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Copper Status of Very Low Birth Weight Infants during the First 12 Months of Infancy[1]

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ABSTRACT. The Cu intake and status of 106 very low birth weight (VLBW) infants (birth weight 1152 ± 251 g, gestational age 29 ± 3 wk, mean ± SD) were determined approximately 1 mo before hospital discharge, at discharge (time 0), and at 3, 6, 9, and 12 mo ± 3 wk corrected for gestational age. Infants were fed either formula plus supplemental Zn/Cu (SUPPL, $n = 29$); formula plus placebo (PLAC, $n = 26$); or a low birth weight formula (LBWF, $n = 26$) or were breast-fed (BRMLK, $n = 25$). Plasma Cu levels in the formula-fed infants increased significantly at each time period with no significant differences between feeding groups. Hair Cu was significantly higher in the SUPPL group compared to the PLAC, LBWF, and BRMLK groups at 3 and 6 mo. Erythrocyte Cu,Zn-superoxide dismutase (CuZnSOD) activity was lowest in the PLAC group. Cu intake was positively correlated with both hair Cu ($r = 0.291$, $p < 0.0001$) and erythrocyte CuZnSOD activity ($r = 0.281$, $p < 0.001$) but not with plasma Cu. An increasing number of formula-fed infants had very low CuZnSOD activity (less than 2 SD below mean) with increasing age ($n = 1$, 2, 8, 11, and 13 infants at times 0, 3, 6, 9, and 12 mo, respectively). At 12 mo, approximately one third of the formula-fed VLBW infants in this study had low Cu status as assessed by CuZnSOD activity. Infants with the lowest CuZnSOD activity were those with the largest weight gains from 0 to 6 mo and were observed in all formula-fed groups. Thus, these data suggest that in formula-fed infants erythrocyte CuZnSOD activity is a more appropriate indicator of Cu status in the VLBW infant than plasma Cu levels and that the fastest growing VLBW infants may be at risk for Cu deficiency. (*Pediatr Res* 32: 183–188, 1992)

Abbreviations

VLBW, very low birth weight
CuZnSOD, Cu,Zn-superoxide dismutase

VLBW infants (<1500 g) are particularly at risk for trace element deficiencies because of reduced tissue stores, especially in the liver, their rapid postnatal growth, and their less developed gastrointestinal tract. This is especially true for Cu, as more than two thirds of the Cu in human fetal liver is accumulated during the last 10–12 wk of gestation (1). These limited stores increase the vulnerability of the VLBW infant to Cu deficiency (2), which is further compounded by their rapid postnatal growth. The net negative Cu balance of the VLBW infant, observed by several researchers (3–6), has been suggested to be due to the immaturity of their gastrointestinal tract, resulting in decreased resorption of endogenous losses (7).

Cu is an essential component of several enzymes including cytochrome *c* oxidase, the terminal component of the electron transport chain that is ultimately responsible for the production of energy in the cell (8); CuZnSOD, which catalyzes the removal of the superoxide radical, thereby protecting cells from potentially harmful oxidizing effects (9); lysyl oxidase, which is involved in collagen cross-linking; and ferroxidases (including ceruloplasmin), which catalyze the oxidation of Fe^{2+} to Fe^{3+} (8). A decrease in the activity of this wide range of Cu-requiring enzymes is probably responsible for the broad range of symptoms seen in Cu deficiency.

Serum Cu levels have traditionally been used to assess Cu status; however, in the VLBW infant the use of serum Cu levels to determine Cu status is complicated by a steady rise in serum Cu as the infant ages. This rise in serum Cu appears to be related to postconceptional age rather than postpartum age (6, 10) and has been reported to steadily increase until 4 mo of age in the VLBW infant (6). Erythrocyte activity of trace element-requiring enzymes, on the other hand, is a good indicator of long-term trace element status and has several advantages for assessing status (11). Erythrocytes are readily obtained and, because of their long half-life, enzyme activity is not responsive to short-term fluctuations in trace element intakes. Thus, the purpose of the present experiment was to determine the Cu status of a group of VLBW infants and assess various measures of Cu status, namely Cu intake, plasma Cu, hair Cu, and erythrocyte CuZnSOD activity. In addition, a second objective was to determine if a Zn and Cu supplement would improve the Cu status of the VLBW infant using these various measures of Cu status. Infants were fed either formula with supplemental Zn + Cu, formula plus placebo, a low birth weight formula, or breast milk.

