

EFFECTS OF AN ANTIOXIDANT-ENRICHED MULTIVITAMIN IN CYSTIC FIBROSIS: RANDOMIZED, CONTROLLED, MULTICENTER TRIAL

Scott D. Sagel¹, MD, PhD, Umer Khan², MS, Raksha Jain³, MD, Gavin Graff⁴, MD, Cori L. Daines⁵, MD, Jordan M. Dunitz⁶, MD, Drucy Borowitz⁷, MD, David M. Orenstein⁸, MD, Ibrahim Abdulhamid⁹, MD, Julie Noe¹⁰, MD, John P. Clancy¹¹, MD, Bonnie Slovis¹², MD, Michael J. Rock¹³, MD, Karen S. McCoy¹⁴, MD, Steven Strausbaugh¹⁵, MD, Floyd R. Livingston¹⁶, MD, Konstantinos A. Papas¹⁷, MD, MPH, Michele L. Shaffer¹⁸, PhD

1. Department of Pediatrics, Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, CO
2. Cystic Fibrosis Foundation Therapeutics Development Network Coordinating Center, Seattle Children's Research Institute, Seattle, WA
3. Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX
4. Department of Pediatrics, Penn State Milton S. Hershey Medical Center, Hershey, PA
5. Department of Pediatrics, University of Arizona, Tucson, AZ
6. Department of Medicine, University of Minnesota, Minneapolis, MN
7. Department of Pediatrics, Jacobs School of Medicine, University at Buffalo, Buffalo, NY
8. Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA
9. Department of Pediatrics, Children's Hospital of Michigan, Detroit, MI
10. Department of Pediatrics, Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, WI
11. Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH
12. Department of Medicine, Vanderbilt University Medical Center, Nashville, TN
13. Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI
14. Department of Pediatrics, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH
15. Department of Medicine, Rainbow Babies and Children's Hospital, Cleveland, OH
16. Department of Pediatrics, Nemours Children's Hospital, Orlando, FL
17. Callion Pharma, Jonesborough, TN
18. Department of Statistics, University of Washington, Seattle, WA

Corresponding author:

Scott Sagel, MD, PhD

Professor of Pediatrics

Children's Hospital Colorado, 13123 E. 16th Avenue, B-395, Aurora, CO 80045

scott.sagel@childrenscolorado.org

Phone: 720-777-2522

Fax : 720-777-7284

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At a Glance Commentary:

Scientific knowledge on the subject: Cystic fibrosis (CF) lung disease is characterized by oxidant/antioxidant imbalance and oxidative stress. Optimizing antioxidant status in CF is an important clinical goal and may positively influence health outcomes.

What this study adds to the field: Supplementation with an oral antioxidant-enriched multivitamin over 4 months, was safe and well tolerated, and led to increased systemic antioxidant concentrations and a transient decrease in systemic inflammation. Although the primary endpoint (change in sputum myeloperoxidase concentration) was not achieved, a clinically meaningful increased time to first pulmonary exacerbation was observed in the antioxidant-treated group.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

ABSTRACT

Rationale: Cystic fibrosis (CF) is characterized by dietary antioxidant deficiencies, which may contribute to an oxidant-antioxidant imbalance and oxidative stress.

Objectives: Evaluate the effects of an oral antioxidant-enriched multivitamin supplement on antioxidant concentrations, markers of inflammation and oxidative stress, and clinical outcomes.

Methods: In this investigator-initiated, multicenter, randomized, double-blind, controlled trial, 73 pancreatic insufficient CF subjects 10 years of age and older with an FEV₁ between 40-100% predicted were randomized to 16 weeks of an antioxidant-enriched multivitamin or control multivitamin without antioxidant enrichment. Endpoints included systemic antioxidant concentrations, markers of inflammation and oxidative stress, clinical outcomes (pulmonary exacerbations, anthropometric measures, pulmonary function), safety and tolerability.

