

Changes in Mineral Micronutrient Status During and After Pulmonary Exacerbation in Adults With Cystic Fibrosis

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 Fibrosis (DISC) Investigators

Abstract

Background: Patients with cystic fibrosis (CF) may be at risk for micronutrient depletion, particularly during periods of illness and infection. The purpose of this study was to investigate serum micronutrient status over time in adults with CF initially hospitalized with a pulmonary exacerbation. **Materials and Methods:** This was an ancillary study of a multicenter trial investigating the role of high-dose vitamin D supplementation in 24 adults with CF (mean age, 29.6 ± 7.3 years). We measured serum concentrations of copper (Cu), iron (Fe), calcium (Ca), magnesium (Mg), potassium (K), and sulfur (S) in subjects at the beginning of a pulmonary exacerbation and again at 3 months. **Results:** Serum concentrations of Cu, Fe, and Ca were significantly lower at baseline compared with 3 months following the pulmonary exacerbation (Cu: baseline, 1.5 ± 0.6 vs 3 months, 1.6 ± 0.6 µg/mL, *P* = .027; Fe: 0.8 ± 0.3 vs 1.3 ± 1.1 µg/mL, *P* = .026; Ca: 9.7 ± 0.8 vs 10.8 ± 2.0 mg/dL, *P* = .024). Serum concentrations of K, Mg, and S did not change over time (K: baseline, 4.9 ± 0.3 vs 3 months, 5.1 ± 0.5 mEq/L; Mg: 1.8 ± 0.2 vs 2.0 ± 0.3 mg/dL; S: 1288.6 ± 343 vs 1309.9 ± 290 µg/mL; *P* > .05 for all). **Conclusion:** Serum concentrations of Cu, Fe, and Ca increased significantly several months following recovery from acute pulmonary exacerbation in adults with CF. This may reflect decreased inflammation, improved food intake, and/or increased absorption following recovery. (*Nutr Clin Pract.* XXXX;xx:xx-xx)

Keywords

micronutrients; cystic fibrosis; copper; iron; calcium; potassium; magnesium; sulfur; lung

Cystic fibrosis (CF) is the most common autosomal recessive, life-shortening disease in Caucasians.¹ While CF primarily affects the respiratory tract resulting in frequent lung infections and progressive lung function decline,² the disease also affects the gastrointestinal tract, leading to various nutrition complications.³ Despite pulmonary function being the primary predictor of mortality,^{3–5} nutrition status plays a critical role in disease progression and survival.^{3,5,6} The risk of nutrition deficiencies in CF is likely attributable to several factors (eg, pancreatic insufficiency leading to fat, protein, energy, and micronutrient malabsorption; bile salt disturbance; chronic inflammation; increased energy needs due to impaired lung function; chronic bronchopulmonary microbial colonization and recurrent lung infection⁷; and decreased nutrient intake, especially during periods of acute illness).³ Thus, nutrition support and counseling have long been an integral part of the multidisciplinary care of patients with CF.⁸ Nutrition management approaches have included anthropometric assessment (typically measures of body mass index [BMI] and body weight over time), measures of selected nutrients (eg, vitamin D, zinc, and retinol), oral caloric and protein supplementation, pancreatic enzyme replacement therapy, appetite stimulants, enteral tube feeding, and micronutrient supplementation.³

