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A pilot study on the safety and efficacy of a novel antioxidant rich formulation in patients with cystic fibrosis

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Abstract

Background: Pancreatic insufficiency and a diminished bile acid pool cause malabsorption of important essential nutrients and other dietary components in cystic fibrosis (CF). Of particular significance is the malabsorption of fat-soluble antioxidants such as carotenoids, tocopherols and coenzyme Q_{10} (CoQ_{10}). Despite supplementation, CF patients are often deficient in these compounds, resulting in increased oxidative stress, which may contribute to adverse health effects. This pilot study was designed to evaluate the safety of a novel micellar formulation (CF-1) of fat-soluble nutrients and antioxidants and to determine its efficacy in improving plasma levels of these compounds and reducing inflammatory markers in induced sputum.

Methods: Ten CF subjects, ages 8 to 45 years old, were given orally 10 ml of the CF-1 formulation daily for 56 days after a 21-day washout period in which subjects stopped supplemental vitamin use except for a standard multivitamin. Plasma obtained at −3, 0 (baseline), 1, 2, 4, and 8 weeks was assayed for β-carotene, γ-tocopherol, retinol, and CoQ_{10} as well as for safety parameters (comprehensive metabolic panel and complete blood count). In addition, pulmonary function was measured and induced sputum was assayed for markers of inflammation and quantitative bacterial counts both prior and during dosing.

Results: No serious adverse effects, laboratory abnormalities or elevated nutrient levels (above normal) were identified as related to CF-1. Supplementation with CF-1 significantly increased β-carotene levels at all dosing time points when compared to screening and baseline. In addition, γ-tocopherol and CoQ_{10} significantly increased from baseline in all subjects. Induced sputum myeloperoxidase significantly decreased and there was a trend toward decreases in PMN elastase and total cell counts with CF-1. There was a significant inverse correlation between the antioxidant levels and induced sputum changes in IL-8 and total neutrophils. Lung function and sputum bacterial counts were unchanged.

Conclusion: The novel CF-1 formulation safely and effectively increased plasma levels of important fat-soluble nutrients and antioxidants. In addition, improvements in antioxidant plasma levels were associated with reductions in airway inflammation in CF patients.

Keywords: Cystic fibrosis; Antioxidant; Malabsorption; Beta-carotene; Inflammatory markers; Coenzyme Q10; Gamma-tocopherol; Induced sputum; Pancretic insufficiency

1. Introduction

Cystic fibrosis (CF) interferes with intestinal digestion and absorption of nutrients and other dietary components. Increased viscosity of pancreatic secretions causes obstruction of pancreatic ducts, leading ultimately to destruction and fibrosis of the exocrine pancreas in 85% of the CF patients

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[1,2]. The resulting failure in secretion of pancreatic digestive enzymes causes steatorrhea and malabsorption of fat-soluble vitamins. Absorption of fat-soluble compounds is further impaired by a diminished bile acid pool due to increased fecal excretion of bile and diminished bile secretion caused by CF-associated liver disease in many CF patients [1,2]. Pancreatic enzyme replacement therapy frequently fails to completely correct malabsorption of lipids because of incomplete intraluminal solubilization and/or reduced mucosal uptake of lipids [3]. This malabsorption often precludes normalization of plasma fat-soluble nutrients and antioxidants such as β-carotene, tocopherols (vitamin E) and coenzyme Q_{10} (CoQ_{10}) with standard nutritional supplements.

This impairment in absorption frequently leads to malnutrition and deficiency of fat-soluble vitamins and antioxidants. Feranchak and colleagues demonstrated that biochemical deficiency of one or more fat-soluble vitamins was present in 45.8% (44 of 96) of patients at diagnosis of CF between ages 4 and 8 weeks [4]. Despite supplementation with ADEK® or other multivitamins, persistent deficiency of either vitamin A (11.1%), vitamin D (12.5%), or vitamin E (57.1%) was common. Furthermore, of those with initial normal vitamin status, deficiency developed subsequently in 4.5% for vitamin A, 14.4% for vitamin D, and 11.8% for vitamin E. Recent studies suggest that other fat-soluble antioxidants such as CoQ_{10}, β-carotene and γ-tocopherol are substantially lower in CF patients than normal controls [5–9].

