Exchangeable zinc pool size at birth is smaller in small-for-gestational-age than in appropriate-for-gestational-age preterm infants

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ABSTRACT

Background: Small-for-gestational-age (SGA) infants are susceptible to postnatal zinc deficiency, but whether this susceptibility is due to intrauterine factors or to high postnatal growth requirements is unknown.

Objective: We hypothesized that the size of the exchangeable zinc pool (EZP), which reflects metabolically available zinc, would be smaller in SGA than in appropriate-for-gestational-age (AGA) infants born prematurely.

Design: Intravenous $^{70}$Zn (45 $\mu$g/kg) was administered to 10 SGA infants (8 boys) with a mean ($\pm$SD) gestational age of 33.3 $\pm$ 1.8 wk and to 11 AGA infants (8 boys) with a mean ($\pm$SD) gestational age of 32.4 $\pm$ 1.2 wk within 24 h of birth. The EZP was determined from isotope enrichment in spot urine collections on days 3–7.

Results: The mean birth weight of the SGA infants was 1.30 $\pm$ 0.2 kg and of the AGA infants was 1.84 $\pm$ 0.3 kg ($P = 0.0001$). The EZP size was significantly smaller in the SGA than in the AGA infants on an absolute basis (13.3 $\pm$ 2.8 and 25.2 $\pm$ 8.1 mg; $P = 0.0002$) and relative to body weight (10.3 $\pm$ 2.5 and 13.9 $\pm$ 4.5 mg/kg; $P = 0.02$). The difference remained significant after adjustment for gestational age and birth weight.

Conclusion: These data provide evidence for differential zinc status at birth between SGA and AGA infants born prematurely at similar stages of gestation and offer at least a partial explanation for the reported benefits of postnatal zinc supplementation. Am J Clin Nutr 2006;84:1340–3.

SUBJECTS AND METHODS

Study design

This was a cross-sectional study that compared the size of the EZP in SGA and AGA infants. The EZP was measured within the first 24 h of birth by using the stable isotope methods described below.

INTRODUCTION

Small-for-gestational age (SGA) infants represent a perturbation in the intrauterine nutritional environment, with alterations in hormonal and metabolic processes and in body composition. SGA infants are highly vulnerable to postnatal complications, including growth faltering, infectious morbidity, and later metabolic complications (1–3).

Zinc is essential for normal growth and development and immune function. Large-scale, well-controlled zinc supplementation studies in developing countries have confirmed postnatal susceptibility to zinc deficiency in SGA infants. Zinc supplementation of primarily breastfed SGA infants during the first year of postnatal life has been associated with significantly improved growth, reductions in infectious morbidity, and reduced mortality (4, 5). Supplementation studies in young, term, appropriate-for-gestational-age (AGA) infants have not consistently shown a benefit of zinc supplements, consistent with the premise that the zinc content of human milk is adequate to meet zinc nutritional needs for the first several months of life for the normal infant (6, 7). The intervention studies provide strong evidence to support the importance of zinc in SGA infants, but the factors or processes responsible for this apparent zinc deficit are unknown.

The etiology of postnatal zinc deficiency may be due to several factors, including higher zinc nutritional requirements to support catch-up growth; inadequate zinc intakes because of exclusive breastfeeding; impaired zinc absorption; increased endogenous losses associated with organ immaturity, especially the gastrointestinal tract; increased losses of endogenous zinc associated with infections; or relatively impaired zinc status at birth. The current study was undertaken to examine potential altered zinc metabolism in the intrauterine environment of infants born SGA.

To evaluate this possibility, we measured the size of the exchangeable zinc pool (EZP; ie, the size of the combined pools of zinc that exchange with zinc in plasma within 3 d; 8) at birth in SGA infants and in AGA infants. We hypothesized that EZP size would be smaller in the SGA infants.
Subjects

Infants who were delivered between ≈32 and 34 wk of gestational age were recruited. Gestational age was determined by the NICU attending physicians using a combination of maternal-fetal dating criteria and neonatal physical examination. Infant weight, length, and head circumference were charted on the Lubchenco growth chart, based on data collected at altitude (10). Infants were considered AGA if their weight-for-gestational-age was above the 40th percentile; the lower limit was selected to define an AGA population that is well-nourished. Infants were considered SGA if their weight-for-gestational-age was below the 10th percentile (10).

Sample size determinations were based on preliminary data from another study (9), from which a mean (±SD) EZP size of 19.6 ± 1.2 mg/kg was observed in 16 preterm infants. For the present study, with 10 infants in each group, power was estimated at 94% to detect a difference of 2 mg/kg, based on a two-sample t test with a type I error rate of 5%.

The principal caregivers of all infants gave their informed consent, and the studies and consent forms were approved by the Colorado Multiple Institutional Review Board at the University of Colorado Health Sciences Center.

Anthropometric measures

Head circumference, midupper arm circumference, scapula and triceps skinfold thicknesses, weight, and length were measured on the first day of life with tape measures and Lange skinfold calipers (Beta Technology Inc). Three measurements were made, and averaged, at the Pediatric General Clinical Research Center by trained neonatal research nurses with extensive NICU experience.

