

## Meeting Zinc, Copper, and Manganese Requirements in the Parenterally Fed Preterm and Full-Term Infant

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Four groups of infants and children have been identified as being at risk of developing acute trace metal deficiency: (a) children with chronic diarrhea (1); (b) children and infants on total parenteral nutrition (2); (c) infants with inborn errors of metabolism; and (d) premature infants (3). The effects of chronic diarrhea on trace metal losses are not discussed here; it should be mentioned, however, that the biliary route is the most important excretory route for manganese and copper; it is also important for zinc and chromium homeostasis. In situations where there are increased losses through the gastrointestinal tract, infants or children may be at increased risk of the early development of multiple trace metal deficiencies. Inborn errors of metabolism are discussed elsewhere in this volume. Numerous cases of both zinc and copper deficiency have been described in infants and children receiving total parenteral nutrition which excluded zinc and copper. For example, at least 12 cases of copper deficiency in preterm infants have been described. These children had the manifestations of copper deficiency, e.g., neutropenia, osteopenia, and anemia (4). Zinc deficiency has also frequently been described in the literature (5). These children have growth delay, immune system dysfunction, exodermal dysfunction, etc. Therefore children receiving prolonged total parenteral nutrition (TPN) are at increased risk for specific trace metal deficiencies. The need to include trace metals in nutrient formulations for those receiving short-term TPN is less clear.

The first generation of TPN formulations were hydrolysates of protein that were "contaminated" with trace metals. Their use was infrequently associated with trace metal deficiencies. The newer formulations, prepared from individually produced crystalline amino acids, are virtually free of trace metals (6). Their use has been associated with clinical manifestations of both copper and zinc deficiency (3,5). It has been documented that there is a significant obligatory urinary loss of zinc, copper, and manganese in infants receiving zinc-, copper-, and manganese-free fluids intravenously (7,8). Therefore zinc, copper, and manganese stores eventually become depleted simply from obligatory excretion if supplements are not given.

## ZINC AND COPPER

The nutritional requirements of the preterm infant are unique. Intakes required to satisfy these needs must be considered independently from the needs of the term infant. The preterm infant is especially at risk of zinc, copper, and manganese deficiency because of limited stores and very rapid growth. Shaw has estimated that two-thirds of the zinc and copper in the fetal body at term are transferred to the fetus during the last 10 to 12 weeks of gestation (3,5). Therefore an infant born 10 to 12 weeks prior to term with increased immediate needs due to rapid protein synthesis and limited endogenous stores must eventually be provided with zinc and copper; otherwise, deficiency signs including growth impairment will become manifest. The need for manganese for the activity of enzymes involved in polysaccharide synthesis can be demonstrated by the impairment of mucopolysaccharide metabolism that is associated with manganese deficiency. The features of manganese deficiency are impaired growth, skeletal abnormalities, depressed reproduction function, and ataxia in the newborn (9). These features seem to be similar in all species studied. Manganese deficiency has not, however, been described in the human.

The timing of the onset of deficiency symptoms is important because the intake dosage necessary to prevent acute deficiency is significantly different from the dose necessary to replete body stores. To prevent deficiency, the infant must be in positive balance (i.e., intake greater than losses). To build up stores, intakes must be considerably larger than losses.

Although it is difficult to match infants receiving TPN with their orally fed counterparts, a comparison is illuminating. Preterm infants fed heat-sterilized pooled human milk have been reported to be in negative zinc balance for the first 60 days of life and negative copper balance until day 35 (10). Similarly, preterm infants retained only small amounts of zinc and copper during the first few weeks of life when fed a variety of cows' milk-based formulas (10). In spite of seemingly inadequate intakes and negative balance, overt signs of zinc and copper deficiency are not observed. Although the concentration of copper in the preterm infant's liver is similar to that of the adult, total stores are markedly limited by the smaller liver size. Nevertheless, it has been suggested that copper stores should be adequate for at least 2 months. It is likely that so long as organ stores of the trace metals are available for mobilization zinc deficiency signs will be averted. As soon as stores are depleted, however, deficiency will rapidly become manifest.