MATERIALS AND METHODS

Subjects. One hundred six infants < 1500 g birth weight (birth weight 1152 ± 251 g; gestational age 29 ± 3 wk; mean ± SD) were recruited for this study from the neonatal intensive care units of the Dr. Charles A. Janeway Child Health Centre, the Grace General Hospital, and St. Clare's Mercy Hospital in St. John's, Newfoundland. The study was conducted prospectively in double-blind fashion and was approved by the Faculty of Medicine Human Investigations Committee. Infants were excluded from the study if they suffered from severe bronchopulmonary dysplasia that required more than 2 wk of oxygen therapy, had hydrocephalus, liver dysfunction, or any congenital malformations. The Fe and Zn status of these infants has been previously reported (12, 13). Approximately 85% of parents of eligible infants approached for this study consented to enroll

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their infants. Of the 106 infants recruited, 84 were formula-fed and 25 were breast-fed. Two formula-fed infants passed away during their hospital stay and one infant was removed to a hospital out of province, leaving 81 formula-fed infants for whom data were available at discharge.

The gestational age of each infant was taken from the last menstrual period of the mother and was also determined by the Dubowitz method (14). If there was a discrepancy of more than 2 wk between the two assessments, the latter was used. Size for gestational age was appropriate if the birth weight fell within 2 SD of weight for gestational age according to the growth curves of Lubchenco (15).

All infants received uniform management as established in the neonatal intensive care unit, and formula-fed infants were fed a premature special care formula containing 3.5 kJ/mL (24 cal/fluid ounce) until they could tolerate a formula containing 3 kJ/mL (20 cal/fluid ounce). At that time (approximately 1 mo before discharge, weight 1850 ± 106 g; gestational age 36 ± 3 wk), infants were assigned using random number tables to receive either *1*) infant formula with whey and supplemental Zn/Cu drops (SUPPL; $n = 29$); *2*) infant formula with whey and water drops (PLAC; $n = 26$); or *3*) an experimental low birth weight formula (LBWF; $n = 26$). A group of infants whose mothers chose to breast-feed were also monitored and served as a reference group (BRMLK; $n = 25$). Some of these infants received special care formula until breast-feeding was fully established. The supplemental Zn/Cu drops [containing 76.5 mmol/L (5 mg/mL) Zn and 15.7 mmol/L (1 mg/mL) Cu] were prepared by adding U.S.P. grade Zn and $CuSO_4$ to deionized water that was autoclaved along with plain deionized water before being added to unmarked 15-mL amber dropper bottles. Formula feedings in hospital were prepared in the formula room so that the nurses did not know to which group an infant was assigned. All infants were fed *ad libitum*. At discharge, parents were provided with the 15-mL amber dropper bottles and were instructed to add 32 drops (1 mL) to each 32-ounce can of formula before preparing individual feedings. Infants fed LBWF did not have any drops added to their formula. All formulas were iron-fortified and were donated by Ross Laboratories (Columbus, OH) in 32-ounce, ready-to-feed cans. Final nutrient compositions of the formulas are provided in Table 1.

Infants in the study were discharged from hospital at a mean weight of 2533 ± 290 g. There were no significant differences in body weight, gestational age, Hb, or ferritin in the different feeding groups at discharge. Study formula and drops were provided for 5 mo after discharge from hospital. After that time, parents were responsible for purchasing their own formula. All subjects were told to restrict food intake to formula only during the first 3 mo in compliance with recommendations of the Canadian Pediatric Society (16) and Health and Welfare Canada (17).

