± .9 (17)	12.2 ± .9 (18)	
Zn in erythrocytes (µg/g Hgb)				
S	29 ± 11 (16)	29 ± 7 (17)	37 ± 6 (15)	G:P = .0001
P	26 ± 12 (18)	20 ± 4 (17)	26 ± 5 (18)	
Serum zinc (µg/dL)				
S	69 ± 20 (18)	113 ± 25** (18)	119 ± 37** (16)	G:P = .003 PxG:P = .001
P	78 ± 24 (18)	80 ± 22 (17)	87 ± 30 (17)	
Serum copper: (µg/dL)				
S	47 ± 17 (10)	71 ± 20 (15)	100 ± 36 (10)	G:P = .004
P	58 ± 23 (15)	104 ± 27 (14)	145 ± 22 (15)	
Total ALP (IU/mL)				
S	332 ± 86 (17)	510 ± 79 (17)	423 ± 143 (13)	G:P = .021
P	340 ± 129 (17)	414 ± 108 (17)	317 ± 61 (17)	
sALP (µg/L)				
S	63 ± 22 (16)	142 ± 29* (16)	93 ± 24 (18)	PxG:P = .005
P	74 ± 24 (14)	121 ± 20 (16)	93 ± 21 (17)	
IGF-I (ng/mL)				
S	66 ± 27 (18)	126 ± 37 (18)	88 ± 30 (16)	NS
P	72 ± 26 (16)	108 ± 48 (14)	81 ± 22 (16)	
IGFBP-3 (mg/L)				
S	1.2 ± .4 (18)	2.7 ± 1.3 (17)	2.9 ± 1.2 (15)	NS
P	1.5 ± .7 (13)	2.6 ± 1.7 (11)	3.0 ± 1.7 (13)	
IGFBP-1 (ng/mL)				
S	34 ± 31 (17)	17 ± 15 (18)	45 ± 21 (16)	NS
P	33 ± 33 (13)	24 ± 18 (12)	44 ± 26 (13)	

ALP indicates alkaline phosphatase; G, Group; PxG, interaction study period × group; NS, not significant. Values are mean ± standard deviation, with number of subjects in parentheses. Tukey HSD (S vs P): ** $P = .01$, * $P = .05$.

nificantly higher in the S group than in the P group, independent of the study period ($F[1,27] = 18.08, P < .0001$). During the first 3 months of supplementation, the S group did not show any changes, whereas in the P group EZn levels decreased slowly. From 3 to 6 months, EZn levels increased in both groups (Table 4).

There was a positive and significant correlation between zinc intake and the concentration of zinc in both serum and erythrocytes at 3 months CA ($r = .58, P = .001$ and $r = .56, P = .002$, respectively) and at 6 months CA ($r = .51, P = .003$ and $r = .70, P = .0001$, respectively). We also found that zinc intake at 3 months CA correlated directly with Δ SDS length from baseline to 3 months CA ($r = .46, P = .009$).

Serum zinc levels at each evaluation point showed a significant correlation with the levels of zinc in erythrocytes (baseline: $r = .41, P = .016$; 3 months CA: $r = .43, P = .01$; 6 months CA: $r = .49, P = .005$). The levels of serum zinc at 3 months CA were also positively correlated with the levels of zinc in erythrocytes at 6 months CA ($r = .51, P = .003$).

During the study period, copper and hemoglobin levels increased in both groups. There were no significant differences between groups in hemoglobin levels. The mean serum copper concentration was lower in the S group than in the P group, independent of the study period ($F[1,16] = 11.67, P = .004$; Table 4). Any infant in the S group presented copper levels below the normal range.

DISCUSSION

The relationship between zinc status and growth in low BW infants has been the focus of attention to

some authors given that these infants have a high risk of zinc deficiency and are frequently growth retarded.²¹⁻²³

Although significant amounts of zinc are stored in bone and muscle, the available pools are too small to provide a metabolic buffer. Thus, serum zinc homeostasis is highly dependent on dietary intake. Using the content of trace elements in human milk as the base and bearing in mind that the absorption of zinc contained in human milk (60%) is higher than the absorption of zinc contained in formula (30%), the limits permitted for zinc content in formula are between .5 and 1.5 mg/100 kcal.¹⁰ We used formulas containing .75 mg of zinc/100 kcal and 1.5 mg of zinc/100 kcal in the P and S groups, respectively.