The earliest case report of acute clinical deficiency in a preterm infant occurred after the infant had received 7 weeks of TPN with no oral supplementation (11). One could argue, therefore, that because acute zinc/copper deficiency is unlikely to develop over the short term, infants receiving brief periods of TPN need not have zinc or copper added to their formulations. In that case, limited stores will become even more depleted, although acute deficiency is unlikely to be a problem. On the other hand, for premature infants receiving long-term TPN who are growing

at a rate equal to the intrauterine growth rate, the appropriate goal is not merely the prevention of acute deficiency but the buildup of body stores.

For the intravenously fed full-term infant, zinc and copper requirements may be estimated by examining the retention of zinc and copper on a diet of human milk. Unfortunately, few balance studies have been completed on the term infant. Cavell and Widdowson (12) and Kleinbaum (13) found that about one-half of their term infants younger than 2 weeks of age were in negative copper balance while receiving pooled human milk. No data are available for older infants. In spite of this apparent negative copper balance, both plasma and hair concentrations of copper increased during the first months of life (13,14). These increases are probably the result of mobilization from organ stores, primarily the liver. Such copper stores are likely to be adequate for at least the first 4 to 6 months of life.

Similarly, Cavell and Widdowson (12) found 9 of 10 term infants to be in negative zinc balance while being fed pooled human milk. Although serum concentrations remain stable, there is a progressive fall in their hair zinc concentration during the first 3 months of life (14,15). Despite the apparent negative zinc balance while being fed human milk and the decline in hair zinc content, acute zinc deficiency does not occur in otherwise healthy full-term infants.

## MANGANESE

Manganese homeostasis appears to be regulated at the excretory level (9). The liver is the key tissue in this regulation, with the bowel serving as an important route of excretion. Biliary excretion is particularly important in adjusting to the manganese load. With intestinal obstruction, manganese excretion is totally stopped (16). The difference in the body's handling of orally versus intravenously injected manganese is worth noting. In adult man, rapid clearance of radioactive manganese occurs (less than 1% of an injected dose is present in the blood after 10 min) (17). The total body excretion of intravenously injected  $^{54}\text{Mn}$  takes place with a half-life of  $37 \pm 7$  days. Orally administered manganese has a half-life of  $10.5 \pm 0.6$  days. This difference in total manganese excretion originates from the different distribution of manganese absorbed from the portal vein system versus manganese injected into the circulatory system. The slower clearance may put the individual receiving intravenous manganese at increased risk of toxicity.

Parenterally fed preterm infants may be at increased risk of manganese toxicity because (a) retention is increased owing to decreased intestinal excretion (less stool is passed); (b) administration by the intravenous route bypasses hepatic control; (c) the blood-brain barrier is premature and probably more permeable; (d) there are no clinical studies or functional parameters available to measure the response to presently recommended intakes of manganese.

The American Medical Association (AMA) has recently published TPN guidelines recommending the inclusion of trace elements in TPN formulations for infants, including premature infants, in order to prevent manganese deficiency (18). Recommended intakes are based on data from a single study of five patients (19).

Although little is known about the metabolism and homeostasis of manganese in infants, from animal studies and a few human experiments, it appears that the safety margin between requirements and toxic doses is extremely narrow, especially in the newborn. As for copper and zinc, one is left with the problem of determining the appropriate intake or goal for the preterm intravenously fed infant.