Table 1. *Composition of test formulas (per L)**

	SUPPL/PLAC	LBWF
Protein (g)	15.7	17.3
Fat (g)	36	37
Carbohydrate (g)	73	71
Energy [kcals (MJ)]	670 (2.8)	670 (2.8)
Ca [mg (mmol)]	490 (12.2)	680 (17.0)
P [mg (mmol)]	345 (11.4)	416 (13.4)
Na [mg (mmol)]	230 (10.0)	230 (10.0)
K [mg (mmol)]	750 (19.2)	1000 (25.6)
Vitamin A (IU)	3200	4000
Vitamin D (IU)	400	550
Vitamin E (IU)	20	30
Vitamin C [mg (μmol)]	100 (568)	200 (1136)
Vitamin B1 [mg (μmol)]	0.65 (2.2)	1.00 (3.3)
Vitamin B2 [mg (μmol)]	1.0 (2.7)	1.5 (4.0)
Vitamin B6 [mg (μmol)]	0.52 (3.1)	0.68 (4.0)
Fe [mg (μmol)]	13 (233)	13 (233)
Zn [mg (μmol)]	11.7/6.7 (179/102)	9 (138)
Cu [mg (μmol)]	1.8/0.76 (28/12)	0.8 (13)

* Values are as reported on product label. Molar values are given in parentheses and have been calculated.

Sample collection and analysis. Data were collected in the hospital at study entry (approximately 1 mo before hospital discharge) (time P) and at hospital discharge (time 0). After discharge, all infants were monitored at the Provincial Perinatal Follow-up Clinic at 3, 6, 9, and 12 mo ± 3 wk corrected for gestational age. Some infants did not attend all scheduled visits; therefore, values were not available for all infants at each sampling time. At each visit possible, a blood sample, a hair sample, a 3-d dietary record, and body weight measurements were collected by a research assistant or by hospital staff. Three-day dietary records were completed by nursing staff in hospital and by parents or guardians before each visit. Each record was verified for accuracy and completeness by a trained research assistant. Unclothed body weights for the preterm infants in hospital and at follow-up clinics were obtained with a calibrated spring balance using the mean of three measurements. For the breast-fed infants, sampling was begun at 3 mo, and only dietary information, body weight measurements, and hair samples were collected.

Heparinized blood samples (0.5–1 mL) were obtained from a heel or finger prick. Samples were centrifuged and separated, and plasma was frozen at −20°C until analysis. Erythrocytes were washed, lysed, stabilized with glutathione (2 mM reduced glutathione in 10 mM potassium phosphate buffer, pH 7.0), and shipped with dry ice to Ottawa for enzymatic assays.

All storage and analytical containers were acid-washed and only purified water was used (> 18 MΩ, Barnstead Purification Systems). Plasma and hair Cu analyses were performed on a Perkin-Elmer (Norwalk, CT) 2380 Atomic Absorption Spectrophotometer with HGA-300 graphite furnace (18). The accuracy of the Cu method was assessed using quality control sera (Dade, Cation-Cal, Baxter Diagnostics Co., Mississauga, Ontario, Canada).

Erythrocyte CuZnSOD (EC 1.15.1.1) activity was determined using an automated xanthine, xanthine oxidase, cytochrome *c* assay (9) and expressed per mg of Hb in the lysate. Hb in the erythrocyte lysate was determined using an automated modification of the method of Beutler (19). Quality control was assayed using pooled rat erythrocytes that had been washed, lysed, and prepared as described above and stored at −85°C.

Dietary data were coded and converted into grams using a database available at the Department of Biochemistry, Memorial University of Newfoundland. Mean daily intakes of Cu were calculated using a modified database consisting of Cu values obtained from company product information, food composition tables, and the literature (20).

One-way analysis of variance was done at each sampling time to assess differences due to gender, size for gestational age, and effects due to feeding group. Two-way analysis of variance was done to determine effects of both feeding group and time. Pearson correlation coefficients were determined to assess relationships between Cu intake, plasma Cu, hair Cu, and erythrocyte CuZnSOD activity. All computations were done using the CSS Statistical software package (StatSoft, Tulsa, OK). Significant differences between means were determined by the least significant difference method at the $p < 0.05$ level of significance.

RESULTS

Body weights of the VLBW infants in this study were not significantly different among the different formula groups at any sampling time, although the BRMLK-fed infants had lower body weight at 3 mo. By 6 mo, there were no differences in body weight between any groups.