Measurements and Main Results: Change in sputum myeloperoxidase concentration over 16 weeks, the primary efficacy endpoint, was not significantly different between the treated and control groups. Systemic antioxidant concentrations (β -carotene, CoQ10, γ -tocopherol, lutein) significantly increased in the antioxidant treated group ($p < 0.001$ for each), while circulating calprotectin and myeloperoxidase decreased in the treated group compared to the control group at week 4. The treated group had a lower risk of first pulmonary exacerbation requiring antibiotics than the control group (adjusted hazard ratio=0.50, $p=0.04$). Lung function and growth endpoints did not differ between groups. Adverse events and tolerability were similar between groups.

Conclusions: Antioxidant supplementation was safe and well tolerated, resulting in increased systemic antioxidant concentrations and modest reductions in systemic inflammation after 4 weeks. Antioxidant treatment was also associated with a lower risk of first pulmonary exacerbation.

Clinical trial registration available at www.clinicaltrials.gov, ID NCT01859390.

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INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by chronic bacterial infection and neutrophil dominated inflammation that leads to the release of vast amounts of proteases and reactive oxygen species (ROS) into the airways¹. Normally, ROS and other oxidants are neutralized by the body's antioxidant defenses. In CF, however, exocrine pancreatic insufficiency and diminished bile acids cause malabsorption of important dietary antioxidants including carotenoids such as beta(β)-carotene, tocopherols (vitamin E), coenzyme Q10 (CoQ10), and selenium. Despite treatment with pancreatic enzymes and supplementation with CF-specific multivitamins, dietary antioxidant deficiencies have been repeatedly demonstrated in individuals with CF, particularly in those with pancreatic insufficiency²⁻⁷. Increased ROS in combination with deficient antioxidant concentrations result in an oxidant-antioxidant imbalance and oxidative stress in CF^{8;9}.

Optimizing antioxidant status in CF is an important clinical goal and may positively influence health outcomes. The results of several trials of oral antioxidant formulations in CF have been previously reviewed^{10;11}. In these studies, circulating antioxidant concentrations were consistently low prior to supplementation and improved with oral treatment. The evidence for clinical effectiveness, however, was mixed and not compelling across studies. A recent Cochrane Review which examined the evidence for dietary antioxidant micronutrients (vitamins E and C, β -carotene, selenium) as treatment for CF lung disease, concluded there has not been a single, well-designed randomized controlled trial in this area¹¹.

A panel of vitamin and antioxidant experts at a CF Foundation sponsored antioxidant workshop recommended conducting carefully controlled trials, using antioxidant "cocktails", and including measurements of biomarkers of inflammation and oxidative stress in such interventional studies¹⁰. In two previous pilot studies, an oral formulation containing several vitamins and antioxidant micronutrients, specifically designed for individuals with malabsorptive disorders including those with CF, safely increased systemic antioxidant levels^{12;13}. While these

were not controlled clinical trials, modest improvements in weight and pulmonary function and small yet significant reductions in airway inflammation were observed over 8-12 weeks, supporting further clinical study of this antioxidant-enriched multivitamin supplement. Therefore, this investigator-initiated, multicenter, randomized, controlled (Phase II) study was conducted to further evaluate the effects of an oral antioxidant-enriched multivitamin supplement on antioxidant concentrations, markers of inflammation and oxidative stress, and clinical outcomes in CF. Some of the results of this study have been previously reported in abstract form¹⁴.

METHODS

Study Design and Population

This was a multicenter, randomized, double-blind, controlled trial conducted from September 2013 to October 2015 at 15 accredited CF care centers across the U.S., and coordinated by the CF Foundation Therapeutics (CFFT) Development Network Coordinating Center (ClinicalTrials.gov identifier: NCT01859390). Institutional review boards at each participating center approved the study and each subject and/or his/her parent provided written informed consent (assent when applicable). Pancreatic insufficient CF subjects (documented by having a spot fecal elastase ≤ 100 $\mu\text{g/g}$ in a stool sample done either historically or at the screening visit) 10 years of age and older with an FEV₁ between 40-100% predicted were eligible to participate. Exclusion criteria were: unwilling or unable to discontinue current oral vitamin and antioxidant supplementation, use of dietary supplements with antioxidants, treatment with ivacaftor/oral corticosteroids/anticoagulant medications, liver enzymes >3 times the upper limits of normal, use of antibiotics for respiratory symptoms within 2 weeks of randomization, recent initiation of new chronic therapy, active treatment for allergic bronchopulmonary aspergillosis or nontuberculous mycobacteria, severe malnutrition (BMI <5 th percentile for subjects <18 years; BMI <18 kg/m^2 for subjects ≥ 18 years), poorly controlled CF-related diabetes (HgbA1c $\geq 7.5\%$), current tobacco smokers, and enrollment in another interventional trial.