Chronic inflammation occurs in CF due to chronic respiratory infections and is characterized by increased production of pro-inflammatory cytokines and inflammatory mediators [10]. Chronic inflammation causes increased free radical production and, in combination with low levels of antioxidants, increases oxidative stress in CF patients. Oxidative stress in CF patients is evidenced by major indicators including reduced oxidative resistance of low density lipoprotein and fatty acids, increased production of malondialdehyde and thiobarbituric acid reacting substances and hemolysis [11–14]. Increased oxidative stress has been associated with decreased pulmonary function in CF. Brown and colleagues demonstrated a correlation between oxidative stress as measured by plasma malondialdehyde and pulmonary function in 34 patients with CF [15]. Furthermore, they showed that decreased plasma antioxidant status (as measured by α-tocopherol, ascorbic acid and sulphydryl proteins) is correlated with decreased pulmonary function.

Others have suggested that antioxidant supplementation could potentially improve pulmonary function in CF patients. In a study of 46 CF patients randomized to low or high dose β-carotene supplementation, Wood and colleagues reported that high dose supplementation improved forced vital capacity [16]. Increases in selenium also correlated with improved forced expiratory volume in one second (FEV_1). Finally, in a placebo-controlled clinical trial of 24 CF patients, Renner and colleagues showed that high dose antioxidant supplementation reduced the duration of antibiotic treatment from 14.5 to 9.8 days over 24 weeks [17].

We conducted this pilot study in CF patients to begin to evaluate the safety and efficacy of a highly bioavailable antioxidant-rich formulation supplying β-carotene, γ-tocopherol, and CoQ_{10}. This formulation utilizes micelle-like particles in order to improve solubilization and intestinal absorption and increase plasma levels of these compounds. Plasma levels of these compounds were compared with baseline values on standard multivitamin supplements used in CF patients. Furthermore, the effects of this formulation on measures of airway inflammation were explored by collecting induced sputum prior to and during dosing.

2. Methods

CF subjects ages 8 to 45 years old, weighing more than 30 kg, with a clinical requirement for pancreatic enzymes and a predicted $FEV_1 > 35\%$, were recruited from the University of Colorado CF Center through clinic visits or phone calls. The diagnosis of CF was confirmed with either an elevated sweat chloride (>60 meq/l), or presence of two disease-causing mutations in the *CFTR* gene and one or more clinical features consistent with CF. Screening criteria included that subjects had to have either a documented serum β-carotene below 0.04 μg/ml when measured or have had no prior measurement performed. Subjects were required to have stable pulmonary disease as defined by both clinical impression and no hospitalizations in the 60 days prior to screening. Subjects were excluded if they had significant liver disease as defined by clinical findings of portal hypertension or cirrhosis or AST, ALT, or GGT > 2x the upper limit of normal. Subjects were also excluded if they had diabetes mellitus requiring insulin therapy, significant anemia (hemoglobin <9 gm/dl), were pregnant or lactating, had oral supplementation with β-carotene or CoQ_{10} in the 4 months prior to the study (other than in ADEK®, SourceCF® or VITAMAX®), or had participated in another interventional clinical trial within the 30 days prior to the screening visit.

Subjects that met screening criteria and signed informed consent at the screening visit had a physical examination, fasting blood drawn for vitamins and nutrient levels, complete blood count (CBC), comprehensive metabolic panel (CMP), serum pregnancy test for females of childbearing years and spirometry. Subjects were instructed to discontinue any nutritional supplement except for a non-CF standard multivitamin and were scheduled to return for a dosing visit in 3 weeks.

At the dosing visit an abbreviated physical examination was conducted, blood was obtained again for vitamins and nutrient levels, CBC, and CMP. Spirometry was repeated and sputum was induced and collected for evaluation of inflammatory mediators. Subjects were given a two month supply of the antioxidant formulation and instructed to ingest a dose of 10 ml along with their current dose of pancreatic enzymes and a standard multivitamin with breakfast each day. The composition of CF-1, produced under cGMP at

Crown Laboratories (Johnson City, TN), is detailed in Table 1.