No significant differences due to gender or size for gestational age were seen in dietary Cu, plasma Cu, hair Cu, or erythrocyte CuZnSOD activity (data not shown); thus, data for all infants were pooled for both gender and for size for gestational age.

The pooled Cu intake and the changes in Cu status of the VLBW infants during the 1st year are shown in Table 2. For the breast-fed infants, only dietary intake, body weight, and hair Cu were determined. Dietary Cu intake in these VLBW infants ranged from 500 to 900 μg/d, with the highest intake seen at 3 mo. This intake then leveled off at 550 to 650 μg/d for the final 6 to 12 mo. When expressed per kg body weight, intakes were highest in hospital (time P) and then declined thereafter for the next year. Plasma Cu in formula-fed infants, in contrast with dietary intakes, significantly rose throughout the study. Hair Cu was fairly constant from study entry (time P) to hospital discharge (time 0) and from 9 to 12 mo. The highest levels of hair Cu were found at 3 mo, followed by those at 6 mo, which corresponded to the times at which Cu intakes were the highest. Erythrocyte CuZnSOD activity in formula-fed infants was highest at the start of the study at times P and 0 and then gradually decreased at 3, 6, 9, and 12 mo.

The Cu intakes of the VLBW infants receiving the different feeding treatments are shown in Table 3. Cu intake (μg/kg/d) of the SUPPL group was greater than that of the PLAC group at 0 and 3 mo. Infants receiving the LBWF had a Cu intake slightly above that of the PLAC group but less than that of the SUPPL group. At 3 mo, the lowest calculated Cu intake was seen in the BRMLK-fed group. After 6 mo, there were no significant differences in Cu intake (μg/kg/d) between any groups.

The effects of the different feeding treatments on Cu status over time are shown in Tables 4 to 6. Plasma Cu levels significantly increased at each time interval, but the differences in Cu intake between the different formula-fed groups were not reflected in differences in plasma Cu levels at times P, 0, 3, and 6 mo (Table 4). The rise in plasma Cu over time was similar in all groups. Hair Cu levels (Table 5) were highest in the SUPPL group compared to all other feeding groups at 3 and 6 mo, with elevated hair Cu levels being displayed in the time period just after the highest Cu intakes. This elevation in hair Cu in the SUPPL group was no longer evident at 9 and 12 mo.

Erythrocyte CuZnSOD activity declined in all formula-fed groups over time (Table 6). There were no significant differences between feeding groups at each time period; however, pooled over the entire study period, the lowest overall CuZnSOD activity was seen in the PLAC group (90 ± 37 U/mg Hb) ($p < 0.05$) compared to the SUPPL and LBWF groups (97 ± 40 and 100 ± 32 U/mg Hb, respectively). During the course of the study, there were an increasing number of infants with low erythrocyte CuZnSOD activity (<50 U/mg Hb); data for these infants are given in Table 7. Infants with low erythrocyte CuZnSOD activity were seen in all formula-fed groups ($n = 3$, 4, and 6 infants from the SUPPL, PLAC, and LBWF groups, respectively), and low activity was confined to these 13 infants. Erythrocyte CuZnSOD activity in these infants was progressively lower at each time interval, whereas in the other infants activity remained constant at 110 ± 26 U/mg Hb throughout the study. VLBW infants with the lowest erythrocyte CuZnSOD activity were those infants with the higher growth rates and lower Cu intake per kg body weight during the first 9 mo (Table 7).

Correlation coefficients for dietary Cu (μg/kg/d), plasma Cu, hair Cu, and erythrocyte CuZnSOD activity were calculated for time 0 on. Dietary Cu was positively correlated with both hair Cu ($r = 0.291$, $p < 0.0001$) and CuZnSOD activity ($r = 0.281$, $p < 0.001$). In contrast, plasma Cu was negatively correlated to both dietary Cu ($r = -0.526$, $p < 0.0001$) and erythrocyte CuZnSOD ($r = -0.284$, $p < 0.0001$).