Field and others¹² maintain that the zinc content in the standard formulas (.5 mg/100 kcal) currently used for feeding preterm infants after discharge may be insufficient and could be related, in part, to the poor growth and development described for many low BW infants. This author found an improved linear growth velocity and motor development score in preterm infants who received a formula containing 1.6 mg of zinc/100 kcal for 6 months.

Castillo-Duran¹³ also found a positive effect of supplementation on longitudinal growth in small for GA infants fed with artificial formula, but not in those who were exclusively breastfed, for at least 4 months. This may be attributed to the lower bioavailability of zinc contained in formula compared with the zinc in human milk, thereby placing artificially fed infants at a higher risk of zinc deficiency. For this reason, the effect of zinc supplementation on artificially fed infants would be more important.

Finally, in the study conducted by Lira et al,¹⁴ zinc supplementation was associated with a reduction in diarrhea prevalence during the first months of life and with a greater weight gain between 4 and 6 months.

In our study both groups were comparable for GA and BW. It is known that many preterm infants develop an energy and protein deficit during their initial period of hospitalization, which can be explained in part by the different clinical evolution of individual infants leading to postnatal growth retardation.^{24,25} These individual variations in growth rate during the first weeks after birth may explain the differences, not statistically significant, observed in our patients' weight and length at 36 weeks PCA (baseline). Although the S group showed lower mean values of length and weight at baseline, we found that these infants had a greater linear growth velocity than infants in the P group during the period of nutritional intervention.

Our study differed from previous works in that we also analyzed the status of IGF-I and its 2 principal BPs: IGFBP-3 and IGFBP-1. We also incorporated a BIA, a simple and noninvasive technique which allows us to estimate body composition in newborns and children.²⁶⁻³⁰ It is based on the fact that the conduction of an applied electrical signal is far greater in fat-free tissues, because of their water and electrolyte content. Thus, impedance (*Z*) could be used to estimate the volume of TBW, which is proportional to length²/*Z*, namely the impedance index.

BIA has been validated by various investigators for the assessment of TBW in term and preterm newborn and infants.^{18,26-29} These studies confirmed that impedance index correlated positively with the size of the body fluid compartments, measured with reference methods (by using the isotopic water or deuterium dilution methods), and is then a significant predictor of TBW. It has also been proved that the impedance index is a superior predictor of TBW compared with either height², or both 1/resistance and height², and that the accuracy of predicting TBW by BIA is significantly improved by the inclusion of additional variables such as weight.¹⁸ The Kushner et al¹⁸ regression equation used in our study offers the advantage of the ability to be applied to all age and weight groups. It is important to ensure that standardized measurement conditions are observed when using published regression equations,²⁹ as in our study, especially the correct electrode positioning, given the small size of these infants. Electrode proximity could result in measurement error because of electrode interaction. The distance of 3 cm, used in our study, has been found to be the minimum required for sufficient separation of the electrodes in infants and young children.²⁶

Our finding of higher mean values of TBW in the S group without significant differences in subcutaneous fat accretion could indicate a positive effect of supplementation on fat-free body mass.

The verification of higher levels of both serum and intra-erythrocyte zinc in supplemented infants confirms the correct application of the treatment, which was made easier in our study by the fact that sup-

plements were administered in the formula and did not require special participation by the parents, guaranteeing the ability to generalize our finding.

The delay in raising zinc levels in erythrocytes and the strong correlation existing between serum levels at 3 months corrected age and the intra-erythrocyte concentration at 6 months suggests that there is an increased requirement for zinc during the catch-up growth period which occurs during the first months of life. This, in turn, may impede its storage in the erythrocytes. Once the rate of growth slows down, the larger amount of zinc given to the S group through their diet allows the intra-erythrocyte deposits to increase.

We added a small amount of copper to the S group formula. Despite this, these infants showed lower serum levels of copper than the P group. These findings support the hypothesis that zinc inhibits copper absorption,¹⁰ and indicates the need to slightly increase the amount of copper in formula containing zinc supplement. At no time during this study did any infant in the S group present copper levels below the normal range.

