A prospective study of serum copper and zinc levels in patients receiving total parenteral nutrition[1, 2]

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ABSTRACT Weekly determinations of serum copper (Cu) and zinc (Zn) were made in eight adult patients receiving total parenteral nutrition (TPN) for 3 to 13 weeks. Serum Cu decreased in all eight patients. Five of eight patients had hypocupremia lasting at least 2 consecutive weeks and three of the five had Cu levels of 30 μg/dl or lower. Low levels of serum ceruloplasmin provided supportive evidence of Cu deficiency in the three patients with the lowest Cu levels. Two patients who had Cu ≤ 20 μg/dl demonstrated declines in hemoglobin which were probably due to Cu deficiency. The mean rate of decline in serum Cu was 10.8 μg/dl/week. After resumption of oral feedings in five patients, the mean rate of increase in Cu was 14 μg/dl/week. The sharpest rise in Cu was seen during the 2nd week after oral feedings were resumed in four of the five patients. Three of eight patients had serum Zn levels less than 70 μg/dl for at least 2 consecutive weeks. Serum Zn decreased at a mean rate of 6.6 μg/dl/week. There was a further decline in serum Zn in three of five patients in whom measurements were made after resumption of oral intake. Concentrations of Zn in TPN solutions varied between 0.63 and 1.0 mg/liter. Cu was undetectable in TPN solutions. *Am. J. Clin. Nutr.* 29: 70–77, 1976.

Total parenteral nutrition (TPN) is an important adjunct in the care of patients who are unable to meet their nutritional needs for prolonged periods by utilizing the digestive tract. Dudrick and colleagues in 1968 described the placement of catheters in large diameter central veins through which hypertonic nutrient solutions could be administered (1). This represented a major advancement, since it assured that enough carbohydrate calories could be given so that the amino acids which were infused simultaneously with the dextrose would be utilized for protein synthesis. However, this advancement in human nutrition has been associated with many complications. The complications which have been described in patients on TPN can be categorized as: *1*) mechanical problems related to catheter placement and maintenance, *2*) infections, and *3*) metabolic abnormalities.

In the United States, TPN solutions contain glucose and synthetic amino acids or protein hydrolysates to which electrolytes, macroelements, and vitamins are added. Trace elements, essential fatty acids, iron, and vitamin K are not provided but may require supplementation in patients on long-term TPN. At present, the Food and Drug Administration has not approved fat emulsions or a trace element mixture for intravenous use.

In 1972, a comprehensive review of the metabolic complications associated with TPN did not mention deficiencies of trace elements (2). In the same year, the first report of human copper (Cu) deficiency during TPN therapy was reported (3). An infant was found to have neutropenia, leukopenia, and anemia after receiving TPN for 6¾ consecutive months. After the anemia was unresponsive to intramuscular iron, studies of copper metabolism disclosed marked hypocupremia and a low serum ceruloplasmin level. All

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TABLE 1
Clinical features of patients

Patient	Age (yr)	Sex	Indication for TPN	Duration of TPN (wk)
1. H. M.	26	M	Severe ulcerative colitis; total proctocolectomy	3
2. S. W.	30	F	Crohn's ileocolitis; total colectomy-ileal resection	5
3. R. A.	31	M	Traumatic rupture of spleen; splenectomy; intraabdominal abscesses	6
4. H. S.	32	M	Enterocutaneous fistula. Postop lysis of adhesions for intestinal obstruction	5
5. E. P.	62	M	Anastomotic leak after hemigastrectomy for gastric ulcer	6
6. C. P.	28	M	Alcoholic pancreatitis with pancreatic pseudocyst	7
7. R. F.	61	M	Perforated duodenal ulcer; intraabdominal abscess; duodenocutaneous fistula	12
8. D. E.	46	F	Short bowel syndrome. Radiation enteritis with enterovaginal fistula	13

hematological parameters were promptly corrected after the administration of oral copper sulfate. Subsequently, other cases of hypocupremia were detected in two infants with protracted diarrhea who were receiving TPN (4). The first reports of Cu deficiency occurring in adults during TPN appeared recently (5, 6). Although serum Cu levels as low as 2 μg/dl were detected, serial measurements of serum Cu were not done to determine how soon hypocupremia, and presumably Cu deficiency, might have been detected after cessation of oral feedings and starting of TPN. In the one patient in whom it was measured, serum zinc (Zn) was also low, at 54 μg/dl (6).