DISCUSSION

Both the Canadian Pediatric Society (21) and the American Academy of Pediatrics (22) have recommended that formula-fed low birth weight infants receive 90 μg Cu/100 kcal (equivalent to 108 μg Cu/kg/d) to avoid Cu depletion, as compared to the recommendation for full-term infants of 60 μg Cu/100 kcal (23). Recommendations as high as 100 to 120 μg Cu/kg/d for VLBW infants have also been made (24), inasmuch as biochemical evidence of Cu deficiency was seen in five of 14 formula-fed VLBW infants fed 80 μg Cu/kg/d (25). Symptoms observed in some of the infants included low neutrophil counts, Hb, red blood cell counts, and reticulocyte counts. The Cu requirement of the VLBW infant is thought to be somewhat higher than that of the full-term infant because fetal liver Cu levels increase from 2.5 to 9 mg during the last 12 wk of gestation (1) and this much greater store of Cu would not be available to the VLBW infant for use.

Cu deficiency has been reported in formula-fed infants, with the majority of cases being in low birth weight or premature infants (for review see Ref. 26). The median age for presentation of Cu deficiency in full-term infants was 8.3 mo (5–18 mo), whereas it was 3 mo in VLBW infants (26). This difference in the onset of Cu deficiency is probably a consequence of the reduced Cu stores of the VLBW infant. In addition, the bioavailability of Cu from cow's milk-based infant formula is less than that of breast milk. In the premature infant, retentions of around 10% have been reported for Cu from formula as compared to 50% from human milk (5, 27). Preterm infants fed formula providing approximately 500 μg Cu/L were in negative (or just barely positive) Cu balance for 4 wk, but even those in positive balance did not approach normal liver *in utero* accretion rates (5). Thus, the formula-fed VLBW infant is at an increased risk for Cu deficiency.

Serum Cu levels are the most common method used to assess Cu status; however, in the VLBW infant a steady rise in serum Cu has been observed (6, 28, 29). This rise in serum Cu is seen

Table 2. *Changes in Cu status over time in VLBW infant**

	Time (mo)†						
	P	0	3	6	9	12	*p* value‡
Weight (kg)	1.9 ± 0.1[a]	2.5 ± 0.3[b]	5.2 ± 0.9[c]	6.8 ± 1.0[d]	8.0 ± 1.2[e]	8.6 ± 1.2[f]	<0.00001
Diet Cu (μg/d)	512 ± 84[a]	518 ± 200[a]	864 ± 392[c]	577 ± 223[a,b]	552 ± 225[a]	647 ± 269[b]	<0.00001
Diet Cu (μg/kg/d)	276 ± 46[d]	205 ± 81[c]	170 ± 83[b]	86 ± 31[a]	72 ± 30[a]	77 ± 35[a]	<0.00001
Plasma Cu (μmol/L)§	8.1 ± 2.5[a]	10.2 ± 3.0[b]	16.1 ± 4.7[c]	18.9 ± 4.2[d]	20.4 ± 5.8[e]	22.4 ± 4.1[f]	<0.00001
Hair Cu (μg/g)‖	16.8 ± 13.0[a]	15.9 ± 12.2[a]	46.2 ± 15.9[c]	25.7 ± 14.3[b]	19.8 ± 9.3[a,b]	19.4 ± 10.9[a]	<0.00001
RBC CuZnSOD (U/mg Hb)§	111 ± 39[c]	116 ± 26[c]	102 ± 29[b,c]	90 ± 36[a,b]	81 ± 32[a]	85 ± 44[a]	<0.0001

* Values are means ± SD; means on the same row with different superscripts are significantly different ($p < 0.05$).

† Data collection times were at study entry (time P), which was approximately 1 mo before hospital discharge (time 0); mean weight at time 0 was 2553 ± 290 g. All other times refer to age in mo corrected for gestational age.

‡ Determined by analysis of variance.

§ Determined in formula-fed infants only.

‖ Hair samples were not available for breast-fed infants at times P and 0.