Subsequent visits at 1, 2, 4, and 8 weeks included repeat complete evaluation except for spirometry and induced sputum which were obtained at either week 4 or 8.

The study was approved by the Colorado Multiple Institutional Review Board and the Pediatric Clinical Translational Research Center (CTRC; formerly GCRC) Scientific Advisory Committee. Informed written consent was obtained from the subject and their parent or guardian (if <18 years old). A Data and Safety Monitoring Board (DSMB) was established to monitor the study.

2.1. Sample collection

2.1.1. Plasma

Whole blood was obtained for routine CBC and chemistries in the hospital laboratory. Additional blood was collected into EDTA tubes and centrifuged at 3000 rpm for 5 min. Specimens were protected from light and the plasma was removed and frozen at −70 °C.

2.1.2. Sputum induction and processing

Sputum was induced by having the subject inhale 3% hypertonic saline for six 2-minute sessions, as previously described [18]. At each 2-minute interval, subjects were instructed to expectorate sputum into one of two containers (one for microbiology and another for inflammatory markers). The sputum specimens were then transported on ice to the laboratories for processing within 20 min. To measure bacterial density (sputum bacterial colony counts, cfu/ml), one container was brought to the Microbiology Laboratory where quantitative microbiology was performed according to a consensus conference on CF microbiology [19]. To determine inflammatory markers in sputum, the second sputum aliquot was processed as previously described [18]. Briefly, after weighing the sputum sample, three times the volume of sterile 0.1% dithiothreitol (DTT) (10% Sputolysin; Calbiochem-Novabiochem Corp., San Diego, CA) and four times the volume of phosphate-buffered saline (Dubelcco's; Gibco BRL, Grand Island, NY) were added to the specimen. The sample was then incubated in a shaking water bath at 37 °C for a total of 15 min and gently mixed using a transfer pipette at 5-minute intervals to ensure complete homogenization. Cytologic examination was performed from an aliquot of homogenized sputum to yield sputum total neutrophil counts. The number of squamous cells were subtracted from the total cell counts to provide the absolute white cell counts. Therefore, total sputum neutrophil counts represented a percentage of the absolute white cell count (exclusive of squamous cells) and were expressed as 10^3 cells/ml. Only samples that weigh greater than 0.5 g and have less than 80% squamous cells were considered adequate and included for further analysis. The remaining homogenized sputum samples were vigorously centrifuged and the supernatants were treated with protease inhibitors, phenylmethylsulfonylfluoride (PMSF) and EDTA (each from Sigma Diagnostics, St. Louis, MO), in order to minimize proteolytic activity. The samples were frozen at −70 °C for later analysis of inflammatory markers.

Table 1
CF-1 formulation

Compound	Amount (10 ml)
Beta-carotene	30 mg
Alpha-tocopherol	200 IU
Gamma-tocopherol	94 mg
Other tocopherols	31 mg
Coenzyme Q_{10}	30 mg
Vitamin D_3	400 IU
Vitamin K_1	300 mcg

2.2. Assays

2.2.1. Vitamins and antioxidants

Plasma was assayed for α- and γ-tocopherol, retinol, β-carotene and CoQ_{10} at the University of Colorado Pediatric CTRC Core Laboratory using a validated HPLC method [20]. 25-Hydroxyvitamin D in plasma was measured with the use of an FDA approved RIA kit from DiaSorin (Stillwater, MN).

2.2.2. CBC and chemistries

Both CBC and clinical chemistries were completed in the main clinical laboratory at The Children Hospital. CBC's were conducted with the use of a Beckman Coulter LH750 Hematology Analyzer. Clinical chemistries were conducted with the use a Dade-Behring Dimension RXL Chemistry Analyzer.

2.2.3. Inflammatory markers

Induced sputum inflammatory markers were analyzed at the CTRC Core Laboratory. Myeloperoxidase (MPO) was analyzed with a BIOXYTECH® MPO-EIA™ kit (OXIS International Inc, Foster City, CA). PMN elastase was analyzed with an ALPCO EIA kit (Salem, NH) and IL-8 was analyzed using an R&D Systems EIA kit (Minneapolis, MN).