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There is extensive documentation regarding the prevalence of fat-soluble vitamin deficiencies in CF and the need for vitamin A, D, E, and K supplementation.⁹⁻¹¹ Other micronutrient deficiencies, however, including mineral and trace element depletion, are not yet well established in individuals with CF, especially during acute exacerbations. One retrospective chart review reported that >20% of adults with CF were deficient in zinc and that subjects with CF with low zinc exhibited worse pulmonary function and a higher prevalence of CF-related diabetes, osteopenia, and osteoporosis.¹² Exacerbations were not found to be associated with lower plasma zinc,¹² but there was no longitudinal follow-up on the same subjects after recovery. In addition, the role of selenium as an antioxidant has been investigated in CF. There are reports of lower total selenium,¹³ selenocysteine, and other selenium compounds¹⁴ in patients with CF compared with healthy controls. This may be due to the factors impairing nutrition status outlined above, increased disposal of selenium, and increased oxidative stress as a result of reactive oxygen species or inadequate absorption of dietary selenium.^{15,16} A review of selenium supplementation in critically ill patients showed that selenium supplementation may reduce mortality.¹⁷ In a CF model, a trial supplementing selenium in combination with vitamin E, D, A, and β -carotene showed that increased plasma levels of selenium were positively correlated with improved lung function measured by forced expiratory volume in 1 second (FEV1), as was β -carotene status.^{17,18} Results from other studies on trace element status in CF and the need to consider specific supplementation are conflicting.¹⁹⁻²¹ One study showed that while serum iron was frequently low in CF, serum levels of calcium, magnesium, copper, and zinc were within the normal range, suggesting that supplementation was not necessary.¹⁹ In contrast, a retrospective study investigating the clinical benefits of zinc supplementation in CF found that CF individuals supplemented with zinc had a decrease in number of infections, an increase in FEV1, and an improvement in energy intake compared with CF controls.²⁰

Although improved nutrition status has been shown to result in a survival advantage in patients with CF,²²⁻²⁴ specific micronutrient status has not been previously investigated in the CF population during times of acute infection and illness, with serial follow-up during the recovery phase. Therefore, the purpose of this study was to compare mineral micronutrient status in adults with CF during and 3 months after hospital admission for an acute pulmonary exacerbation.

Subjects and Methods

Study Population

This was an ancillary study to a randomized, double-blind, multicenter, placebo-controlled trial investigating high-dose vitamin D₃ (250,000 IU) in adults with CF admitted to the hospital with a pulmonary exacerbation (Vitamin D for Enhancing

the Immune System in Cystic Fibrosis [DISC] NCT01426256). Subjects were a subset of the participants in DISC recruited at the Emory University Hospital site who were enrolled between January 2012 and January 2014. Inclusion criteria included patients diagnosed with CF, age ≥ 16 years, admission for a pulmonary exacerbation within the past 72 hours, and ability to tolerate oral medications. Exclusion criteria included serum 25(OH)D >55 ng/mL or <10 ng/mL, dose of more than 2000 IU of vitamin D above intake via multivitamin supplements, past intake of more than 2000 IU vitamin D or its equivalent weekly dose (14,000 IU/wk) for more than 1 week at any time within the past 60 days, pregnancy or plans to become pregnant during the course of the study, history of disorders associated with hypercalcemia including parathyroid disease, hypercalcemia, history of nephrolithiasis within the past 2 years, chronic kidney disease worse than stage 3, use of oral or intravenous glucocorticoid within the month prior to enrollment, history of lung transplantation, hospice care, FEV1% $<20\%$, significant hepatic dysfunction, use of cytotoxic or immunosuppressive drugs, history of AIDS, history of illicit drug abuse, enrolled in a study within 1 month of enrollment, and inability to complete study procedures.

Blood was collected and serum processed at entry (while hospitalized for acute pulmonary exacerbation) and again 3 months later. Subjects were enrolled within 72 hours of admission for baseline and were seen ± 28 days from the 3-month time point.

The study was approved by the Emory Institutional Review Board and overseen by an independent data safety monitoring board. Subjects provided written informed consent to store all blood samples and to collect information on nutrition blood markers.

Micronutrient Analysis

Blood was collected in a fasting state at baseline during an inpatient pulmonary exacerbation and again at 3 months following recovery. Serum was collected in trace element-free tubes (BD Vacutainer, catalog No. 368380), centrifuged at 1300 relative centrifugal force for 15 minutes at 4°C, and stored at -80°C . Serum trace element and mineral concentrations were measured using inductively coupled plasma optical emission spectrometry (ICP-OES).²⁵ One hundred microliter samples of isolated serum were digested by addition of 0.25 mL OmniTrace 70% HNO₃ (EMD Chemicals) and incubated overnight at 60°C with 150 to 200 rpm orbital shaking. The acid lysates were then diluted to 5% HNO₃ with OmniTrace water (EMD Chemicals), clarified by centrifugation (3000g for 10 minutes), and introduced via a pneumatic Seaspray nebulizer using argon carrier gas into a Vista Pro ICP-OES (Varian Inc). The ICP-OES was calibrated using National Institute of Standards and Technology (NIST)–traceable elemental standards and validated using NIST–traceable 1577b bovine liver reference material and Seronorm Trace Elements Serum Level