Table 3. *Dietary intake of Cu by different feeding groups**

Time†	Feeding group‡ SUPPL	PLAC	LBWF	BRMLK
		μg/d		
P	473 ± 65 (26)[a]	513 ± 61 (26)[a,b]	547 ± 104 (27)[b]	ND
0	752 ± 127 (29)[b]	367 ± 73 (27)[a]	415 ± 83 (26)[a]	ND
3	1329 ± 304 (24)[c]	664 ± 109 (22)[b]	695 ± 165 (22)[b]	350 ± 55 (6)[a]
6	664 ± 210 (25)[b]	547 ± 197 (17)[a,b]	495 ± 246 (20)[a]	573 ± 200 (11)[a,b]
9	595 ± 226 (22)[a,b]	550 ± 248 (18)[a,b]	473 ± 164 (22)[a]	671 ± 281 (7)[b]
12	737 ± 333 (19)[b]	669 ± 277 (16)[a,b]	545 ± 184 (22)[a]	667 ± 235 (9)[a,b]
		μg/kg/d		
P	256 ± 33 (26)[a]	274 ± 37 (26)[a]	299 ± 56 (26)[b]	ND
0	302 ± 49 (28)[c]	139 ± 24 (26)[a]	166 ± 26 (26)[b]	ND
3	269 ± 64 (21)[c]	125 ± 27 (20)[b]	142 ± 50 (22)[b]	72 ± 19 (6)[a]
6	98 ± 34 (23)	76 ± 26 (14)	80 ± 30 (17)	80 ± 23 (9)
9	78 ± 29 (19)	72 ± 37 (13)	62 ± 22 (20)	83 ± 43 (6)
12	89 ± 44 (16)	69 ± 31 (13)	69 ± 29 (18)	81 ± 30 (6)

* Values are means ± SD (*n*); means on the same row with different superscripts are significantly different ($p < 0.05$). ND, not determined.

† All infants except the BRMLK group were fed premature formula (Special Care, Ross Laboratories) from birth to time P, which was approximately 1 mo before hospital discharge (time 0); mean weight at time 0 was 2553 ± 290 g. All other times refer to age in mo corrected for gestational age.

‡ See text for full details of feeding groups.

Table 4. *Plasma Cu (μmol/L) of VLBW infants fed different diets**

Time	Feeding group SUPPL	PLAC	LBWF
P	7.9 ± 2.1 (26)	8.3 ± 2.7 (27)	8.1 ± 2.8 (24)
0	9.6 ± 2.9 (28)	10.0 ± 2.9 (25)	11.0 ± 3.1 (24)
3	16.0 ± 5.0 (22)	17.3 ± 4.8 (21)	14.9 ± 4.1 (18)
6	18.3 ± 3.9 (25)	18.4 ± 4.3 (22)	20.2 ± 4.3 (18)
9	18.3 ± 5.5 (23)[a]	20.9 ± 6.2 (19)[a,b]	22.5 ± 5.3 (18)[b]
12	20.9 ± 3.1 (17)[a]	22.7 ± 3.7 (19)[a,b]	23.7 ± 5.1 (17)[b]

* Values are means ± SD (*n*); means on the same row with different superscripts are significantly different ($p < 0.05$); times and feeding groups are the same as in Table 3. Plasma Cu was not determined in the BRMLK group.

even when the infant is in net negative Cu balance (3, 30). It appears that serum Cu in the VLBW infant reflects the capacity of the immature liver to synthesize ceruloplasmin, not Cu status, thus rendering it a poor indicator of Cu status (31). The very low plasma Cu levels in the normal neonate are thought to increase slowly toward normal adult levels during the first 3 mo of postnatal life (32). The results of the present study demonstrate that this is a much slower and longer process in the VLBW infant: plasma Cu levels continue to increase for the 1st year of postnatal life and beyond 3 mo, similar to term infants levels, they are higher than the normal adult values of 15.7 μmol/L (100 μg/dL) that we see in our laboratory (33).