In a prospective study designed to determine time of onset and clinical significance of Cu and Zn deficiencies in adult patients on TPN, weekly determinations of serum Cu and Zn were made in eight hospitalized patients receiving TPN for 3 to 13 weeks.

Methods

Blood samples were collected before initiation of TPN or soon after TPN therapy was started. Over a 4-month period, we collected weekly serum samples from all patients who were potential candidates for long-term TPN. The clinical features of the eight patients who received TPN for a minimum of 3 weeks and who are the subjects of this report are shown in Table 1. At the beginning of the study, patients D. E. and R. F. had already received TPN for 4 and 7 weeks, respectively. Both of them were severely deficient in copper and are included in this report.

Blood was drawn from an antecubital vein into plastic syringes (Monject). Serum was separated within 3 hr and refrigerated in acid-washed plastic vials until Cu and Zn determinations were performed. All serum samples were assayed within 7 days of collection. Analyses of the serum for Cu and Zn were performed by atomic absorption spectroscopy (Unicam SP-90) after dilution of serum 1:1 with deionized water. Serum ceruloplasmin was performed by the method of Henry et al. (7).

TABLE 2
Total parenteral nutrition solution[a]

Constituent	Amount
Fluid	1,000 ml
Amino acids	26.7 g
Dextrose	267 g
Sodium	40 mEq
Potassium	28.3 mEq
Magnesium	5.3 mEq
Calcium	6.7 mEq
Chloride	60.9 mEq
Sulfate	5.3 mEq
Phosphate (HPO_4)	13.3 mEq
Gluconate	6.7 mEq

[a] Contents listed are in 1 liter for our average adult patient receiving three bottles/day. In addition, ⅔ ampules of M.V.I. (U.S. Pharmaceutical) and ½ vial of Folbesyn (Lederle) are added to one bottle of six.

The composition of the TPN solution used in our hospital is listed in Table 2. A total of 18 samples of TPN solutions were collected at random from the pharmacy on 2 separate days. The Cu and Zn analyses of these TPN samples were performed on the day of collection. Six of the 18 samples contained dextrose, amino acids, and electrolytes; six contained dextrose, amino acids, electrolytes, and Folbesyn; and six contained glucose, amino acids, electrolytes, Folbesyn, and another multivitamin infusion (M.V.I.—USV Pharmaceutical) which, in addition to water-soluble vitamins, contains vitamins A, D, and E.

Results

Figure 1 shows the concentration of Cu in the serum during TPN therapy and, for five patients, after resumption of oral intake. Serum Cu decreased in all patients during TPN therapy. Five of the eight patients developed hypocupremia lasting at least 2 consecutive weeks. Two of the five patients (H. S. and E. P.) had mild decreases in serum Cu which were first detected 3 and 6 weeks, respectively, after TPN was begun. Three patients (D. E., R. F., and H. M.) had severe hypocupremia. Patients R. F. and D. E. were the most deficient in Cu; unfortunately we did not obtain blood samples before starting TPN therapy on these two patients since both had received TPN for several weeks before we began this study.

In the six patients in whom initial measurements of serum Cu were made either before TPN was started or during the 1st week of therapy, the mean rate of decline of serum Cu during TPN therapy was 10.8 μg/dl/week (range 5.6 to 20 μg/dl). Cu levels increased slightly during the 1st week of TPN in three patients (H. S., E. P., and C. P.) but decreased during the 2nd week. Hypocupremia for at least 2 consecutive weeks was not detected in three patients; however, one such subject (S. W.) had a serum Cu level of 55 μg/dl at the end of 4 weeks of TPN and another (C. P.) had Cu values between 75 and 80 μg/dl between weeks 3 and 6.

