Copper Enzyme Activities in Cystic Fibrosis Before and After Copper Supplementation Plus or Minus Zinc

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One laboratory reports low activities for 2 blood copper enzymes in subjects with cystic fibrosis (CF), which suggests that moderate copper deficiency is common in this state. The present study attempted to confirm this proposition in 3 ways: repeat the measures for 1 of the 2 copper enzymes (superoxide dismutase) in a new group of CF patients (males and females, N = 38), add another copper enzyme measure (plasma diamine oxidase) that has high sensitivity to copper status, and test if copper enzyme activities in CF patients rise by copper supplementation. The last test was performed plus or minus zinc supplementation since poor zinc status may contribute to poor copper status. The results for the first 2 aims supported the idea of poor copper status, as low activities were found for CF subjects for 2 copper enzyme activities, erythrocyte superoxide dismutase and plasma diamine oxidase (although normal activities were obtained for another copper enzyme, plasma ceruloplasmin, both as U/mL plasma or U/mg ceruloplasmin immunoreactive protein). For the last aim, copper enzyme activities were not altered by copper supplementation (6 weeks, 3 mg copper/d as copper-glycinate), plus or minus concurrent zinc supplementation (30 mg zinc/d as zinc-glycinate). Therefore, CF may cause a tendency to moderate copper deficiency, which may be due to abnormal copper metabolism not easily corrected by increased copper and/or zinc intake.
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INDIVIDUALS with cystic fibrosis (CF) are often monitored for signs of some nutritional deficiencies, but not usually copper deficiency. If copper deficiency is present, even in moderate form, it could affect health and longevity in CF patients. One reason is that infection is a major concern in CF,[1] and immune function is affected by even marginal copper deficiency in rats and mice.[2] Another issue is that 3 copper metalloenzymes show antioxidant actions.[3] In CF, pulmonary oxidant stress is considered a major cause of mortality.[4] Although copper deficiency certainly is not the only factor that can cause problems with immune function or oxidant stress, it can be a contributor.[3]

Percival's group provides evidence that people with CF are prone to moderate copper deficiency based on low activities for 2 blood cell copper enzymes, cytochrome C oxidase and superoxide dismutase.[5,6] The influence of factors other than copper status on these 2 enzyme activities in blood cells is poorly characterized, but these activities have been found to react to small changes in copper status in humans and experimental animals.[7-15] In contrast to these findings, serum copper values are reported as normal or high in CF.[5,6,16] However, the latter does not necessarily indicate good copper status. Physiological stress raises values for serum copper because stress elevates serum contents of the protein ceruloplasmin, which contains most of the copper in serum.[17] Our laboratory has found that in rats, inflammatory stress can produce normal or above normal values for serum copper and/or ceruloplasmin activities, despite moderate copper deficiency based on low dietary copper intake, low activities for various copper enzymes, and a high ceruloplasmin activity response to bolus copper administration.[9-11] A similar phenomena may occur in humans with rheumatoid arthritis.[7] They can show high values for ceruloplasmin activities despite moderate copper deficiency based on low ratios of ceruloplasmin activity to ceruloplasmin protein, plus low erythrocyte superoxide dismutase activities that increase with copper supplementation.[7]

Therefore, although the ceruloplasmin data are inconclusive for elucidating typical copper status in CF, the low activities for 2 other copper enzymes[5,6] raise a concern. This concern could be increased by 3 observations: (1) extend previous findings of low copper enzyme activities to a larger number of subjects in an additional geographical area (differences in location may involve different tendencies in dietary and pharmacological treatment of CF patients); (2) extend the results found for 2 copper enzyme activities to another enzyme activity with high sensitivity to changes in copper status; and (3) show that low copper enzyme activities are reversed by copper supplementation.

The present study tested for these 3 possibilities. For the second possibility, plasma diamine oxidase activities were used. These activities show sensitivity to small changes in copper status that exceed those seen so far for other copper enzymes.[8,12,18,19] For the third possibility, copper was given plus or minus zinc supplementation. Although these 2 minerals are typically considered antagonistic,[3] one study finds that moderate zinc deficiency in humans may actually compromise copper status.[14] Since CF patients may be prone to moderate zinc deficiency, [20] production of adequate zinc status may help produce adequate copper status.