Isotope preparation and administration

Zinc oxide powder enriched with 70Zn was obtained from Trace Science International (Ontario, Canada) and prepared as previously described (11, 12). An accurately weighed dose of enriched isotope solution was administered intravenously to each subject, providing ≈45 μg 70Zn/kg body weight. Isotope was administered via an intravenous catheter, which was already in place, over approximately a 1-min interval and the syringe was rinsed 2–3 times with 0.5 normal saline. All isotope was administered in the presence of research personnel, before initiation of the feeds (enteral or parenteral).

Specimen collection

Amount of isotope excreted in the urine was determined in spot urine sample collections over 5 d. An initial basal sample was collected before isotope administration. Two spot urine samples with a typical volume of ≈20 mL were obtained on days 3–7 after isotope administration. Urine samples were collected by first cleansing the subject’s perineum with deionized water and filter paper and applying a custom-made, zinc-free urine bag. Urine was drained from the bag via a small plastic catheter tube placed inside and attached to the urine bag (9). Once collected, the urine was stored at −20 °C until analyzed.

Sample preparation and analyses

Preparation of individual urine samples included wet digestion with concentrated nitric acid and hydrogen peroxide. Dried samples were then ashed on a hot plate, reconstituted in ammonium acetate buffer, and chelated to remove major minerals (13). Zinc concentrations were then measured with the use of a Perkin-Elmer 2380 atomic absorption spectrophotometer (Norwalk, CT). Zinc isotope enrichment in urine was determined from isotope ratio measurements by inductively coupled plasma mass spectrometry (VG Plasma Quad 3; VG Elemental, Cheshire, United Kingdom).

Data analyses

The EZP is defined as the estimate of the total size of the combined pools of zinc that exchange with zinc in plasma within 3 d. The EZP was calculated by dividing the mass of intravenous isotope dose by the enrichment value at the y intercept of the linear regression of a semilogarithmic plot of urine enrichment data from days 3 to 7 after isotope administration. This method of estimation of EZP size relies on treatment of the individual zinc pools in the EZP as a single homogeneously mixed pool and assumes that losses also occur at a monoexponential rate. Compared with determination of the EZP by complex compartmental modeling, the urine extrapolation method used in the present study likely results in an overestimation in the actual EZP size of ≈ 25% (8). After initial plotting of log-transformed data, all regressions were submitted to evaluation by least median of squares regression to systematically identify data outliers (PROGRESS, Antwerp Group on Robust and Applied Statistics, University of Antwerp, Antwerp, Belgium). This "robust regression" method resulted in elimination of 0–2 points out of the 10 points available for each infant; from the final regression, EZP was calculated.

Mean differences in EZP size were compared by using Students t tests, where α = 0.05. Analysis of covariance allowing for unequal variances was also used to compare EZP size between groups while controlling for birth weight and gestational age. Univariate correlations between EZP, birth weight, and anthropometric variables were evaluated by Pearson’s correlations. Statistical analyses were performed with the use of SAS version 8 (SAS Institute Inc, Cary, NC).

RESULTS

Descriptive data for the 2 groups of infants are presented in Table 1. The ponderal index, based on measurements by the GCRC research nurses on day 1 of life, was significantly higher in the AGA group, consistent with greater intrauterine wasting in the SGA group. Dietary zinc intakes before isotope administration were minimal for all subjects. Thirteen of the infants had no enteral intake and were maintained on intravenous dextrose and electrolyte fluids (with no zinc or other nutrients). One SGA infant received 4 oz (118.28 mL) of premature infant formula; 8 (4 SGA, 4 AGA) other infants received an average of 1 oz (29.57 mL) of infant formula before the isotope dose. From the enteral feeds, the estimated amount of zinc absorbed would account for 1–2% of total EZP size. Four infants in the SGA group
The group difference in EZP size remained significant when adjusted for both birth weight and gestational age (Table 1).

When EZP was adjusted for birth weight, the difference between the 2 groups remained significant: 10.3 ± 4.5 mg/kg for the SGA and AGA infants, respectively (P < 0.001). When EZP was adjusted for gestational age (wk), EZP/kg was significantly lower in the SGA infants (13.3 ± 2.8 mg/kg) than in the AGA infants (25.2 ± 8.1 mg/kg) (P < 0.001; Figure 1). When EZP was adjusted for birth weight, the difference between the 2 groups remained significant: 10.3 ± 2.5 and 13.9 ± 4.5 mg/kg for the SGA and AGA infants, respectively (P = 0.02).

The group difference in EZP size remained significant when adjusted for both birth weight and gestational age (P < 0.05).

Absolute size of EZP was significantly positively correlated with birth weight for both groups combined (r = 0.56, P = 0.01). Gestational age was negatively correlated with EZP size per kg (EZP/kg) for both groups (r = −0.50, P = 0.02). The EZP size of the infants who received zinc from enteral or parenteral sources was not different in either group compared with those who received no exogenous zinc. EZP/kg was not significantly correlated with the ponderal index for the groups separately or combined. Similarly, the EZP/kg was not correlated with either triceps or subscapular skinfold-thickness measurements.