2.2.4. Spirometry

Pulmonary function tests (PFTs) were performed using the Sensomedics Vmax body box (VIASYS Healthcare Inc., Conshohocken, PA) with KOKO software. Knudsen normal values were used to calculate the percent of predicted for each subject. Spirometry was performed and the forced expiratory curves were evaluated for acceptability in accordance with American Thoracic Society recommendations.

2.3. Statistical analysis

A Sign Test was used to test the differences in the mean antioxidants prior to (Visits 1 and 2, "pre") and during dosing

Table 2
Patient characteristics

	Enrolled ($n=10$)
Age (years)	16.7±4.9
Weight (kg)	50.5±11.0
Height (cm)	157±13.4
BMI (kg/m^2)	20.2±2.38
FEV_1 (% predicted)	86.8±22.3
Creatinine (mg/dl)	0.8±0.2
Total Bilirubin (mg/dl)	0.5±0.3
AST (units/l)	23±12
ALT (units/l)	51±9
Albumin (g/dl)	3.8±0.24
Pancreatic enzyme use	100%

FEV_1, forced expiratory capacity in 1 second; AST, aspartate amino transferase; ALT, amino alanine transferase. Values are mean±standard deviation.

(Visits 3–6, "post"). Differences in inflammatory mediators pre- and post-treatment were assessed using the signed-ranks test. An antioxidant score was calculated by summing the normalized changes of the antioxidants from baseline to the time of final induced sputum (CoQ_{10}, β-carotene, γ-tocopherol). The change in each antioxidant was divided by the value of that antioxidant at the time of the final induced sputum: [CoQ_{10}(sputum)–CoQ_{10}(base)]/CoQ_{10}(sputum)+[β-carotene(sputum)–β-carotene(base)]/β-carotene(sputum)+[γ-tocopherol(sputum)–γ-tocopherol(base)]/γ-tocopherol(sputum). Pearson correlation coefficients were calculated to test for association. Data are presented as median or mean±standard error of the mean unless otherwise stated. A significance level of 0.05 was considered to be statistically significant.

3. Results

Fourteen subjects with CF underwent screening from which 10 subjects met inclusion and exclusion criteria. Data from all 10 subjects were available for analysis of plasma vitamin and antioxidant levels and safety parameters. Seven subjects completed the study with both pre- and post-dosing induced sputum and all vitamin and antioxidant time points. Characteristics of the subjects that were enrolled in the study are presented in Table 2.

3.1. Safety

There were 21 adverse events in 8 subjects, 3 of which were categorized as serious adverse events (SAE) involving 2 subjects with respiratory exacerbations judged to be unrelated to CF-1 (Table 3). One subject with an SAE had not been dosed with CF-1 and the other had been on the supplement for 1 week duration at the time of the adverse event. Two events (nausea/upset stomach) could possibly be related to the formulation. There were no notable changes in the clinical laboratory parameters including comprehensive clinical chemistries and complete blood counts. No vitamin or antioxidant levels measured exceeded the laboratory's reported normal range.

3.2. Plasma vitamins and antioxidants

β-Carotene plasma levels at screening and baseline were near or below the limits of detection (0.03 μg/ml) in all subjects. CF-1 significantly increased plasma β-carotene post-dosing ($p<0.01$) at a supplementation level of less than

Table 3
Adverse events

Subject	Events	Severity	Relationship	Outcome/Comments
2	Nasal congestion Increased cough Lung sounds — crackles RLL Abdomen slightly distended	Mild	Not related	Resolved other than nasal congestion and dose not adjusted
4	Occasional headache (seasonal allergies) Green nasal drainage	Mild	Not related	Resolved and dose not adjusted
5	Increased cough (SAE) Shortness of breath (SAE)	Moderate	Not related	Patient withdrew from the study after one week on therapy
6	Increased cough Diminished lung sounds at bases	Mild	Not related	Resolved and dose not adjusted
7	Pulmonary exacerbation (SAE)	Severe	Not related	Patient withdrew prior to dosing
10	Nauseated Back pain Nasal congestion Increased cough Increased tightness in the chest	Mild	Nausea possibly related, others not related	Nausea resolved and dose not adjusted; increase cough, tightness and nasal congestion not resolved
11	Nasal congestion Increased cough Upset stomach	Mild	Upset stomach possibly related, others not related	Upset stomach resolved and dose not adjusted; increase cough not resolved
14	Croupy cough Clear nasal drainage	Mild	Not related	Resolved and dose not adjusted

SAE = severe adverse event.