After resumption of oral feeding in five patients, the mean rate of increase in serum Cu was 14 μg/dl/week (range 7 to 20 μg/dl). The sharpest rise in Cu was seen during the 2nd week after oral feedings were started in four of the five patients. In R. F. the sharpest rise was 20 μg/dl during the 1st week after he began eating (Fig. 2). R. F. and D. E. underwent surgery after 5 and 10 weeks, respectively, of TPN; the increase in serum Cu concomitant with the operations probably represents Cu received through blood transfusions.

Analyses for serum ceruloplasmin were done in the three patients (H. M., D. E., and R. F.) with severe hypocupremia. At times when serum Cu was 30 μg/dl (H. M.), 30 μg/dl and 22 μg/dl (R. F.), and 12 μg/dl (D. E.), the corresponding ceruloplasmins were 116, 102, 45, and 41 units, respectively (normal = 280 to 570). No patient had historical or biochemical evidence of liver disease to suggest an inability to synthesize ceruloplasmin, nor was proteinuria present to suggest urinary loss of copper-binding proteins. None of the patients were taking estrogens and only H. M. and S. W. were receiving corticosteroids.

Three of eight patients (R. A., C. P., and D. E.) had serum Zn levels less than 70 μg/dl for at least 2 consecutive weeks (Table 3). D. E. was found to have a Zn level of 45 μg/dl when first measured during the 7th week of TPN therapy. Her serum Zn level remained less than 50 μg/dl for 4 weeks until it rose concomitantly with multiple blood transfusions given at the time of an operation during the 10th week. Soon after the operation she resumed oral feedings. The other two patients (R. A. and C. P.) developed low serum Zn levels after 4 and 8 weeks, respectively.

Serum Zn was determined before starting TPN or during the 1st week of therapy in six patients. Four of these six subjects main-

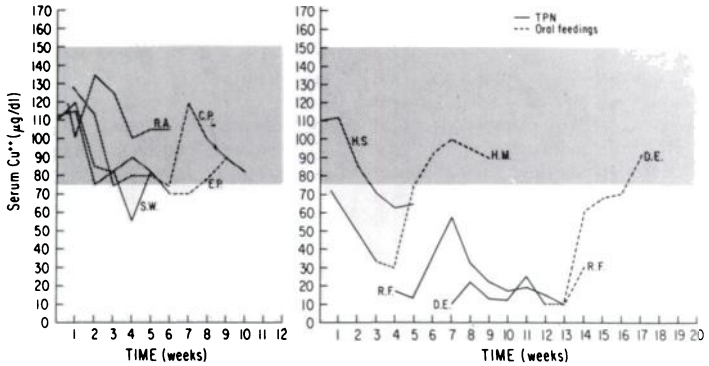

FIG. 1. Serum Cu at weekly intervals during TPN therapy (——) and after resumption of oral intake (-----).

tained zinc levels within the normal range in spite of receiving TPN from 3 to 10 weeks; however, zinc levels declined into the low normal range on at least one determination in all patients. Serum Zn decreased at a mean rate of 6.6 μg/dl/week (range 0.33 to 20 μg/dl) in these six patients.

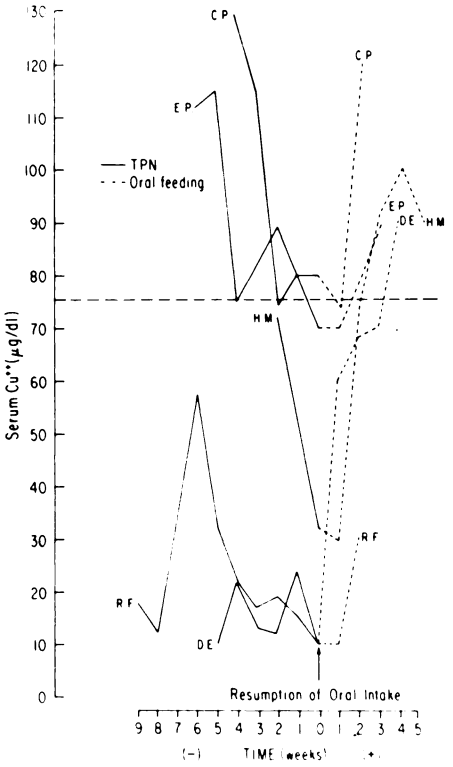

FIG. 2. Serum Cu during TPN therapy (——) and after resumption of oral intake (-----) in five patients. *Horizontal axis* shows time in weeks that patients were on TPN (–) and when receiving oral feedings (+). Lower range of normal for serum Cu (75 μg/dl) is depicted as (– – –).