MATERIALS AND METHODS

Subjects

Males and females with CF were recruited through Children's Hospital, Columbus, OH (age 24 ± 8 years; range, 12 to 48). This study was approved by human subject institutional review boards of Ohio State University and Children's Hospital. The subjects, their parents, or legal guardians signed informed consent. All subjects had pancreatic insufficiency and took replacement enzyme and all consumed the multivitamin ADEK (Scandipharm, Birmingham, AL). Subjects were

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Submitted May 19, 2003; accepted July 22, 2003.

Supported by a grant to R.A.D. from the Cystic Fibrosis Foundation.

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0026-0495/04/5301-0033$30.00/0

doi:10.1016/j.metabol.2003.07.017

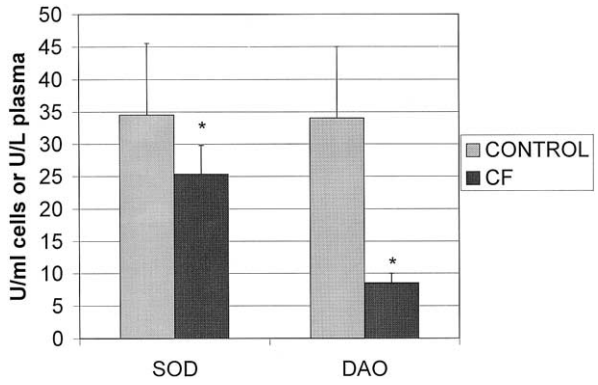

Fig 1. Activities of erythrocyte superoxide dismutase (SOD) and plasma diamine oxidase (DAO) in subjects with CF and controls. Values are means ± SD. SOD is expressed as U/mL packed red blood cells × 10^{-2}. DAO is expressed as U/L plasma. For controls, n = 30; for CF patients, n = 38. *Significantly different from controls (unpaired *t* test, *P* < .05)

excluded if they had diabetes, renal failure, or advanced pulmonary disease with forced expiratory volume in 1 second (FEV_1) less than 40%, or if pregnant. Recruited subjects (N = 48) were randomly assigned to 1 of 4 treatment groups (n = 12 per group): copper (3 mg of copper/d as copper-glycinate from Albion Laboratories, Clearfield, UT), zinc (30 mg of zinc/d as zinc-glycinate from Albion), copper + zinc, or placebo (maltodextran). Supplements were taken as 1 capsule per day for 6 weeks. Copper- and zinc-glycinate had altered metalloenzyme activities and more general measures of health in studies of other types of subjects.[7,8,18,21] Before and after the supplementation period, blood was drawn into heparin-vacuum tubes. Subjects who began the study were excluded if hospitalized during participation (including for infection), or if they had acute problems such as diarrhea for 3 or more consecutive days. Following 10 such exclusions, a total of 38 subjects remained.

Subjects were not fasted before blood draw, but generally had not eaten for at least 2 hours prior to blood draw. Blood was centrifuged at 3,500 × *g* at 4°C. Plasma was stored at −70°C. Erythrocytes were extracted and stored at −20°C for superoxide dismutase analysis as described previously.[7,9] For comparison purposes, blood was drawn from healthy males and females (n = 30) in the same age range as the subjects with CF. The control subjects were recruited as part of other studies performed in our laboratory.

Enzyme Assays

Ceruloplasmin activity was measured by oxidase activity toward p-phenylenediamine (Sigma Chemical Co, St Louis, MO) as described by Rice.[22] Units were arbitrarily designated as the absorbance change multiplied by 0.01. Ceruloplasmin protein was assessed using radial immunodiffusion (RID) plates (The Binding Site Ltd, Birmingham, England) according to the manufacturer's instructions. In our laboratory's experience, duplicate samples generally give nondetectable differences for the RID and under 4% for the activity assay, with no detectable differences for either assay when run on different days (unpublished data). Erythrocyte Cu-Zn superoxide dismutase activity was measured by the modified pyrogallol autoxidation assay.[9] In our laboratory's experience, a single sample generally gives nondetectable differences when the assay is repeated on the same day or several months later when the sample extracts are stored at −10°C (unpublished data). Plasma diamine oxidase activity was analyzed by a slight modification of the colorimetric assay of Takagi et al.[12,23] Results are analyzed in comparison to a standard curve using commercial diamine oxidase, which can bring some consistency to the assay.