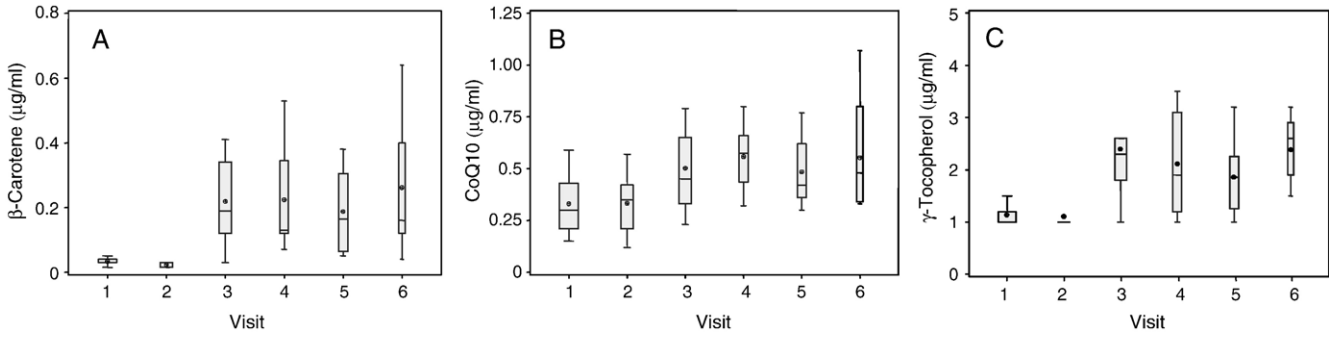

Fig. 1. Box and whisker plots for serum levels of A) β-carotene (μg/ml), B) CoQ_{10} (μg/ml) and C) γ-Tocopherol (μg/ml) at each of the six study visits. Supplementation with CF-1 commenced after visit 2. Whiskers denote minimum and maximum observations, box signifies 25th percentile, median, and 75th percentile with mean represented by a ⊕. Box width varies with number of observations.

0.6 mg/kg (average wt ~50.5 kg) (Fig. 1A). There was no significant change in plasma retinol in these subjects.

CoQ_{10} was below the lower limit of the normal range (<0.29 μg/ml) in 40% (4 of 10) subjects at screening and 33% (3 of 9) of subjects at baseline. Supplementation with CF-1 significantly increased mean CoQ_{10} plasma levels post-dosing when compared to the pre-dosing values ($p<0.01$) (Fig. 1B). CoQ_{10} plasma levels were normalized in all subjects following treatment with CF-1.

γ-Tocopherol was below detectable limits (<1.0 μg/ml) in 60% (6 of 10) of subjects at screening and 78% (7 of 9) of subjects at baseline. Supplementation with CF-1 significantly increased mean γ-tocopherol plasma levels post-dosing when compared to pre-dosing values ($p<0.01$) (Fig. 1C). γ-Tocopherol levels were in the detectable range in all subjects following treatment with CF-1 (no normal range established).

3.3. Induced sputum inflammatory markers

Measures of inflammatory mediators in induced sputum were available from 7 subjects who had induced sputum collected at visit 2 (immediately prior to dosing) and either week 4 or 8 (Fig. 2). Myeloperoxidase decreased significantly ($p=0.04$) post-dosing as compared to baseline. There were no changes in IL-8. PMN elastase values were approximately 35% lower and total and absolute neutrophils were 65% lower at week 4 or 8 when compared to baseline, although statistically non-significant. Baseline plasma CoQ_{10} was inversely correlated with induced sputum total neutrophil counts ($R=-0.68$ $p=0.04$), myeloperoxidase ($R=-0.73$ $p=0.02$) and IL-8 ($R=-0.68$ $p=0.04$) (Fig. 3). The greatest impact on sputum inflammatory mediators was seen when evaluating the effect of the "antioxidant score", a measure of the change in multiple antioxidant plasma levels from pre-and post-dosing (Fig. 4). There was a significant inverse correlation in the antioxidant score and changes in IL-8 ($R=-0.82$, $p=0.03$) and total neutrophils ($R=-0.80$, $p=0.03$). There was a trend toward a correlation between the antioxidant score and the changes in myeloperoxidase ($R=-0.66$, $p=0.11$).