An interesting finding in three of five patients in whom measurements were made after resumption of oral intake was a significant decline in zinc levels during the 1st week of oral feeding. The mean rate of decline in the three patients (H. M., E. P., and D. E.) was 42 μg/dl/week with a range of 24 to 72 μg/dl. After the initial decline upon restarting oral feedings, the serum Zn returned to normal levels during the 2nd week of oral feedings in all three patients.

Three of eight patients (S. W., R. F., and H. M.) received topical zinc oxide for care of their wounds. Cutaneous absorption of Zn probably helped maintain normal Zn levels.

All patients benefited from TPN therapy. Every patient gained weight in spite of significant catabolic states, and periodic urinary nitrogen determinations in six of eight subjects confirmed that they were in positive nitrogen balance. Serum albumin levels were low in all patients before starting TPN but increased to normal or near normal in all patients before cessation of TPN.

Decreases in hemoglobin from 11.6 to 7.8 (R. F.) and 11.7 to 9.1 (D. E.) occurred over 8 and 5 weeks, respectively, during which time serum Cu levels were very low. Serum folate and vitamin B_{12} levels were normal in both patients, as was the serum iron in R. F. Patient D. E. had a serum iron of 41 μg/dl with a total iron binding capacity of 215 μg/dl (19% saturation); her bone marrow revealed hypoactive erythroid and granulocytic series, but stainable iron was normal and there were no ringed sideroblasts. Nei-

TABLE 3
Serum Zn (μg/dl) at weekly intervals

Patient	Before TPN	Week																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
H. M.[a]	165	154	138	105	75	112	132	105[c]	110	112								
S. W.[a]	95	122	95	88	70	80												
R. A.[b]	94	100	75	76	66	50	46											
E. P.	94	88	134	156	160	124	92[c]	68	85	85								
H. S.	160	145	145	180	176	120												
C. P.[b]		100	80	74	100	85[c]	121	85[d]	70	66	60							
R. F.[a]					132	128	85	140	136	143	104	80	104	80[c]	80	100		
D. E.[b]								45	42	45	44	68	122[c]	50	70	85	85	90

[a] Patients who received topical zinc oxide. [b] Patients with serum Zn less than 70 μg/dl for 2 or more consecutive weeks. [c] Resumption of oral intake. [d] Restart TPN.

ther patient had neutropenia; however, mild leukopenia (white cell counts 3200 to 4300) was present in D. E.

Concentrations of Zn in TPN solution varied between 0.63 and 1.0 mg/liter. Zn was not intentionally added; therefore, Zn in the solutions represented contamination with Zn during preparation. Cu was undetectable in the TPN solutions.

Discussion

Copper and zinc are widely distributed in foods, and dietary deficiencies of these trace elements in healthy persons in the United States must be very uncommon. Most American diets provide at least 2 mg of Cu/day, an amount known to maintain equilibrium of Cu in balance studies. The mixed diet consumed by American adults contains an average Zn content of 10 to 15 mg/day, which in healthy adults will achieve equilibrium or positive balance of Zn (8).

