Sequential Concentrations of Copper and Ceruloplasmin in Serum from Preterm Infants with Rickets and Fractures

Winston W. K. Koo, Paul Succop, and K. Michael Hambidge

Concentrations of copper (Cu) and ceruloplasmin in serum were measured serially in 49 preterm infants with mean (± SEM) birth weights of 979 ± 33 g and gestational ages of 28.4 ± 0.3 weeks at three, six, nine, and 12 months postpartum. Serial radiographic studies showed 17 infants with (group A) and 32 infants without (group B) rickets or fractures. Cu and ceruloplasmin concentrations in serum also were measured in 21 healthy term infants (group C) with birth weights 3668 ± 98 g at three, six, and 12 months postpartum. Analyses of covariance of serial changes in these serum variables—taking into account such potential covariates as differences in gestational age, birth weight, initial weight and length, changes in weight and length during the study, the duration of parenteral nutrition, and increased enteral copper intake—showed that patients of preterm infants had significantly lower concentrations of Cu in serum up to age six months and ceruloplasmin up to age three months (P < 0.001) when compared with term infants. By one year of age, Cu and ceruloplasmin concentrations in serum in all groups had increased significantly (P < 0.001), into the adult range, and were not significantly different among groups. These data document a maturation lag in copper metabolism in small, preterm infants. Changes in concentrations of Cu and ceruloplasmin in serum were significantly correlated (r = 0.92, P < 0.001) but were not significantly different between preterm infants with and without rickets or fractures at each age.

Additional Keyphrases: bone · metabolism · pediatric chemistry

Abnormal skeletal changes, including osteopenia, widened metaphyses, fractures, and (or) rickets (R/F), occur frequently in small (low birth weight) preterm infants (1–3). Although calcium and phosphorous deficiencies are thought to be important in the pathogenesis of these skeletal problems (1, 4), similar radiographically documented abnormalities have been reported to accompany very low concentrations of Cu in serum (5–7). However, reports of Cu concentrations in serum are infrequent in preterm infants with metabolic bone disease.

One longitudinal study of infants with various degrees of prematurity showed that Cu concentrations in serum increase after birth and reach adult values by about six months postpartum; in some instances, by the age of one year these exceed adult values (8). However, there are no data on the serial changes of Cu concentrations in serum in small, preterm infants beyond the first three months postpartum. Data are also lacking on changes in serum concentrations of ceruloplasmin, an α2-glycoprotein that is the primary carrier for Cu in the circulation (9, 10). In our study, we documented the serial changes in Cu and ceruloplasmin concentrations in serum in small preterm and term infants during the first year after birth, and tested two hypotheses about Cu and ceruloplasmin concentrations in serum: (a) They are lowest in very-low-birth-weight (<1500 g, VLBW) infants who display radiographic changes consistent with osteopenia and R/F and are in the intermediate range in VLBW infants without R/F when compared with healthy term infants at the same postnatal age, and (b) these values tend to converge in the three groups as postnatal age increases.

Materials and Methods

Subjects and Procedures

Forty-nine VLBW infants with mean (± SEM) birth weights of 979 ± 33 g and gestational ages of 28.4 ± 0.3 weeks were the subjects of the present study. Serial radiographic studies were used to document the presence (group A, n = 17) or absence (group B, n = 32) of

6 Nonstandard abbreviations: VLBW, very low birth weight; R/F, rickets and (or) fractures; and PN, parenteral nutrition.
R/F at three-month intervals during the first year postpartum. The initial radiographs were made at ages three and six months because of previous reports of the occurrence of R/F between these ages (1–3). All screening radiographs and radiographs taken for clinical purposes were reviewed for R/F, based on previously described criteria (1, 3). Radiographic abnormalities included four infants with fractures, six infants with rickets, and seven infants with rickets and fractures. No new skeletal abnormalities were noted at or after the screening radiograph taken at six months, and all cases of R/F were healed by age 12 months. Details of clinical and radiographic changes are presented elsewhere (3). None of the investigators knew the skeletal radiographic status of the infants before enrollment in the study, and radiographs were reviewed without knowledge of the biochemical data. In addition, 21 healthy term infants (group C) with birth weights of 3668 ± 98 g were enrolled in this study. Radiographic study was not performed for term infants.