1 and Level 2 (Sero, Billingstad, Norway). Elements (copper, iron, calcium, potassium, zinc, magnesium, and sulfur) were queried in triplicates, with a detection range between 0.005 and 50 parts per million and coefficient of variation for assay precision typically ranging between 5% and 10%. Cesium (50 ppm) was used for ionization suppression, and yttrium (5 ppm) was used as an internal standard for all samples. All reagents and plasticware were certified or routinely tested for trace metal work. Elemental content data were summarized using native software (ICP Expert; Varian Inc) and normalized to serum volume. Quality controls showed no significant contamination in the microcentrifuge tubes of all micronutrients with the exception of zinc, hence the exclusion of zinc in the micronutrient analysis.

Statistical Analysis

Descriptive statistics were reported for all variables. Continuous data are presented as means \pm SD, and categorical data are presented as percentages. Paired *t* tests were used to compare the differences in serum micronutrient concentrations between baseline and 3 months following recovery. In addition, *t* tests were used to compare clinical differences between groups that increased in copper, iron, and calcium and groups that decreased in copper, iron, and calcium. All tests were 2 sided, with a *P* value of $<.05$ considered significant. A simple linear regression was used to analyze vitamin D and baseline iron interactions. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

Baseline demographics and clinical characteristics for the 24 participants are described in Table 1. The gender of the subjects was balanced between male and female. Most subjects were Caucasian, and their mean BMI was lower than the 2008 CF Foundation Nutrition Guidelines target BMI (≥ 22 kg/m² for females and ≥ 23 kg/m² for males).²⁶ The mean FEV1 (percentage predicted) indicated moderate pulmonary impairment. Ninety-two percent of participants were pancreatic insufficient, and approximately one-third had CF-related diabetes. Approximately half of the cohort was taking multivitamin supplements.

Micronutrient Status

Mean serum concentrations of trace elements (copper and iron) and minerals (calcium, potassium, magnesium, sulfur) at baseline and 3 months are described in Table 2. Participants exhibited a significant increase in copper, iron, and calcium over time. A total of 4.2% of subjects during the hospital admission for pulmonary exacerbation were deficient in copper (defined as <0.8 μ g/mL),³⁰ and 4.2% were deficient in calcium (defined as corrected calcium <8.4 mg/dL).³¹ At 3 months, 4.2% were

Table 1. Baseline Demographic and Clinical Characteristics of Participants at the Time of Enrollment.

Characteristic	Mean \pm SD or n (%)
Age, y	29.6 \pm 7.3
Female, n (%)	12 (50)
BMI, kg/m ²	21.4 \pm 3.9
Caucasian, n (%)	20 (83)
FEV1, % predicted	51 \pm 15
Pancreatic insufficiency, n (%)	22 (92)
Cystic fibrosis-related diabetes, n (%)	7 (29)
F508del/F508del, n (%)	11 (46)
Total number of respiratory infections in past 12 mo	2.3 \pm 1.6
Multivitamin use, n (%)	13 (54)
Calcium supplement, n (%)	8 (33)
Vitamin D supplement, n (%)	4 (17)

deficient in copper and no one was deficient in calcium. There were no statistically significant differences in baseline BMI, baseline 25(OH)D, age, sex, number of respiratory infections in the past 12 months, or change in FEV1 from baseline to 3 months between the subjects who exhibited increased serum concentrations in copper, iron, and calcium and the subjects in whom calcium, copper, and iron decreased.

Discussion

In this study, we present data showing that serum concentrations of the trace elements copper and iron and the mineral calcium are significantly lower in adults with CF at the beginning of treatment for pulmonary exacerbation with IV antibiotics compared with 3 months postexacerbation. Serum levels of the other micronutrients measured (potassium, magnesium, and sulfur) did not significantly differ during an exacerbation and following recovery at 3 months. During the pulmonary exacerbation, subjects exhibited a higher prevalence of calcium deficiency.