Copper deficiency in humans occurs only under unusual circumstances. Most recognized cases of copper deficiency have been in children. Rapidly growing premature infants and children with chronic malabsorption who are maintained on copper-poor cow's milk have developed copper deficiency (9, 10). Infants with marasmus frequently develop marked hypocupremia concomitant with their rehabilitation exclusively with milk diets. Before refeeding, the infants are probably depleted of Cu stores because of chronic diarrhea, low dietary Cu intake, and lack of ligands for facilitating Cu transport. Cordano et al. (11) suggest that upon refeeding, the low Cu content of mild formulas is not adequate to support accelerated growth during recovery; hence the most marked decline in Cu in infants with marasmus occurs after refeeding. Menkes' kinky hair syndrome is characterized by extremely low concentrations of Cu in serum, liver, and brain, and apparently results from an incomplete block in the intestinal transport of Cu (12, 13). Clinical manifestations in infants with Menkes' syndrome are scorbutic type bone changes, seizures, pili torti, arterial intimal abnormalities, and temperature instability. The nephrotic syndrome may be associated with urinary excretion of ceruloplasmin, a protein which is normally not found in urine, and the urinary Cu loss is in direct proportion to the amount of proteinuria. Low serum Cu and ceruloplasmin result, but it is not known whether tissue stores of Cu are depleted (14). Hypocupremia associated with malabsorption or protein-losing enteropathy might result from impaired absorption of Cu or excessive fecal losses of ceruloplasmin or both (15).

Copper deficiency in patients receiving TPN solutions to which trace elements had not been added has been observed in an infant, an adolescent, and adults (3–6). Serum Cu levels of 2 to 32 μg/dl were first detected at 2½ to 18 months after initiation of TPN therapy. Leukopenia, neutropenia, and/or anemia were present in all patients. Administration of either oral or intravenous copper sulfate resulted in prompt correction of the hematological abnormalities. In the infant described by Karpel and Peden who had received "more than adequate amounts of ascorbic acid," scorbutic type bone lesions were probably due to decreased activity of ascorbate oxidase, a cuproenzyme. As observed by others, the bony defects improve and may completely resolve after Cu supplementation (3, 16).

A decrease in serum Cu during TPN therapy occurred in all eight of our patients. Five of eight subjects had hypocupremia for at least 2 consecutive weeks, and three of the five had Cu levels of 30 μg/dl or lower. Low levels of ceruloplasmin, an α_2-globulin which binds approximately 95% of circulating Cu, provided supportive evidence of copper deficiency in the three patients with the lowest copper levels. The hepatic cellular concentration of Cu is thought to influence the synthesis of this protein in the liver, and Cu deficiency is accompanied by a low serum concentration of ceruloplasmin (17, 18).

Two of our three patients who developed severe hypocupremia had features in common with the patients described by Vilter et al. and Dunlap and colleagues (5, 6). Patient D. E. had chronic diarrhea and a 40-pound weight loss associated with a short bowel syndrome resulting from partial resection of her small intestine. As did one of the patients described by Dunlap et al., R. F. may have lost excessive amounts of biliary Cu through his high-output duodeno-cutaneous fistula. H. M. had severe ulcerative colitis with 15 watery stools/day. His serum Cu (73 μg/dl) was in the low normal range before starting

TPN and declined to 33 μg/dl after 3 weeks of TPN. It is likely that all three of the patients had depleted body stores of Cu before initiating TPN because of poor dietary intake and/or excessive losses of Cu in feces or fistula drainage. As observed in H. M., a rapid decline in serum Cu after starting TPN therapy is analogous to the hypocupremia that develops in infants with marasmus who are refed exclusively with copper-poor milk formulas (11).

Cu levels less than 20 μg/dl are associated with impaired hematopoiesis in swine and humans (5, 6, 19). Both of our patients who had serum Cu levels below 20 μg/dl demonstrated declines in hemoglobin. Although Cu deficiency almost certainly contributed to the anemia, we do not know whether it was the only or major cause since copper supplements were not given to determine erythropoietic response. The anemia of Cu deficiency is thought to result from a reduced rate of red cell synthesis, shortened erythrocyte survival time, and impaired transferrin formation from ferrous iron caused by inadequate concentrations of ceruloplasmin, a feroxidase enzyme (5, 19, 20). As observed by Vilter et al. in their patient with anemia which corrected after copper supplements, D. E. had a low serum iron when stainable bone marrow stores of iron were normal. This is consistent with a defect in the release of iron from tissue stores (6).