In order to determine appropriate manganese intakes for the parenterally fed preterm and full-term infant, 19 human infants were provided with manganese-supplemented or manganese-free parenteral nutrition (8). All were newborn infants, either full-term or premature of similar postnatal age. All infants received a complete formulation parenterally because of their inability to tolerate oral nutrients. The formulation included adequate amounts of protein, energy, minerals, and trace elements other than manganese. Following a 72-hr adaptation period, a 72-hr collection of urine was completed and the urine analyzed for manganese by absorption spectrophotometry using a carbon rod. Manganese intakes were  $47.8 \pm 5.4$   $\mu\text{g}/\text{kg}/\text{day}$  versus  $0.8 \pm 0.5$   $\mu\text{g}/\text{kg}/\text{day}$  in the unsupplemented group. The range of intake was between 35 and 70  $\mu\text{g}/\text{kg}/\text{day}$ . Manganese excretion was unrelated to intake and similar in the two groups; it was also independent of gestational age. Manganese retention represented 98.2% of intake in the manganese-supplemented group.

In addition to the infants just described, an additional group of five infants had their urine collected while awaiting the start of intravenous feeding. These infants were receiving only electrolytes and glucose by vein. Their excretion of manganese (obligatory excretion) was  $1.63 \pm 1.07$   $\mu\text{g}/\text{kg}/\text{day}$ . Results from this group and the unsupplemented parenterally fed group indicate that the obligatory excretion of manganese is approximately 1 to 2  $\mu\text{g}/\text{kg}/\text{day}$  in an intravenously fed infant not passing any stool.

### ESTIMATING REQUIREMENTS

Having now determined approximate rates of retention, it is possible to estimate requirements. In order to prevent deficiency, one simply must replace ongoing losses. In the case of manganese, this goal can be achieved with an intake of 1 to 2  $\mu\text{g}/\text{kg}/\text{day}$ . In order to determine the rate of manganese infusion that repletes body stores, one must look at the literature for estimates of rates of intrauterine manganese accretion. The only published data on rates of intrauterine manganese accretion from infants who died at various gestational ages is a Ph.D. thesis written by Claire Casey (*personal communication*). Using these values, one may calculate that a 1-kg infant would store manganese at approximately 10  $\mu\text{g}/\text{kg}/\text{day}$ , and an older infant would store somewhere closer to 2 to 3  $\mu\text{g}/\text{kg}/\text{day}$ . Therefore to replete stores in a 1-kg infant at rates of intrauterine manganese accretion, intakes of approximately 10  $\mu\text{g}/\text{kg}/\text{day}$  would be appropriate. Compared to the AMA recommendations, this value is within the range of recommended intakes (2 to 10  $\mu\text{g}/\text{kg}/\text{day}$ ).

One may conclude that: (a) there is almost complete retention of intravenous infused manganese; and (b) the repletion of manganese stores may be achieved

with intravenous intakes of approximately 10  $\mu\text{g}/\text{kg}/\text{day}$ . To prevent deficiency, considerably smaller intakes would suffice.

Turning now to zinc and copper, in order to determine the intravenous zinc and copper quantities necessary to build up body stores in the preterm infant and achieve positive retention in the full-term infant, balance studies were completed in 38 preterm, full-term, and full-term small-for-gestational-age (SGA) infants who received complete intravenous formulations excluding zinc and copper (7). The study design was similar to the design previously described for manganese. Zinc as zinc sulfate and copper as  $\text{CuCl}_2$  were added to individual infant formulations in quantities ranging from 91 to 824  $\mu\text{g}/\text{kg}/\text{day}$  for zinc and 8 to 92  $\mu\text{g}/\text{kg}/\text{day}$  for copper. Samples of infusions as well as of urine, stool, and aspirate were collected for 72 hr and analyzed for zinc and copper by atomic absorption spectrophotometry. Zinc and copper retention correlated significantly with intake ( $r = 0.89$  and  $0.82$ , respectively,  $p < 0.01$ ) and were independent of gestational age, postnatal age, and birth weight. In full-term and full-term SGA infants, intakes of zinc and copper greater than 150 and 16  $\mu\text{g}/\text{kg}/\text{day}$ , respectively, were adequate to replace ongoing losses and prevent acute deficiency. The dosage for copper is similar to the current AMA recommendations; the zinc dosage is 50% higher. Preterm infants receiving zinc at 438  $\mu\text{g}/\text{kg}/\text{day}$  and copper at 63  $\mu\text{g}/\text{kg}/\text{day}$  achieve intrauterine retention rates. These dosages are significantly higher than the AMA recommendations. Both trace elements can be delivered by peripheral or central line without complications.