Statistical Methods

Statistical analysis was done using the Jump 3.1 program (SAS Institute, Cary, NC), with significance at $P < .05$. For each parameter, values for the subjects with CF, prior to supplementation or placebo, were compared to control values by unpaired, 2-tailed Student's *t* test. In addition, pre-supplement or pre-placebo values were compared with post-supplement or post-placebo values, respectively, by paired, 2-tailed Student's *t* test.

RESULTS

Compared to a group of healthy subjects, individuals with CF showed low activities for the copper enzymes erythrocyte superoxide dismutase and plasma diamine oxidase (Fig 1). However, these enzyme activities in the subjects with CF were not elevated by 6 weeks of copper supplementation, with or without concurrent zinc supplementation (Figs 2 and 3). A pooling of the results for copper supplementation alone with those of the copper + zinc supplementation also showed no copper effect on the activities of either enzyme (Fig 4). The lack of effect was seen not only in mean results, but also in most individuals.

The zinc in the supplement appeared to be bioavailable since supplementation with zinc (±copper), but not placebo or copper alone, elevated plasma zinc values (18 ± 5 μmol/L before zinc ± copper *v* 25 + 8 μmol/L after zinc + copper, $P < .05$, paired *t* test).

For another copper enzyme, ceruloplasmin, CF patients and controls showed similar activities (Table 1). The same was true for ceruloplasmin activity/immunoreactive protein ratios (Table 1), which can be affected by copper status.[24] These normal values for ceruloplasmin activity and activity/protein ratios were unchanged by copper supplementation, plus or minus zinc (data not shown).

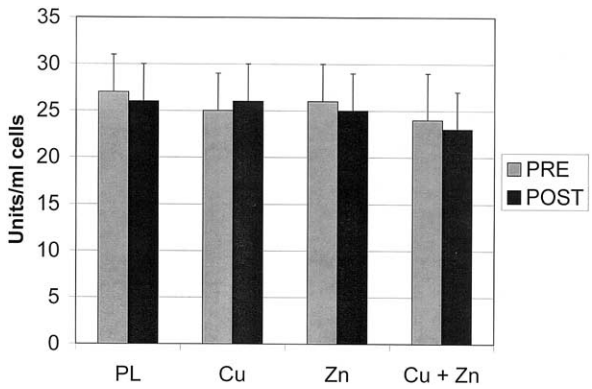

Fig 2. Supplementation effects of either placebo (PL), copper (Cu), zinc (Zn) or Cu + Zn on activities of erythrocyte superoxide dismutase in subjects with CF. Values are means ± SD expressed as U/mL packed red blood cells × 10^{-2}. For placebo, n = 8; for copper, n = 9; for zinc, n = 10; and for copper + zinc, n = 11 . There were no significant differences caused by any supplementation treatment (paired *t* test, *P* > .05).

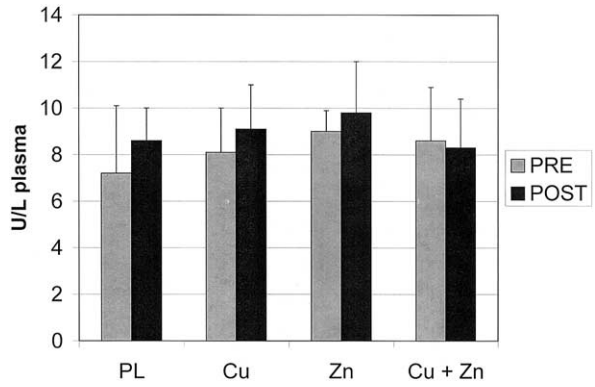

Fig 3. Supplementation effects of either placebo (PL), copper (Cu), zinc (Zn) or copper + zinc on plasma diamine oxidase activities in subjects with CF. Values are means ± SD expressed as U/L plasma. There were no significant differences caused by any supplementation treatment (paired *t* test, *P* > .05).