Copper is an essential trace metal that functions as a cofactor for various enzymes^{32,33} throughout several systems affecting neurologic, skeletal, and circulatory functions.^{33,34} Studies have found low copper enzyme activities (erythrocyte superoxide dismutase^{35,36} and plasma diamine oxidase³⁵) in patients with CF, suggesting abnormal copper homeostasis and metabolism as a cause of moderate copper deficiency.³⁵ A case-control study in North India showed a higher prevalence of copper deficiency in children with CF compared with controls and, similar to our study, lower levels of copper during periods of exacerbation.³⁷ Copper deficiency may also affect the immune system^{38,39} and play a role in antioxidant actions,⁴⁰ affecting mortality in CF. However, studies show variable results on the need to supplement copper given the widely varying results ranging from increased plasma copper and ceruloplasmin activity,⁴¹ to decreased serum copper levels in CF populations.³⁷ In addition,

Table 2. Serum Concentrations of Trace Elements and Minerals at Baseline and 3 Months.

Micronutrient	Baseline ^a	3 Months ^a	Reference Range ^b	Percentage Increase	P
Copper, µg/mL	1.5 ± 0.6	1.6 ± 0.6	0.32–2.93 ^c	10.3	.027
Iron, µg/mL	0.8 ± 0.3	1.3 ± 1.1	0.07–3.57	73.9	.026
Calcium, mg/dL	9.7 ± 0.8	10.8 ± 2.0	6.9–12.4	11.1	.024
Potassium, mEq/L	4.9 ± 0.3	5.1 ± 0.5	2.5–6.8	2.6	.21
Magnesium, mg/dL	1.8 ± 0.2	2.0 ± 0.3	1.82–2.31 ^d	6.2	.07
Sulfur, µg/mL	1288.6 ± 343.3	1309.9 ± 289.8	N/A	1.7	.83

^aValues reported as mean ± SD.

^bReference ranges are from 2001 to 2002 National Health and Nutrition Examination Data (NHANES²⁷), unless otherwise stated.

^cNHANES data 2011–2012.²⁸

^dNHANES I.²⁹

individuals with CF are not regularly monitored for copper deficiency, and during acute illness, serum copper levels may be artificially elevated by inflammation (due to increases in the major copper-carrying protein ceruloplasmin), making serum copper results less reliable.¹⁵ Thus, further investigation into serum copper and ceruloplasmin levels, concomitant markers of inflammation (eg, C-reactive protein), in addition to copper-related enzyme activity during adult CF pulmonary exacerbation would help both to define the potential need for oral copper and to inform future efficacy studies of oral copper supplementation.

We found that serum iron concentrations were significantly lower at baseline, during a pulmonary exacerbation, compared with 3 months postexacerbation. Decreased concentrations of bioavailable iron during an exacerbation may have implications for anemia in CF. Iron deficiency anemia has been shown to be prevalent in patient populations with advanced pulmonary disease.⁴² In CF, anemia is associated with worse measures of lung function and vitamin deficiency, defined as deficiency in 2 or more of the following vitamins: A, D, or E.⁴³ During acute illness of CF, the acute-phase reactant ferritin may be artificially elevated due to inflammation⁴⁴; however, serum-bioavailable iron may still be low because of increased hepcidin levels, a hormone that prevents iron release from macrophages, thus disrupting normal iron recycling.⁴⁵ Although it is recommended that iron status be monitored yearly in CF,⁴⁶ supplementation during lung exacerbations in CF is still debated, as studies have suggested the role of iron in increasing infection and enabling the growth of bacteria such as *Pseudomonas aeruginosa*.⁴⁷ A recent double-blind, randomized, placebo-controlled trial, however, showed that a low 6-week dose of ferrous sulfate could improve hypoferrremia without significantly effecting sputum iron, the Akron Pulmonary Exacerbation Scores, or the sputum microbiome.⁴⁷ Low serum iron in CF may be due to 2 main causes: iron deficiency or anemia of chronic disease (the second most prevalent cause of anemia after iron deficiency), in which total body iron stores are intact but bioavailable iron is low.⁴⁸ In the case of iron-deficiency anemia, in which an absolute iron deficiency

exists, iron supplementation is recommended.⁴⁸ In anemia of chronic disease, patients with a normal ferritin level should not be given iron supplementation, and the main treatment should focus on treating the underlying disease.⁴⁸ Further investigation into the effects of iron supplementation in anemia of chronic disease in CF is needed to make recommendations on oral iron supplementation.