An interaction between zinc and iron has been described, associated with the intake of large amounts of zinc.³¹ However, it is unlikely to happen with the addition of zinc to the infants foods. Our finding of a similar increase in hemoglobin levels in both S and P groups, through the study period, supports this hypothesis.

It is known that zinc is required for normal bone growth and development. Recent studies in human osteoblast-like cells indicate that zinc increases the half-life of sALP, which is essential for normal bone formation and mineralization.³² However, to our knowledge, this is the first study which has addressed the effect of zinc supplementation on serum levels of this enzyme *in vivo*. We have found that serum sALP levels increase from baseline to 3 months CA in both groups, being significantly higher in the S group than in the P group. We also found that at 3 months CA, the levels of sALP correlated with length, weight, and cephalic perimeter increase during the study period.

In our study, the increase in serum IGF-I levels during the period of rapid growth was higher in zinc-supplemented infants than in the P group, although differences between groups were not significant. Therefore, we have not been able to demonstrate in our study any significant effect of zinc supplementation on IGF-I levels, probably because of the small size of the sample or the fact that zinc could be required for some aspect of growth regulation at the cellular level, which is independent of the effects observed in circulating IGF-I.

In our study, zinc intake in the diet was strongly related to growth during the first months of life. On the other hand, the improvement in linear growth and in TBW, estimated by BI, found in the supplemented group during the follow-up period, as well as our finding of significantly higher levels of sALP, indicates that zinc supplementation has a positive effect on linear growth in premature infants at least until 6 months. This effect was even more pro-

nounced during the first 3 months, the period during which the infants were exclusively formula-fed and the intake of zinc in the S group was greater. Another possible explanation is that during the first months of life, the period of catch-up growth for premature infants, the response to zinc supplementation would be greater.

We will be following up on the mental development and growth of these children at 9 months, 12 months, and 4 years to evaluate the long-term effect of zinc supplementation. On the other hand, the biological effect of zinc on growth remains to be established.

ACKNOWLEDGMENTS

This study was supported by grants from the Council for Education, Culture and Sport of the Government of the Canaries (project no. 1997/050).

We thank the medical and nursing staff of the Neonatal Unit of the University Hospital of the Canaries for their cooperation. We also thank J. R. Murguía and M. Bain for their help in the preparation of the manuscript.