DISCUSSION

Based on the present study and 2 past studies from another laboratory, people with CF tend to show low activities for 3 copper enzymes (one of these in multiple blood cell types).[5,6] These studies have encompassed 58 subjects in 2 geographical locations. These enzyme activities have included plasma diamine oxidase, erythrocyte superoxide dismutase, and white blood cell cytochrome c oxidase, all of which have shown high sensitivity to relatively moderate changes in copper status in both humans and experimental animals.[7-15,18,19] These changes in copper enzyme activities have occurred at the same time as effects such as alterations in a blood clotting factor, cardiovascular related parameters, and indices of oxidant stress. Therefore, low copper enzyme activities suggest strongly that CF produces a tendency for moderate copper deficiency. The role of dietary copper intake in this tendency has not been ascertained in this or other studies. However, the absence of an effect of copper supplementation on copper enzyme activities

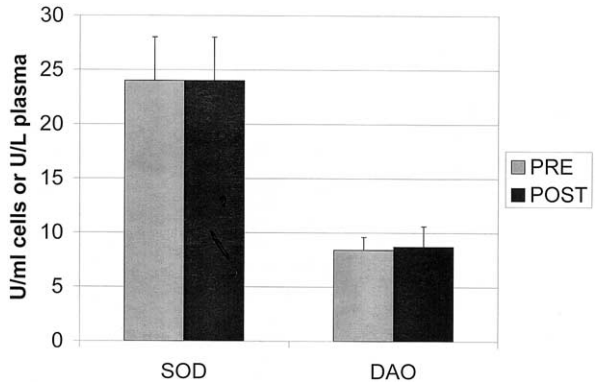

Fig 4. Pooled data from supplementation with copper and copper + zinc: effects on activities of erythrocyte superoxide dismutase (SOD) or plasma diamine oxidase (DAO) in subjects with CF. Values are means ± SD for 38 subjects and are U/mL packed red blood cells × 10^{-2} (SOD) or U/L (DAO). There were no significant differences caused by supplementation (paired *t* test, *P* > .05).

Table 1. Ceruloplasmin Values in CF Patients and Controls

Subjects	N	Activity (U/L)	Immunoreactive Protein (mg/L)	Ratio Activity/Protein (U/mg)
Controls	30	138 ± 64	350 ± 55	0.39 ± 0.06
CF	38	167 ± 63	379 ± 154	0.45 ± 0.10

NOTE. There were no significant differences between CF patients and controls (unpaired *t* test).

in the present study showed that diet alone cannot be a full explanation for the apparent moderate copper deficiency. In fact, this lack of copper supplementation effect, along with the normal ceruloplasmin activities and activity to protein ratios could be said to contradict the conclusion that people with CF are prone to moderate copper deficiency.

In regard to the first issue, it could be speculated that CF does not produce copper deficiency, but rather affects copper enzymes by altering copper protein synthesis, copper protein degradation, or in the case of plasma diamine oxidase, rate of entry of a copper protein into the plasma. The last consideration is known to be affected by intestinal or renal injury, but they raise, not lower entry rates.[12,25] As far as protein synthesis or degradation of these copper enzymes, very little is known in regard to these enzymes in blood. Thus, it can be speculated that factors other than copper status affect the relevant enzyme synthesis or degradation rates, but this cannot be confirmed at present. The issue of degradation is complex since copper saturation of superoxide dismutase may affect its degradation rate in blood cells.[26] This could explain why superoxide dismutase protein levels are low in white blood cells from adults with CF.[6]

Abnormal copper protein synthesis in individuals with CF, although possible, seems an unlikely explanation for the low copper enzyme activities. Generally, processes that regulate protein synthesis affect groups of proteins with functional relationships and/or location similarities. In contrast, the 3 copper enzymes examined thus far for CF are very diverse in function.[3] Moreover, one of these enzymes was analyzed in 3 different blood cell types, and another of these enzymes was analyzed in the plasma. Therefore, although it cannot be absolutely stated that poor copper status explains the low blood copper enzyme activities in CF, it is a very reasonable explanation.