All of the subjects had bacteria chronically cultured from their sputum including 7 with Pseudomonas aeruginosa, 5 with Staphylococcus aureus, and 2 with Hemophilus influenza. Antibiotic use included: azithromycin (6 subjects), inhaled tobramycin (2 subjects), doxycycline (1 subject), ciprofloxacin (1 subject), itraconazole (1 subject) and trimethoprim/sulfamethoxazole (1 subject). There was no significant change in sputum bacterial colony counts or pulmonary function tests throughout the study.

4. Discussion

This pilot study demonstrated that this novel antioxidant preparation, CF-1, effectively increased selected serum

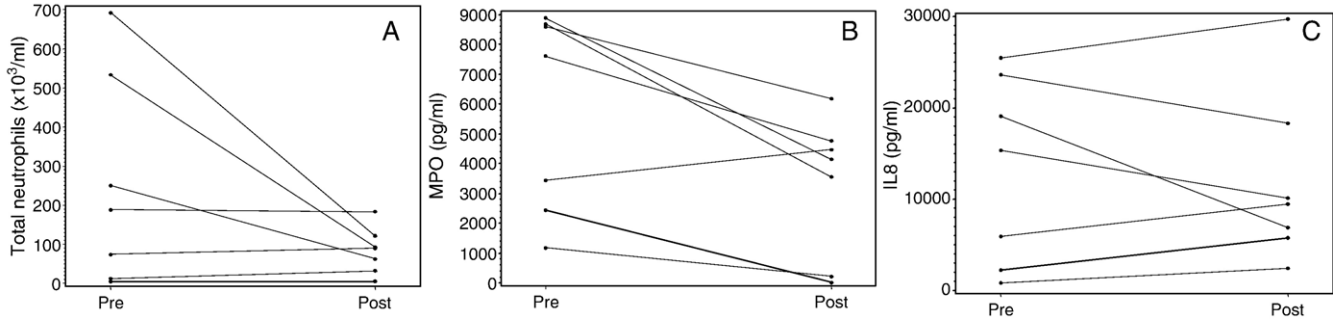

Fig. 2. Measures of inflammatory mediators in induced sputum collected at Pre-dosing visit of CF-1 antioxidant solution (visit 2) and Post-Dosing (week 4 or 8). Panel A) Total Neutrophils (10^3/ml), $p=0.04$, B) Myeloperoxidase (pg/ml) $p=$NS and C) IL-8 (pg/ml), $p=$NS.

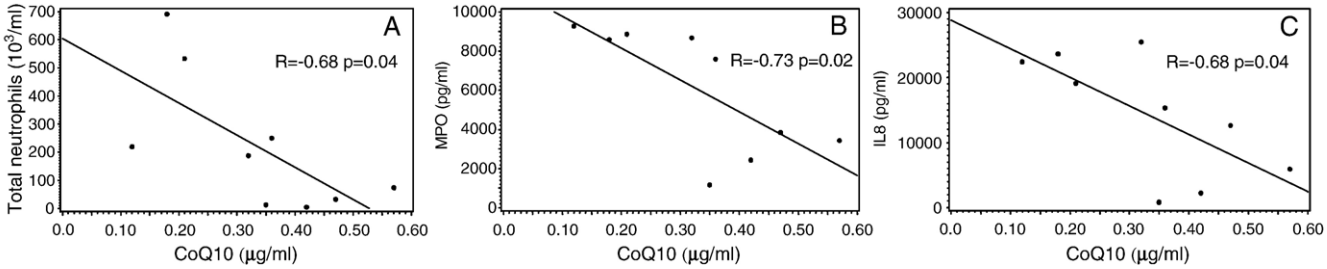

Fig. 3. Inflammatory mediators at baseline are inversely correlated with CoQ_{10}. (µg/ml), Panel A) Total neutrophils (10^3/ml) $R=-0.68$, $p=0.04$; Panel B) MPO (pg/ml) $R=-0.73$, $p=0.02$, and Panel C) IL-8 (pg/ml), $R=-0.68$, $p=0.04$.