REFERENCES

- Díaz-Gómez NM, Domenech E, Barroso F. Influencia de la GH y de la nutrición sobre el crecimiento en el periodo neonatal. *An Esp Pediatr.* 1997;46:41-46
- Díaz-Gómez NM, Domenech E, Barroso F. Breast-feeding and growth factors in preterm newborn infants. *J Pediatr Gastroenterol Nutr.* 1997;24:322-327
- Le Roith D. Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N Engl J Med.* 1997;336:633-640
- Roth HP, Kirchgessner M. Influence of alimentary zinc deficiency on the concentration of growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin in the serum of force-fed rats. *Horm Metab Res.* 1994;26:404-408
- Hambidge M. Human zinc deficiency. *J Nutr.* 2000;130:1344S-1349S
- Dreosti IE. Zinc and the gene. *Mutat Res.* 2001;475:161-167
- Black M. Zinc deficiency and child developmental. *Am J Clin Nutr.* 1998;68(suppl):4S-9S
- Cossack ZT. Effect of zinc level in the refeeding diet in previously starved rats on plasma somatomedin C levels. *J Pediatr Gastroenterol Nutr.* 1988;7:441-445
- Browning JD, MacDonald RS, Thornton WH, O'Dell BL. Reduced food intake in zinc deficient rats is normalized by megestrol acetate but not by insulin-like growth factor-I. *J Nutr.* 1998;128:136-142
- Klein CJ. Nutrition requirement for preterm infant formulas. *J Nutr.* 2002;132:1395S-1577S
- Uauy R, Olivares M, González M. Essentiality of copper in humans. *Am J Clin Nutr.* 1998;67:952S-959S
- Friel JK, Andrews WL, Matthew JD, et al. Zinc supplementation in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr.* 1993;17:97-104
- Castillo-Durán C, Rodríguez A, Venegas G, Alvarez P, Icaza G. Zinc supplementation and growth of infants born small for gestation age. *J Pediatr.* 1995;127:206-211
- Lira PIC, 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(suppl):418S-824S
- Goldberg GR, Black AE, Jebb SA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify underrecording. *Eur J Clin Nutr.* 1991;45:569-581
- Mataix J, Mañas M, Llopis J, Martínez E, eds. *Tabla de Composición de Alimentos Españoles.* 3rd ed. Granada: Universidad de Granada; 1998
- Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate D. McCance and Winddowson's. *The Composition of Foods.* 5th ed. Londres: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food; 1991
- Kushner RF, Schoeller DA, Fjeld CR, Danford L. Is the impedance index significant in predicting total body water? *Am J Clin Nutr.* 1992;56:835-839
- Largo RH, Walli R, Duc G, Fanconi A, Prader A. Evaluation of perinatal growth. *Helv Paediatr Acta.* 1980;35:419-436
- Hernández M, Castellet J, García M y cols. *Curvas de Crecimiento.* Madrid, Spain: Editorial Garsi; 1985
- Díaz-Gómez NM, Domenech E, Barroso F. Elementos traza y factores de crecimiento en el periodo perinatal. *An Esp Pediatr.* 1996;44:351-356
- Obladen M, Loui A, Kampmann W, Renz H. Zinc deficiency in rapidly growing preterm infants. *Acta Paediatr.* 1998;87:685-691
- Sazawal S, Black RE, Menon VP, et al. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial. *Pediatrics.* 2001;108:1280-1286
- Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low weight infants. *Pediatrics.* 1999;104:280-289
- Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics.* 2001;107:270-274
- Fjeld CR, Freundt-Thurne J, Schoeller DA. Total body water measured by ¹⁸O dilution and bioelectrical impedance in well and malnourished children. *Pediatr Res.* 1990;27:98-102
- Mayfield SR, Uauy R, Waidelich D. Body composition of low-birth-weight infants determined by using bioelectrical resistance and reactance. *Am J Clin Nutr.* 1991;54:296-303
- Tang W, Ridout D, Modi N. Assessment of total body water using bioelectrical impedance analysis in neonates receiving intensive care. *Arch Dis Child.* 1997;77:F123-F126
- Raghavan CV, Super DM, Chatburn RL, Savin SM, Fanaroff AA, Kalhan SC. Estimation of total body water in very-low-birth-weight infants by using anthropometry with and without bioelectrical impedance and H₂ [¹⁸O]. *Am J Clin Nutr.* 1998;68:668-674
- Díaz-Gómez NM, Hernández JM, González NL, Domenech E, Cortabaria C, Clemente I. Análisis de la composición corporal en el recién nacido de madre diabética. *An Esp Pediatr.* 2000;52(suppl 4):209-210
- Whittaker P. Iron and zinc interactions in humans. *Am J Clin Nutr.* 1998;68(suppl):442S-446S
- Hall SL, Dimai HP, Farley JR. Effects of zinc on human skeletal alkaline phosphatase activity in vitro. *Calcif Tissue Int.* 1999;64:163-172

The Effect of Zinc Supplementation on Linear Growth, Body Composition, and Growth Factors in Preterm Infants

N. Marta Díaz-Gómez, Eduardo Doménech, Flora Barroso, Silvia Castells, Carmen Cortabarría and Alejandro Jiménez

Pediatrics 2003;111;1002-1009

DOI: 10.1542/peds.111.5.1002

This information is current as of October 19, 2004

Updated Information & Services

including high-resolution figures, can be found at:
<http://www.pediatrics.org/cgi/content/full/111/5/1002>

References

This article cites 29 articles, 14 of which you can access for free at:
<http://www.pediatrics.org/cgi/content/full/111/5/1002#BIBL>

Citations

This article has been cited by 2 HighWire-hosted articles:
<http://www.pediatrics.org/cgi/content/full/111/5/1002#otherarticles>

Subspecialty Collections

This article, along with others on similar topics, appears in the following collection(s):
Nutrition & Metabolism
http://www.pediatrics.org/cgi/collection/nutrition_and_metabolism

Permissions & Licensing

Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
<http://www.pediatrics.org/misc/Permissions.shtml>

Reprints

Information about ordering reprints can be found online:
<http://www.pediatrics.org/misc/reprints.shtml>

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™