A transiently low serum Zn level may not indicate Zn deficiency of body tissues. Halsted et al. (21) speculate that it may represent a redistribution of intravascular Zn which is loosely bound to albumin. Low serum Zn and a reciprocal hypercupremia sometimes occur with stressful situations such as myocardial infarction, surgery, or inflammatory diseases (21). This reciprocal relationship of Zn and Cu may in part be explained by leukocyte endogenous mediator (LEM), a heat-labile, low molecular weight protein which appears in plasma within 2 hr after initiating an infection (22). Intraperitoneal injection of LEM into rats produces a rapid accumulation of ^{65}Zn in liver while plasma ^{65}Zn declines. In contrast, LEM produces significant increases in serum Cu and ceruloplasmin concentrations (23).

Although all eight patients in this series demonstrated a decrease in their serum Zn during TPN therapy, only three had low serum levels for at least 2 weeks, during which time there were no other conditions present which are associated with hypozincemia. Therefore, we think that decreased serum Zn for that interval of time represented Zn deficiency. Only D. E. had a marked hypozincemia; it persisted until she received multiple blood transfusions in preparation for surgery. Zn deficiency has resulted from malabsorption (24), and in this patient chronic malabsorption associated with her short gut syndrome probably contributed to her deficiency.

An initial decrease in serum Zn after resuming oral intake was observed in three of five patients. Others have observed that after protein and zinc depletion, the administration of Zn by mouth resulted in a further decline in serum Zn (25). Spencer believes that a fall in serum Zn after giving oral Zn is suggestive of Zn deficiency and reflects the pulling of plasma Zn into tissues (26). A shift of Zn from the intravascular to the intracellular compartment might represent utilization of Zn by anabolic tissues. Since endogenous Zn secretion into the small bowel is primarily via the pancreatic juice (27), the loss of Zn in pancreatic secretions stimulated by oral feedings may partly explain the decline in serum Zn after resumption of oral intake.

Deficiencies in Cu and Zn in patients treated with TPN may result from: *1*) decreased intake, *2*) increased urinary excretion, and, in some patients, *3*) excessive losses in feces or from enterocutaneous fistulas. Cu was undetectable, as determined by atomic absorption spectorphotometry, in the TPN solutions we use. Zn concentrations in the solutions varied from 0.63 to 1.0 mg/liter. Therefore, the average adult receiving 3 liters of TPN/day received a maximum Zn intake of only 3 mg daily or 20% of the Recommended Daily Allowance (8). Stegink et al. found that heat sterilization of TPN solutions containing glucose and either amino acids or protein hydrolysates results in glucose-amino acid complexes (28). A 2- to 5-fold increase in urinary Zn, Cu, and iron excretion in the urine resulted when the glucose-amino acid complexes were present, presumably from a chelation effect by the sugar-amino acid complex. Solomons et al. studied one patient during TPN therapy and found normal levels

of urinary Cu and Zn (29). The major excretory route for Zn is via the feces, whereas Cu is excreted primarily by way of the biliary tree (27). Increased losses of both of these elements may occur in malabsorption, inflammatory bowel disease, or enterocutaneous fistulas and lead to Cu and Zn deficiencies.

Many patients receiving TPN have cutaneous wounds. TPN can provide adequate calories and protein which are necessary to maintain positive nitrogen balance and heal wounds more efficiently. However, Cu and Zn are also important in synthesis of connective tissue. As shown by ^{65}Zn uptake in incised wounds of rats, Zn migrates rapidly into granulation tissue and Zn sulfate therapy has been reported to accelerate healing of chronic wounds in humans (30). In Zn-deficient rats, the tensile strength of healing surgical incisions is significantly depressed compared to controls (31). Zn deficiency in rats results in impaired DNA synthesis leading to a reduction in the ability of the fibroblast to synthesize collagen (32, 33). Cu also plays an important role in connective tissue integrity. Amine oxidase, a cuproenzyme, is necessary for the conversion of lysine to desmosine and isodesmosine, the amino acids required for elastin cross-linkage. Lysyl oxidase, an enzyme required for cross-linking of collagen, is also Cu-dependent. Cu deficiency results in impaired elastin and collagen synthesis (30). Thus, impaired wound healing may be a deleterious effect of Cu and Zn deficiencies in postoperative patients receiving TPN.

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