Blood samples were collected at three-month intervals on four occasions in VLBW infants and at three, six, and 12 months postpartum in term infants. All infants were clinically stable at the time of assessment, and no visible hemolysis was noted in any serum samples. Needles, syringes, and storage containers were checked at random and were free of detectable Cu. Serum samples were stored at −70 °C and measured for Cu and ceruloplasmin in batches. The technician who performed the biochemical measurements was unaware of the group to which the infant was assigned.

We measured the Cu concentration in serum by flameless atomic absorption spectrophotometry, using a Model 5000 with HGA 500 graphite furnace and AS40 autosampler (all from Perkin-Elmer, Norwalk, CT) (11). Serum (20 μL) was diluted with 500 μL of 10 mmol/L HNO₃ before Cu measurement. Standards were made up from dilution of the stock solution (1 g/L; Baker Scientific Co., Phillipsburg, NJ) in 10 mmol/L HNO₃ and type I reagent-grade water. Inter- and intraassay coefficient of variations (CVs) were 2% and 6%, respectively. The detection limit for this assay is 1.6 μmol/L (1 μmol/L = 6.36 μg/dL). The normal reference range for adults is 13.4–19.0 μmol/L. The value obtained from the National Institute of Standards and Technology Reference Serum 8419 (certified Cu concentration = 11.8 ± 1.6 μmol/L) by use of the same analytical technique was 12.6 ± 0.2 μmol/L (mean ± SD).

Ceruloplasmin concentration in serum was determined by a colorimetric, enzymatic assay (12) with an interassay CV of 5%. The normal reference range for adults is 1.57–3.21 μmol/L (1 μmol/L = 13.4 mg/dL).

The nutrient intakes of infants in this study are reported elsewhere (3). Infants in group A received standard parenteral nutrition (PN) solution longer than those in group B (40 ± 15 vs 15 ± 3 days, P <0.05). Standard PN solutions deliver 3.1 μmol of Cu per kilogram of body weight per day (13). The enteral nutrients used were similar for infants in groups A and B and included their own mother’s milk, preterm infant formula, and standard cow milk formula. However, group A infants also received increased enteral Cu longer than group B infants (42 ± 13 vs 20 ± 5 days, P <0.05), in the form of preterm infant formula (Cu 267.6 μmol/L, Similac Special Care; Ross Laboratories, Columbus, OH) or powdered human milk fortifier (Mead Johnson Co., Evansville, IN) that provided an additional Cu at 63 μmol/L of human milk. All infants in group C received only enteral feedings with standard cow milk formula, with Cu at 94.4 μmol/L (Similac; Ross Laboratories), throughout infancy. Cereals and solids were usually introduced during the second six months at the discretion of the parents and primary-care physician. This study was approved by the Review Board for Human Investigation, Children’s Hospital Medical Center and University of Cincinnati Medical Center, and written informed consent was obtained from a parent of each subject in this study.

Statistical Methods

Cu and ceruloplasmin concentrations in serum and the postnatal ages at each blood sampling were transformed to their natural logarithm to account for deviations from linearity in the serial determinations and also to approximately normalize these statistical distributions. Serial changes with age for each serum variable were estimated for each infant by random coefficient regression analyses (14, 15). Analyses of covariance were used to determine whether differences in the serial changes of Cu concentrations in serum among groups were significant. These analyses took into account potential covariates, including differences in gestational age, birth weight, initial weight and length, changes in weight and length during study, the duration of PN, increased enteral Cu intake, and a term for interacting gestational age and R/F status. The relationship between serum Cu and ceruloplasmin was estimated by a linear-regression model.