**DISCUSSION**

The lower EZP in the SGA infants at birth provides at least a partial explanation for the apparent vulnerability of this group of infants to zinc deficiency. The hepatic stores of zinc at a gestational age of ≈31 wk for AGA infants have been calculated to be ≈8 mg Zn (14), which would account for ≈40% of the total EZP as measured in this study. The urine extrapolation method of calculating EZP has been concluded to overestimate the EZP size by ≈25% (8), bringing the actual mean EZP size for the AGA group to 18 mg. This neonatal hepatic store is thought to be utilized during the first 2–3 mo after delivery, an estimate based largely on the measurement of zinc and metallothionein concentrations in the livers of preterm and term infants at autopsy (6, 14, 15). This suggests that these stores from the liver may be utilized at an average rate of ≈100 μg Zn/d. If the 50% reduction in EZP in the SGA infants was evenly distributed between the several zinc pools, this would equate to the EZP, this implies a reduction in neonatal hepatic stores of zinc to 4–5 mg. If utilized at the same rate in the SGA infants as it does in the AGA infants, neonatal hepatic stores would be exhausted after ≈1 mo of postnatal life. Alternatively, absorption would need to increase to compensate for the deficit. Although this might be possible to achieve, given the generous amount of highly available zinc in human milk in the early postpartum months, there is currently no evidence that either the premature or term infant compensates for a higher requirement with higher absorption efficiency (9, 16, 17). Thus, the data reported in this study suggest that the hepatic zinc reserves would be available to the SGA infant for a much shorter time after delivery and to a considerably lesser extent than for the AGA infant.

The size of the EZP is thought to represent metabolically active zinc and was previously found to be correlated with dietary zinc intake in adults (8), in breastfed and formula-fed infants aged 3–5 mo (16), and in older breastfed infants who are consuming complementary foods (18). We did not specifically control for dietary intake after delivery but rather assumed that intake of human milk or formula, and thus of zinc, during the first 24 h would be minimal. Although some of the subjects did receive some exogenous zinc before isolette administration, the amounts were very small. We previously reported a mean EZP of ≈20 mg/kg in 2-wk-old AGA premature infants born at ≈31 wk gestation who were fed either premature infant formula or fortified human milk (9). Hence, once a consistent dietary zinc intake is established, EZP size increases directly with dietary intake in AGA premature infants as it does in adults (8) and older infants (16). Data are currently unavailable to determine whether SGA premature infants would have a different response in EZP size once nutrition support, including zinc, was initiated.

Although the EZP has not been extensively evaluated specifically as an index of zinc status, controlled depletion studies in adults have shown that the size of these combined rapidly turning over pools decreases (8, 19). Likewise, zinc supplementation in adults has been reported to increase the EZP size (20), although the findings have not been consistent (19; NF Krebs, unpublished observations, 2005). The observation that the difference in pool size was present at birth supports an initial substantial deficit in the tissue zinc concentrations of the SGA infants, which may pose an increased risk of postnatal zinc deficiency.

The apparent low zinc status of the SGA infants at birth coincides with an evident immediate need for higher than normal dietary zinc intakes starting in the neonatal period to facilitate catch-up growth and full development of the immune system. A longitudinal, community-based study in India (5) showed decreased infectious morbidity and mortality in zinc-supplemented

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**TABLE 1**

Descriptive data for subjects in 2 groups

<table>
<thead>
<tr>
<th>Gestational age (wk)</th>
<th>Birth weight (kg)</th>
<th>Ponderal index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-for-gestational-age infants (n = 2F, 8M)</td>
<td>33.3 ± 1.8</td>
<td>1.30 ± 0.2</td>
</tr>
<tr>
<td>Appropriate-for-gestational-age infants (n = 3F, 8M)</td>
<td>32.4 ± 1.2</td>
<td>1.84 ± 0.3</td>
</tr>
</tbody>
</table>

1 All values are ¯x ± SD.
2,4 Significantly different from small-for-gestational-age infants: P = 0.0001. 4P = 0.004.
3 Ponderal index = weight (g) × 100/length (cm).
SGA infants born at term. In Chile, zinc supplementation of SGA infants initiated in the neonatal period was associated with more rapid growth (4). The results of these previous studies indicate the benefits of zinc supplementation in SGA infants and, in conjunction with the observations in the present study, support a relatively higher dietary zinc requirement postnatally in SGA infants to attain normal growth and development. Susceptibilities to infectious diseases and to inadequate intakes of human milk are also potential contributing factors to a zinc deficit in young SGA infants. Longitudinal data on zinc homeostasis in breastfed SGA infants, from birth onward, are not available but are necessary to establish optimal strategies to prevent zinc deficiency in this vulnerable population group.

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NFK, JLW, LVM, and KMH participated in the study design and data interpretation. JLW and DJR implemented the study. NFK had principal responsibility for the data analysis. NFK, KWF, and KMH drafted the manuscript. None of the authors had any conflicts of interest related to this study.

REFERENCES