Calcium is an essential mineral that is necessary for proper mineralization of bone and as a second messenger in several cellular processes. Inadequate calcium economy has been demonstrated to be associated with osteopenia, osteoporosis, and an increased risk of fracture.⁴⁹ Calcium also plays an important developmental role in muscle contractions and proper functioning of the nervous system.⁵⁰ Given the high prevalence of suboptimal calcium intake and its importance for many processes in CF, daily calcium supplementation in addition to vitamin D intake is recommended for CF individuals.⁵¹ Calcium supplementation in the context of acute illness of CF, however, is not well investigated. In colorectal adenoma, it has been shown that calcium supplementation may decrease proinflammatory markers such as C-reactive protein, interleukin-6, interleukin-8, and interleukin-1β.⁵² Further investigation is needed to determine the role of calcium supplementation during acute exacerbations of CF. It is not known if correcting these mild depressions in calcium would translate into improved clinical outcomes; however, depressions in calcium may also reflect poor protein nutrition status, given that calcium is highly protein bound. This analysis reports total serum calcium concentrations, which includes ionized calcium as well as protein-bound calcium. Vitamin D deficiency may also contribute to low serum calcium concentrations, as vitamin D is an important nutrient to enhance the intestinal absorption of calcium. As this was a subanalysis of an ongoing high-dose vitamin D supplementation trial, it is possible that increases in calcium were due to improvements in vitamin D status.

Strengths of this study included the collection of blood during the fasted state and longitudinal follow-up. All subjects had blood drawn within the initial 72 hours of hospitalization for a

pulmonary exacerbation, and the minerals were analyzed using inductively coupled plasma optical emission spectrometry. As this study was conducted ancillary to an ongoing vitamin D trial, a limitation is that we cannot account for vitamin D as a potential confounder influencing micronutrient status. Low micronutrient levels at baseline could be attributed to either low dietary intake or malabsorption due to the exacerbation. Another limitation was the small sample size and the observational nature of the study, which made it difficult to determine cause and effect between micronutrient status and the pulmonary exacerbation.

In conclusion, serum levels of calcium, copper, and iron were significantly decreased during exacerbations of CF compared with 3 months after recovery. Our data suggest the potential need to investigate the effect of oral supplementation of these depleted micronutrients during acute lung exacerbations in CF and to study their role during lung infection and acute illness.

Statement of Authorship

M. J. Lee, J. A. Alvarez, E. M. Smith, J. F. Chmiel, P. M. Joseph, R. E. Grossmann, A. Gaggar, T. R. Ziegler, and Vin Tangpricha contributed to the conception/design of the research; M. J. Lee, J. A. Alvarez, J. F. Chmiel, P. M. Joseph, R. E. Grossmann, T. R. Ziegler, and V. Tangpricha contributed to the analysis and interpretation of the data; A. Gaggar contributed to the acquisition and interpretation of the data; M. J. Lee, J. A. Alvarez, E. M. Smith, and D. W. Killilea contributed to the acquisition, analysis, and interpretation of the data; E. M. Smith contributed to the analysis of the data; M. J. Lee, J. A. Alvarez, and V. Tangpricha drafted the manuscript; M. J. Lee, J. A. Alvarez, E. M. Smith, D. W. Killilea, J. F. Chmiel, P. M. Joseph, R. E. Grossmann, A. Gaggar, T. R. Ziegler, and V. Tangpricha critically revised the manuscript; M. J. Lee, J. A. Alvarez, E. M. Smith, D. W. Killilea, J. F. Chmiel, P. M. Joseph, R. E. Grossmann, A. Gaggar, T. R. Ziegler, and V. Tangpricha agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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