Plasma zinc and copper concentrations in both full-term and preterm infants remained within the normal range, even at the highest and lowest intakes. Values for zinc and copper, however, were lower in the SGA group despite similar intakes. This result is different from another study which showed higher copper concentrations in orally fed SGA infants compared to preterm and full-term AGA infants (20). Although not measured, it is possible that serum ceruloplasmin concentrations were lower in the SGA group because of an inadequate amino acid supply in this rapidly growing population.

Prior to determining appropriate intakes for trace elements, one must be clear on the goals one is aiming at. For example, for the preterm infant I arbitrarily assigned the goal of duplication of intrauterine accretion rates. For the full-term infant, I suggest that retention rates similar to those of full-term infants fed pooled human milk are appropriate. Based on these assumptions, the studies just described demonstrate that infants receiving trace metals in appropriate quantities by peripheral vein infusion can meet these arbitrarily assigned goals.

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## DISCUSSION

*Dr. Dorner:* If you look at manganese values in human milk reported within the last 10 years, you will find that they decreased by a factor of 10. Now we have heard that they are at a level of 5 ppb; we also found these values. I fear that 10 years from now we will have even lower values. With respect to totally parenterally fed infants we should keep this in mind and especially what you, Dr. Zlotkin, said about manganese toxicity.

Elements mainly excreted by the bile and not by the urine are also excreted in high concentrations by sweat. These data are rather old and are derived from adults. Is it not necessary to take into account the dermal loss in the patients reported here? Did these infants have no stools? What about bowel movements before and after balancing? How did you manage to obtain complete 72-hr urine specimens in preterm infants?

*Dr. Zlotkin:* The only way that one can collect samples from small babies over a prolonged period of time is by having a designated individual in charge specifically of collecting samples, as opposed to being in charge of looking after the babies. I think that if it cannot be done, especially for the trace elements where contamination is such a major problem, it probably is not worth trying. In terms of your earlier questions, I was talking about the apparent zinc/copper/manganese balance. I know of no data on the concentration of manganese in the sweat of young infants; and as difficult as it is to collect urine for a 72-hr period, I shudder at the thought of having to try and collect any sweat that might be passed from these infants. In practical terms it would be impossible. In terms of stool output, the

amount of stool passed after a 3-day period on parenteral nutrition is minimal. Originally we measured the concentration of nutrients in the stool, but because the stool volume is so small we stopped doing it.

*Dr. Senterre:* I would like to stress that metabolic balance studies show that very-low-birth-weight infants fed human milk or infant formula are all in negative zinc balance for weeks. The fecal loss is always higher than the intake, and the urinary excretion of zinc is not negligible, about 100  $\mu\text{g}/\text{kg}/\text{day}$ . I did not observe any problem in those infants. It is almost the same with copper; the fecal excretion of copper is higher than the intake, but, in contrast to zinc, urinary excretion is rather low, between 2 and 6  $\mu\text{g}/\text{kg}/\text{day}$ . Preterm babies on total parenteral nutrition for 2 to 3 weeks, in fact, lose a lesser amount of zinc and copper than when they are fed orally. Do we really need to infuse zinc and copper? If we do, we must conclude that not only parenterally but also orally fed infants must be supplemented. The second point is: What amounts? Once the regulatory role of the placenta is out, I do not think we must necessarily try to mimic the intrauterine accretion rate in very-low-birth-weight infants. In my neonatal unit in parenterally fed very-low-birth-weight infants, I give zinc and copper only to cover the losses and not to try to have an intrauterine accretion rate. Finally, I think it is also important to stress that trace element requirements, like protein requirements, are related to the rate of growth, and I prefer to advise an intake which is related to energy and not a fixed daily dosage. We usually infuse about 200  $\mu\text{g}$  of zinc and 20  $\mu\text{g}$  of copper per 100 kcal.