antioxidant levels of CF subjects. CF-1 improved plasma levels of β-carotene, γ-tocopherol, and CoQ_{10} in pancreatic insufficient cystic fibrosis subjects. All 7 subjects who completed the study had plasma levels of these antioxidants within the normal range at the end of the study. Most subjects normalized plasma levels within the first week of use of this formulation. Other than poor taste (the formulation was not optimized for taste), few adverse events and no serious adverse events appeared related to CF-1.

The technology in CF-1 enhances absorption and enables normalization of blood levels without having to use excessively high doses. CF-1 significantly increased plasma β-carotene at all time points despite the large degree of individual variability in β-carotene absorption among both healthy individuals and those with malabsorptive problems [13,16,17,21–23]. Studies of β-carotene supplementation in CF patients have shown varying results, with 120 to 240 mg of β-carotene required in one study and 2.1 mg/kg needed in another to produce modest increases in serum levels. Doses of 0.5–1.0 mg/kg with certain β-carotene/starch water-dispersible formulations were necessary to achieve increased serum levels [13,17,22,23]. Finally, Wood et al. found little to no increase in plasma levels with supplementation [16]. It is important to note that although β-carotene levels were normalized in this study, there was no significant increase in vitamin A.

Current recommended supplementation of vitamin E in CF patients consists only of α-tocopherol. While supplementation with high levels of α-tocopherol alone increases blood levels [24,25] this strategy may result in further imbalances in malabsorbing CF patients because such supplementation has been shown to deplete γ-tocopherol in the blood and tissues. In this study we evaluated vitamin E as mixed tocopherols. The increase in the blood levels of γ-tocopherol may be particularly important for CF patients due to its function as a scavenger of reactive nitrogen species and its synergistic effects with α-tocopherol [26]. Cystic fibrosis is characterized by a neutrophil dominated airway inflammation. Activated neutrophils release oxidants, proteases, and cytokines, further sustaining and increasing the inflammatory response and causing direct injury to the lungs. Improved antioxidant capacity with γ-tocopherol, especially if present in the lungs, could potentially decrease oxidant-mediated damage and limit the cytokine-mediated neutrophil recruitment.

Non-antioxidant mechanisms may also mediate the physiologic actions of antioxidants, thus explaining why some studies have not shown a decrease in oxidative stress parameters while showing positive correlations between micronutrients and pulmonary function. There is growing understanding of the non-antioxidant properties of the tocopherols in the inhibition of PKC and NFkB and the reduction of C-reactive protein levels, 5-lipoxygenase, tyrosine-kinase as well as cyclooxygenase-2 [27]. β-Carotene enhances cell-mediated and humoral immune response through various mechanisms. Although it is not known