Statistical analyses were performed by using the CLINFO program of the National Institutes of Health, General Clinical Research Center, and the University of Cincinnati Computer Center Amdahl 5880 mainframe computer. Unless otherwise stated, all values are given as mean ± SEM. Statistical significance was based on a P value of 0.05 for each estimated value.

Results

At the onset of study, Cu concentrations in serum in VLBW infants with and without R/F were similar, and both groups of VLBW infants had lower Cu concentrations in serum than did term infants (P <0.001); for ceruloplasmin concentrations the relationship among groups was the same as for Cu (Table 1). Very low concentrations of Cu in serum were found only at age three months in two infants with R/F (5.3 and 5.5 μmol/L) and in three infants without R/F (5.2, 5.8, and 6.1 μmol/L). Correlating with the very low Cu concentrations in serum were low ceruloplasmin concentrations in serum: 0.45 and 0.37 μmol/L, respectively, for
the two infants with R/F and 0.45, 0.52, and 0.60 μmol/L, respectively, for the three infants without R/F.

With increasing postnatal age, Cu and ceruloplasmin concentrations in serum increased significantly (P <0.001) in all groups but were no longer significantly different between groups by the age of one year (Table 1). The changes in Cu in serum were correlated with changes in ceruloplasmin concentrations in serum throughout the study (r = 0.92, P <0.001). None of the potential covariates for Cu and ceruloplasmin concentrations in serum that we entered into the analyses was statistically significant.

**Discussion**

With the availability of graphite-furnace atomic absorption spectrophotometry in clinical medicine, concentrations of various trace metals, including Cu, are being measured in serum with increasing frequency in the evaluation of patients with metabolic bone disease. Data are available on changes of Cu concentrations in serum during early infancy (7, 8, 16–22), but few reports have provided serial data beyond six months postpartum (8, 20, 22). Cu concentrations in serum for preterm infants were reported in only one series (8) of infants, whose birth weights were about twice those of the subjects in our study. Our data therefore may be useful in the interpretation of Cu concentration in serum from small preterm infants.

We found that at three months postpartum, the Cu concentrations in serum of VLBW infants were significantly lower than those from infants born at term, a finding consistent with the data in the literature (7, 8, 16–18). Grouping the infants by R/F status (present vs absent) and birth weights (<1500 g vs >1500 g) minimized any effect attributable to gestational age. That is, any effect that might be attributable to gestational age is represented in this study by the factors R/F status and birth weight. The three-month difference in mean gestational ages between the VLBW infants and term infants is reflected by an approximate three-month lag in both Cu and ceruloplasmin concentrations in serum in VLBW infants as compared with those in infants born at term. Therefore, our data support reports that Cu concentrations in serum are related to gestational age (7, 16), at least for the first few months after birth.

Consistent with reports in the literature for preterm (7, 8, 16–18) and term (19–22) infants, our data demonstrated a significant postnatal increase in Cu concentrations in serum. Regardless of the infants' gestational ages, the Cu concentrations in serum increased significantly from about two months after birth (7, 8, 16–20, 22) and reached adult values by late infancy (8, 20, 22).

Variation in the Cu content of infant milk formula does not influence Cu concentrations in serum in preterm (18) or term (19, 22) infants. Copper in human milk appears to be better absorbed than that contained in infant milk formulas (23, 24). However, even VLBW infants have the capacity for intestinal absorption of Cu and positive copper balances are reported in VLBW infants during the neonatal period (23–25). For infants receiving PN for less than two months, variation in the copper intake from 0.31 to 0.63 μmol/kg per day does not affect Cu concentrations in serum (26). Infants receiving PN for fewer than five weeks may have increased Cu concentrations in serum despite receiving copper-free PN solution (27). Dietary factors apparently do not significantly affect Cu concentrations, postnatal age being the major determinant of Cu concentrations in serum.