Erythrocyte CuZnSOD is a good indicator of Cu status in adults and, in contrast with serum Cu, it is not affected by such physiologic factors as estrogen levels (33) and responds to changes in Cu status before any differences in serum Cu or ceruloplasmin occur (8, 34, 35). There is little information on the use of erythrocyte CuZnSOD activity to assess Cu status in VLBW infants, and it is not known whether it rapidly increases along with plasma Cu levels. A good correlation between erythrocyte CuZnSOD and plasma Cu levels was seen in eight Cu-deficient full-term infants (mean age 14 mo) before and after 120 d Cu supplementation, and these authors suggested that erythrocyte CuZnSOD activity is a good measure of Cu status (36). These infants, however, were beyond the period of the rapid rise in plasma Cu that occurs in infancy. The results of the present study demonstrate that erythrocyte CuZnSOD activity in the VLBW infant, in contrast with plasma Cu levels, did not increase dramatically during the 1st year, but rather, activity decreased by about 25% during this time period. Whether this decrease in CuZnSOD activity reflects nonoptimum Cu status or whether it is also a physiologic response cannot be determined with certainty from this study. However, several pieces of evidence suggest that some of these VLBW infants may have suboptimal Cu status as indicated by their erythrocyte CuZnSOD activity. CuZnSOD activity tended to be lower in the PLAC group compared to the SUPPL or LBWF groups. In addition, those infants with the lowest CuZnSOD activity (<50 U/mg Hb) had a higher growth rate during the first 6 mo of the study compared to the other infants ($p < 0.002$) and subsequently had lower Cu intakes per kg body weight (87 ± 45 *versus* 125 ± 82 μg/kg/d, *p*

Table 5. *Hair Cu (μg/g) of VLBW infants fed different diets**

Time	Feeding group SUPPL	PLAC	LBWF	BRMLK
P	12.6 ± 4.2 (7)	14.3 ± 9.8 (6)	24.2 ± 19.8 (6)	ND
0	14.7 ± 5.5 (9)	13.4 ± 9.3 (7)	20.5 ± 20.8 (6)	ND
3	66.0 ± 0 (7)[d]	56.0 ± 0 (10)[c]	37.0 ± 10.3 (11)[b]	26.7 ± 6.0 (7)[a]
6	39.9 ± 11.0 (10)[b]	17.8 ± 14.2 (10)[a]	18.2 ± 8.1 (9)[a]	27.0 ± 0 (4)[a]
9	18.6 ± 8.1 (16)[a,b]	20.6 ± 7.1 (13)[a,b]	17.6 ± 11.7 (14)[a]	25.4 ± 9.3 (7)[b]
12	21.3 ± 15.9 (13)[b]	21.1 ± 6.4 (12)[b]	20.7 ± 10.2 (16)[a,b]	12.8 ± 6.5 (10)[a]

* Values are means ± SD (*n*); means on the same row with different superscripts are significantly different ($p < 0.05$); ND, not determined. Times and feeding groups are the same as in Table 3.

Table 6. *Erythrocyte CuZnSOD activity (U/mg Hb) of VLBW infants fed different diets**

Time	Feeding group		
	SUPPL	PLAC	LBWF
P	123 ± 53 (9)	97 ± 32 (9)	114 ± 15 (5)
0	122 ± 17 (8)	108 ± 32 (11)	123 ± 25 (5)
3	99 ± 32 (11)	101 ± 33 (9)	112 ± 17 (8)
6	82 ± 37 (12)	85 ± 38 (11)	109 ± 31 (7)
9	75 ± 31 (13)	74 ± 36 (11)	93 ± 29 (10)
12	97 ± 49 (10)	80 ± 46 (11)	80 ± 41 (13)

* Values are means ± SD (*n*); times and feeding groups are the same as in Table 3. Erythrocyte CuZnSOD was not determined in the BRMLK group.

Table 7. *Characteristics of VLBW infants with low erythrocyte CuZnSOD activity (<50 U/mg Hb)*