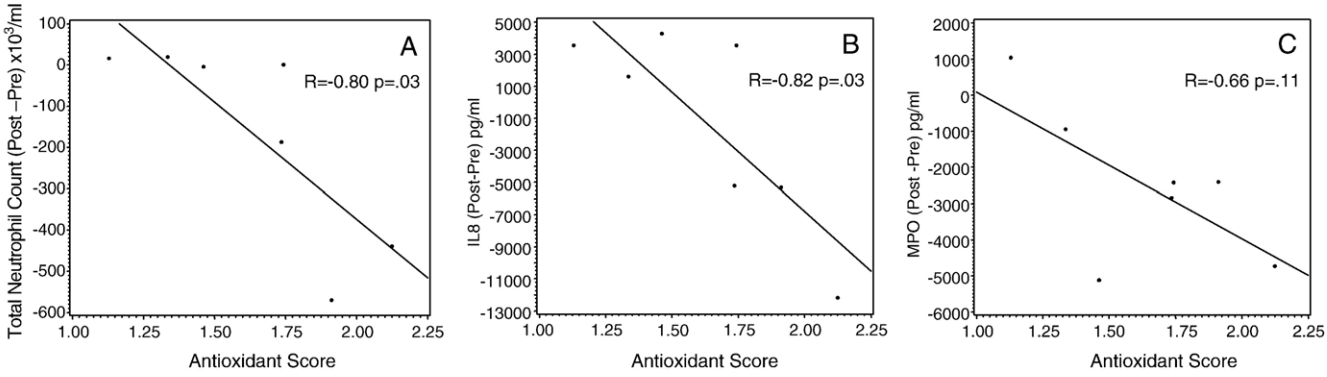

Fig. 4. Antioxidant score $[(_{post}CoQ_{10}-_{pre}CoQ_{10})/_{pre}CoQ_{10}+(_{post}\beta\text{-carotene}-_{pre}\beta\text{-carotene})/_{pre}\beta\text{-carotene}+(_{post}\gamma\text{-tocopherol}-_{pre}\gamma\text{-tocopherol})/_{pre}\gamma\text{-tocopherol}]$ is inversely correlated with change in inflammatory mediators after treatment with CF-1. Panel A) Total neutrophils (10^3/ml) $R=-0.80$, $p=0.03$; Panel B) IL-8 (pg/ml) $R=-0.82$, $p=0.03$ and Panel C) MPO (pg/ml) $R=-0.66$, $p=0.11$.

whether CoQ_{10} reduces inflammation in CF, it merits evaluation because of its antioxidant potential and the low blood levels observed in CF patients.

Somewhat surprisingly for a pilot study of this size, induced sputum inflammatory markers were inversely correlated with antioxidant status. The decrease in induced sputum myeloperoxidase and the observed decreases in PMN elastase and total cell counts after starting CF-1 suggest a beneficial effect on lung inflammation and support the need for further evaluation in a larger controlled clinical trial. These inflammatory markers have been shown to correlate with FEV_1 in clinically stable CF children and may serve as surrogate measures of lung inflammation and ultimate pulmonary decline [28,29].

In conclusion, this pilot study shows that CF-1 can significantly increase and normalize plasma levels of fat-soluble antioxidants including β-carotene, γ-tocopherol, and CoQ_{10} in malabsorbing CF patients. This study also suggests a possible antioxidant-mediated improvement in selected sputum inflammatory markers. Further research is needed to explore the effect of fat-soluble antioxidant supplementation in decreasing lung inflammation and potentially leading to improved clinically relevant health outcomes in CF patients.

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Conflict of Interest Statement:

Konstantinos Papas: I serve as the Medical Director of Yasoo Health Inc. (YASOO) and, as such, I am salaried and own stock in the company. YASOO manufactured the prototype antioxidant formulation that was investigated in this clinical trial. YASOO developed this formulation with the support of a Small Business Innovation Research grant from the National Institutes of Health This formulation is now is being developed into an antioxidant rich complete dietary supplement for cystic fibrosis patients with the support of a Cystic Fibrosis Therapeutic Development grant. I assisted with the design of the trial especially in all aspects related to the safety and handling of the formulation and dosing of the patients, interpretation of the results and the preparation of the manuscript in collaboration with the investigators at the University of Colorado Health Sciences Center and the Children's Hospital.

The conduct of the clinical trial, including design, patient screening and selection, implementation, monitoring, sample collection, sample analysis, database creation and statistical analysis, was under the full control of the investigators at the University of Colorado Health Sciences Center and the Children's Hospital. This research center has a long and distinguished record in cystic fibrosis research. Statistical analysis of the data was completed by Dr. Marci Sontag, Instructor of Preventive Medicine and Biometrics. Clinical coordination and data management was completed by Ms. Churee Pardee, Advanced Practice Nurse. All analyses of blood and induced sputum were conducted at the Children's Hospital.

Ronald Sokol: Dr. Sokol serves on the Scientific Advisory Board of YASOO and has received stock options in this capacity.

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