*Dr. Zlotkin:* Certainly the dosage you use will keep your infants generally in positive balance. I started my talk by giving the four stages that were mentioned in terms of the development of deficiency, and I think it is a philosophical decision on where one intervenes—whether one intervenes at a time just before a clinical deficiency becomes evident or well before that, at the stage when stores are becoming depleted. There is no obvious answer to that question: It is a matter of philosophy—certainly the safety of the infant is the main point. Up to this point, the philosophical approach that I have taken is to try to replete the stores at the level they would have been during the last trimester of pregnancy. Similarly, I am sure that the system you are using would not get you into trouble. When we talk about the preterm infant, it is very difficult to decide what is absolutely right because from a teleological point of view the infant should not be outside the womb; therefore there are no “normal” values for that infant. Therefore we unfortunately are forced to state arbitrarily what is a normal value. I think the argument is a philosophical one, and if you can show me that your way is as good and safe as mine I will take your way; and if I can show you the same, I would hope that you would say the same thing.

*Dr. Senterre:* How do you solve the problem when the baby is orally fed because at that time it is not possible to get a positive balance. I do not provide a very high intake of zinc and copper as has been advised. Once preterm babies reach about 37 weeks' gestation, the absorption of zinc and copper improves dramatically and the urinary excretion decreases so babies can compensate later for early negative zinc and copper balances.

*Dr. Zlotkin:* In general terms I would not supplement the preterm baby who is orally fed. I would only do it on a specific case basis as the need arises.

*Dr. Delange:* I have a short question on disorders possibly caused by deficiency in manganese in humans. Kaelli (*Soc Exp Biol Med* 1970;135:216) reported that iodine-deficient rats developed goiters more frequently and more severely if they were also manganese-deficient. The mechanism was unknown. This was interesting to us because we had a chance to survey a population on an isolated island in the midst of the Kivu Lake which was uniformly submitted to an extremely severe degree of iodine deficiency. Still, only one part

of that population had goiter. One of the only differences between the goitrous and the nongoitrous populations was that the nongoitrous population had a higher manganese intake than the goitrous one because the soil on which they were living was of volcanic origin. Does anyone have additional information on the possible relationship between manganese intake and thyroid function in humans?

*Dr. Hambidge:* I have two comments with respect to Dr. Zlotkin's talk. The first of these concerns the obligatory losses of zinc during total parenteral nutrition, which I suggest depend very much on the particular amino acid solution that is used. We compared urinary zinc excretion rates in very-low-birth-weight preterm infants receiving different proprietary intravenous amino acid solutions and found different urinary zinc excretion rates. I do not have an explanation for this. It is not connected with the amount of zinc in the infusate, and we found that whether supplemental zinc in various quantities is added to the infusate makes no difference to the urinary excretion rate. This is in line with the findings of Wolman and colleagues in adults several years ago. I do not think it can be explained on the basis of amino acid composition of these intravenous infusates because the amino acids are very similar. I suspect it is something else in these particular preparations. With these results in mind, we cannot take Dr. Zlotkin's data and apply it across the board, but we will need to determine the losses for each individual amino acid solution used. My second point is that I would take very strong issue on Dr. Zlotkin's attitude about the possibility of trace element deficiencies not occurring in free-living communities, including normal term infants and children, because I think it is incompatible with the facts: It is incompatible with what we know about iron deficiency, about iodine and selenium deficiency in certain geographic areas, and about zinc deficiency.

*Dr. Zlotkin:* Dr. Hambidge's points are very well taken. Because amino acids bind with metals in a specific way, one might expect that the amino acid content would have had an effect on trace metal excretion in the urine.

*Dr. Van Caillie:* We are all convinced we should give zinc and copper. What about selenium? Do you supply it? In the commercially available electrolyte solutions in Europe, we do not have selenium at this moment. In 1977 I lost one child after 18 months of TPN from a cardiac arrest and a second one in 1981. Again, I am convinced we should have given selenium. I know Dr. Lombeck thinks it is dangerous. What do you do in practice?