Parameter	Time	Low SOD*† (<50 U/mg Hb)	Normal SOD† (110 ± 26 U/mg Hb)	*p* value
Erythrocyte SOD (U/mg Hb)	0	43 (1)	119 ± 21 (24)	
	3	43 ± 5 (2)	108 ± 23 (28)	
	6	41 ± 6 (8)	110 ± 21 (22)	<0.001
	9	41 ± 9 (11)	99 ± 19 (24)	
	12	38 ± 10 (13)	114 ± 29 (21)	
Diet Cu (μg/d)	0	500 (1)	509 ± 211 (23)	
	3	900 ± 283 (2)	900 ± 399 (22)	
	6	517 ± 204 (8)	624 ± 254 (17)	NS
	9	567 ± 296 (9)	496 ± 213 (21)	
	12	636 ± 266 (11)	540 ± 135 (15)	
Diet Cu (μg/kg/d)	0	168 (1)	194 ± 80 (21)	
	3	161 ± 81 (2)	172 ± 88 (20)	
	6	73 ± 30 (6)	92 ± 32 (14)	<0.03
	9	75 ± 38 (7)	68 ± 31 (18)	
	12	82 ± 33 (9)	63 ± 26 (14)	
Body weight (kg)	0	2.97 (1)	2.58 ± 0.25 (21)	
	3	5.89 ± 1.21 (2)	5.50 ± 1.16 (24)	
	6	7.27 ± 0.32 (9)	6.67 ± 1.20 (18)	<0.002
	9	8.18 ± 0.44 (8)	7.90 ± 1.57 (21)	
	12	8.59 ± 1.02 (9)	8.60 ± 1.68 (18)	

* Less than 2 SD below the normal range.
† Values are means ± SD (*n*).

< 0.03), although total Cu intakes (μg/d) were not significantly different. Finally, for those infants with low CuZnSOD activity, the activity was progressively lower at each sampling time, with the majority first displaying low CuZnSOD activity at 6 mo. Excluding the infants with low CuZnSOD activity from the data in Table 2, erythrocyte CuZnSOD activity for the infants remained constant throughout the study at 110 ± 26 U/mg Hb.

The results of this study confirm the results of others (37, 38): dietary supplements of Cu do not accelerate or improve the increase in plasma Cu seen in VLBW infants. Hillman *et al.* (37) have shown that an increase in formula Cu from 40 to 167 μg/dL for 8 wk did not result in an elevation in serum Cu or ceruloplasmin, nor did a supplement of 500 μg Cu/d for 4 mo increase serum Cu in VLBW infants (38). These authors have suggested that, had the supplement lasted longer, differences may have been seen. However, in the study reported here, supplements were given for 6 mo (time P to 5 mo), and, even with this extended supplementation period, plasma Cu levels did not increase. Both hair Cu and to a smaller degree erythrocyte CuZnSOD activity increased with Cu supplementation. Hair Cu levels increased at 3 mo and were more than doubled by 6 mo. This large increase was no longer evident after the supplements were discontinued. The large elevation in hair Cu levels reflected Cu intakes, with these increases evident later than at the start of the supplements. This is to be expected because hair mineral levels are known to reflect dietary intakes retrospectively (20). Cu supplementation had only a small effect on erythrocyte CuZnSOD activity, although this was not unexpected if the activity was already saturated in most infants.

Even though one group of infants also received Zn in the supplement, it is unlikely that the Zn supplement adversely affected their Cu status. In a previous study with adult subjects, we found that a ratio of Zn to Cu of at least 25 to 1 was needed before any decrease in erythrocyte CuZnSOD activity was observed (34).

In conclusion, these data demonstrate that erythrocyte CuZnSOD activity in the VLBW infant does not show the physiologic 3-fold increase during the 1st year that is observed with plasma Cu levels. The only parameters that were positively associated with dietary Cu intake were erythrocyte CuZnSOD activity and hair Cu levels. Thus, we suggest that erythrocyte CuZnSOD activity is a more appropriate indicator of Cu status in the VLBW infant than plasma Cu levels. Using erythrocyte CuZnSOD activity to assess Cu status, the results of this study suggest that up to one third of the formula-fed VLBW infants in the present study may have had suboptimal Cu status between 6 mo and 1 y. Low hair Cu levels in the breast-fed infants at 12 mo were also noted in this study. Further study, including CuZnSOD data, would be required to more fully assess the Cu status of the breast-fed infants. Six mo to 1 y also coincides with the time during which both formula-fed and breast-fed infants were switched to cow's milk containing approximately 0.1 mg Cu/L, which is much lower than the Cu content of the infant formula. In the VLBW infant, the use of Cu supplements or a formula with supplemental Cu for longer than 6 mo may be beneficial.

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