Our data showed that serial concentrations of Cu in serum were similar in VLBW infant groups studied at each postnatal age, regardless of the radiographically determined skeletal status and whether the blood samples were obtained during acute or healing phases of bone abnormalities. Furthermore, none of the infants in this study had the extremely low Cu concentrations in serum (<4.7 μmol/L) found in infants with typical Cu deficiency states (5–7), presumably because of the relatively brief period of administering Cu-containing PN solutions and the early attempts at enteral feeding for our patients.

It is not surprising that concentrations of ceruloplasmin and Cu in serum are highly correlated, because ceruloplasmin is the primary carrier for Cu in the circulation (6, 7). At the beginning of this study the mean ceruloplasmin concentrations in serum in all

### Table 1. Serial Changes in Serum Copper and Ceruloplasmin Concentrations in VLBW Infants

<table>
<thead>
<tr>
<th>Group</th>
<th>93 ± 2⁰</th>
<th>189 ± 3</th>
<th>277 ± 2</th>
<th>371 ± 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>9.8 ± 0.6(14)b,c</td>
<td>16.8 ± 1.1(14)</td>
<td>20.6 ± 1.1(16)</td>
<td>22.0 ± 0.9(11)</td>
</tr>
<tr>
<td>μmol/L</td>
<td>10.5 ± 0.8(31)</td>
<td>16.5 ± 0.8(28)</td>
<td>22.0 ± 1.1(26)</td>
<td>25.5 ± 1.1(26)</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>17.5 ± 1.1(20)</td>
<td>22.2 ± 1.3(21)</td>
<td>—</td>
<td>23.3 ± 1.2(20)</td>
</tr>
<tr>
<td>μmol/L</td>
<td>0.97 ± 0.10(12)</td>
<td>1.79 ± 0.17(13)</td>
<td>2.16 ± 0.14(15)</td>
<td>2.39 ± 0.16(11)</td>
</tr>
<tr>
<td>C</td>
<td>1.49 ± 0.10(11)</td>
<td>1.94 ± 0.13(11)</td>
<td>—</td>
<td>2.46 ± 0.10(12)</td>
</tr>
</tbody>
</table>

* Group A = VLBW infants with rickets/fractures, Group B = VLBW infants without rickets/fractures, Group C = term infants.

* Mean ± SEM.

* Number in parentheses = number of infants.

* Serum copper and ceruloplasmin increased significantly with postnatal age for each group: P <0.001 (random coefficient regression).

* Significantly higher than for other groups at the same postnatal age: P <0.001 (by analyses of covariance).

Copper: 1 μmol/L = 6.36 μg/dL; ceruloplasmin: 1 μmol/L = 13.4 mg/dL.
infants were below the adult reference range and were lower in preterm infants than in term infants. However, ceruloplasmin concentrations in serum increased with postnatal age in all infant groups and were similar to adult values by the age of one year. These trends are consistent with other reports based on colorimetric (16, 17) and immunodiffusion (20, 22) measurements of ceruloplasmin. Our data therefore support the thesis that infants, particularly preterm infants, have a maturational lag in the hepatic synthesis of ceruloplasmin.

We conclude that Cu and ceruloplasmin concentrations in serum in VLBW infants during the acute and healing phases of metabolic bone disease are similar and cannot be used to distinguish between infants with and without radiographic skeletal abnormalities. Our data make it difficult to support the presence of overt Cu deficiency in VLBW infants with R/F. However, because small preterm infants have low body stores of Cu at birth (28) and have high demands for copper during the period of rapid postnatal growth (29), one should not dismiss Cu deficiency as a nutritional problem that may potentially lead to metabolic bone disease.

Supported by grants from NIH 5R01 HD18505, NIH RR123, NIH RR58 (CLINPO), NIADDKD 5R22AM12432, RR69, W.W.K.K. was the recipient of the NIH Clinical Associate Physician Award 3M01 RR0123-21S1.

References