*Dr. Zlotkin:* I wish you had not asked that question. The answer is: We do give selenium. The reason I wish you had not asked the question is that I really cannot give an excellent justification for its use. My poor justification would be that we know the infants born prematurely are born with low stores and therefore are at risk of deficiencies. In addition, like you, we have observed that selenium levels and glutathione peroxidase levels decrease with increasing time on TPN. Again, I do not think either of these reasons are convincing. A third and perhaps most important unconvincing reason would be that since adding selenium to the TPN regimen we have never seen a case of selenium deficiency, although during the past 2 years we have seen a couple of cases of biotin deficiency. The only reason I bring up biotin is because it is an example of a nutrient that we were not adding to the formulation because we did not think it was necessary until we had a couple of children who became extremely ill and nearly died before we recognized that we were in fact leaving out an essential nutrient. So, based on those terrible reasons, we in fact do include selenium.

*Dr. Golden:* You were giving 80 calories/kg?

*Dr. Zlotkin:* Yes. Eighty nonprotein calories per kilogram.

*Dr. Golden:* To me that would be an energy-deficient diet; if I give a child an energy-deficient diet, I do not expect to get retention of anything. The major determinant of

retention in my experience is growth rate, and I get a very close experimental relationship between the rate of growth and the rate of retention of individual nutrients in children.

*Dr. Zlotkin:* There are pretty good data around to show that the intravenously fed baby in an incubator in a thermoneutral environment needs a lot fewer calories than we previously appreciated. These infants gained weight at a rate similar to that of intrauterine weight change on 80 nonprotein calories per kilogram. I am convinced that intravenously fed infants in a thermoneutral environment who are passing no stool and who are inactive need a lot fewer calories than previously was appreciated. Indirect calorimetry evidence shows that at around 60 cal/kg/day infants are in neutral energy balance. They can certainly grow fairly well on 80 nonprotein kcal/kg/day.

*Dr. Golden:* We can disagree about these figures for premature infants; but in terms of using your data to make recommendations of how much should or should not be added, I thoroughly agree with whoever said that they should be related to the energy intake; indeed, I would go even further and say they should be added to the *additional* energy above the maintenance requirement so that they are then directly related to the rate of weight gain.

*Dr. Hurley:* I was very interested in your data on manganese and especially the observation of retention of manganese during the early neonatal period. Dr. George Cotzias showed that in suckling mice during the first 17 days of life there was absolutely no excretion of manganese. In fact, he could increase the manganese concentration in these mice by feeding their mothers a high manganese diet which was reflected in the milk. We thought that was a very interesting and mysterious observation, and it is exciting that you have reported a similar finding in the human. In regard to your comments about the lack of toxicity of manganese, though, I think it may be a little premature to make that statement. Although manganese taken by the oral route is relatively nontoxic, intravenously it can have very toxic effects. In addition to the toxic effects of manganese on the brain, we have been investigating the effects of manganese on carbohydrate metabolism. Both manganese deficiency and excess manganese can influence carbohydrate metabolism. This may be very important during that critical neonatal period.

*Dr. Hambidge:* From the Toronto experience and the Denver experience with traditional balance studies, it can be concluded that the breast-fed premature infant does somewhat better than the formula-fed infant with respect to zinc status. The suggestion that the breast-fed infant is at greater risk from zinc deficiency is misleading.

*Dr. Zlotkin:* The Toronto experience in terms of the absorption of metals from breast-fed infants is similar to yours. My point about the infant at risk would be that this baby had received some total parenteral nutrition without trace metals and had been in the hospital for a fairly long time without getting any supplementary trace metals; therefore stores would be depleted.

*Dr. Aggett:* No, the infant had been delivered at 32 weeks' gestation and had no special care at all. I would like to also make a point that this type of "transitory" zinc deficiency has been observed in full-term infants.