As far as the issue of normal ceruloplasmin-related measurements in CF, it can be noted that though such measurements are often applied to copper status assessment, the relationships are not always clear. Serum or plasma ceruloplasmin activities are low with severe copper deficiency in humans or experimental animals.[3,9-11,24,27] The low activities occur due to copper's role in ceruloplasmin enzyme activity plus a high turnover rate of apo-ceruloplasmin (ceruloplasmin protein not saturated with copper).[3,24] In contrast to results with severe copper deficiency, low ceruloplasmin activities are not always seen in humans with moderate deficiencies.[7,13] Thus, the normal values seen here for subjects with CF do not rule out a moderate copper deficiency. In fact, the normal values may actually comprise a sign of moderate copper deficiency. The reason is that CF is associated with inflammatory stress,[28] and such stress raises

ceruloplasmin protein synthesis.[3,9-11] Therefore, CF should produce high plasma ceruloplasmin activities unless poor copper status cancels out the effects of inflammation. Our laboratory has shown such behavior in rats with chemically induced inflammation.[9-11]

If, in the CF patients, a moderate copper deficiency did in fact prevent inflammation-induced elevation in ceruloplasmin activities, then there could be low ceruloplasmin activity to protein ratios. Copper is needed for ceruloplasmin enzyme activity but not for ceruloplasmin protein synthesis.[3,24] Thus, during copper deficiency, there can be abnormally high amounts of apo-ceruloplasmin, which lacks, or is low in enzymatic activity. However, this was not the case for the people with CF since they showed normal ceruloplasmin activity to protein ratios. However, another consideration is that the apoceruloplasmin turnover rate greatly exceeds that of holo-ceruloplasmin.[24] Therefore, apo-ceruloplasmin molecules with little or no copper or enzyme activity may not accumulate to any great extent in subjects with CF. Previous work on the effects of poor copper status on ceruloplasmin activity to protein ratios has yielded inconsistent results. On one hand, low ratios are seen in severely copper deficient rats as well as in humans with rheumatoid arthritis, who show signs of moderate copper deficiency.[7,24] Low ratios are also seen in renal dialysis patients and human volunteers consuming high-dose vitamin C, though in these 2 cases, the copper status of the subjects is uncertain.[29,30] On the other hand, normal ratios are seen in trauma patients who show low activities for erythrocyte superoxide dismutase and plasma diamine oxidase.[31]

If moderate copper deficiency is commonly associated with CF, then it can be asked: why did copper supplementation not correct this situation? The type of supplement used here, copper glycinate, has impacted copper enzyme activities in three previous studies from our laboratory,[7,8,32] as well as in studies from other laboratories.[18,21] The lack of effect seen here may indicate that little of the ingested copper was absorbed in the CF patients who typically have digestive difficulties.[33] The present study did not test for this possibility. Even so, this study did find that zinc glycinate was well absorbed, at least based on plasma zinc values. Since both minerals were given with the same ligand, and since there is some overlap in copper and zinc absorption pathways,[34] possibly, the bioavailability of the zinc is evidence that the copper was also fairly bioavailable. This idea was not tested. It could not have been tested by measuring plasma copper. Plasma copper values are mainly reflective of ceruloplasmin-bound copper, and ceruloplasmin levels are affected by multiple factors including inflammation and hormonal fluxes.[3]

Possibly, the copper supplement given here was absorbed reasonably well, but the copper was not incorporated effectively into the appropriate enzymes. Such a defect could involve chloride metabolism, which is thought to be the primary area of defect in CF.[33] In yeast, insertion of copper into enzymes is influenced by chloride.[35] Therefore, there is the possibility that the abnormal chloride metabolism of CF may cause inefficient copper incorporation into enzymes. If that is true, then in people with CF, effective copper enzyme activation may require specific copper complexes that incorporate well into enzymes despite abnormal chloride metabolism.

Zinc supplementation had no effect on the copper enzyme activities measured. One study suggests that poor zinc nutritional status can impair copper status,[14] although this has not been verified in any other studies. Other studies have typically shown either an antagonistic relationship, or no effect of zinc intake on copper enzyme activities.[3,36-38]

In conclusion, moderate copper deficiency appears to be a problem in people with CF, but correcting the problem seems to require more than just increasing total copper intake.

ACKNOWLEDGMENT

The authors thank Elizabeth Joseph for her fine technical assistance.

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