The trial entailed four study visits (see Figure E1 in the online supplement). Following the screening visit, participants were instructed to discontinue their current vitamin supplementation and take a control multivitamin without antioxidant enrichment (Table E1), two softgel capsules once daily for 4-8 weeks. At Visit 2 (baseline visit), subjects were randomized 1:1 to receive either the antioxidant-enriched multivitamin ('treated' group) or continue on the control multivitamin ('control' group), two softgels once daily for 16 weeks (identical in appearance and taste to ensure blinding). An adaptive randomization algorithm was employed based on stratification factors for: age (10-17 years, ≥ 18 years), FEV₁ % predicted (40-70%, >70-100%), chronic use of inhaled antibiotics, and chronic use of azithromycin. A fasting blood draw for antioxidant and vitamin concentrations and markers of inflammation and oxidative stress, anthropometric measures, and pulmonary function were obtained at baseline, 4 and 16 weeks. Subjects were instructed to hold their vitamin formulations and fast for at least 8 hours prior to these study visits and blood draws, so that the most recent dose of the vitamin formulations was taken the prior day and >12 hours prior to the scheduled blood draw. Induced sputum and urine specimens were collected at baseline and week 16 for markers of inflammation and oxidative stress. Biochemical measurements were performed in the CFFT Center for Biochemical Markers at the University of Colorado (detailed in the online supplement).

Primary and Secondary Outcomes

The primary endpoint was the difference between treatment groups in the change in sputum myeloperoxidase (MPO) from baseline to week 16. Secondary endpoints included change from baseline to weeks 4 and 16 in systemic antioxidant concentrations, systemic and sputum markers of inflammation and oxidative stress, vitamin levels, change from baseline in FEV₁ % predicted, weight, body mass index (BMI), time to first protocol-defined pulmonary exacerbation, and number of exacerbations. Safety and tolerability were monitored throughout the study.

Statistical Analyses

The study was designed to enroll 80 participants in order to detect a \log_{10} difference between groups of 0.60 ng/mL (SD=0.87) in sputum MPO with 80% power based on a two-sided $\alpha=0.05$, assuming an attrition rate of 15%¹². All analyses were based on a modified Intent-to-Treat (m-ITT) population defined as all randomized participants who received at least one dose of study drug. The primary endpoint was assessed using linear regression to model 16 week change in \log_{10} sputum MPO, adjusting for treatment group and randomization stratification factors. Secondary endpoint models for biochemical markers were adjusted similarly. Cox proportional hazards models were fit to estimate hazard ratios for time to first protocol-defined pulmonary exacerbation and graphically displayed using Kaplan-Meier estimates. Two sample t-tests were used to compare all continuous variables by study group, while differences in proportions were tested using the Fisher's Exact test with confidence intervals (CI) estimated using the Newcombe-Wilson method without continuity correction. Poisson regression with an offset for follow up time was used to calculate rate ratios, CIs and corresponding p-values. No adjustments for multiple comparisons were made and all testing was performed using a two-sided, 0.05 significance level; all analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) or R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Subjects

Of the 120 screened participants, 74 met the eligibility criteria and 73 were randomized, 36 to the treated group and 37 to the control group (Figure 1). Reasons that the goal of randomizing 80 subjects was not achieved are provided in the online supplement. All randomized subjects received at least one dose of study drug constituting the m-ITT population. Baseline

demographics and clinical characteristics for the randomized subjects are shown in Table 1. The two groups were well matched. Two participants from each group withdrew from the study. The two control subjects and one treated subject withdrew per subject decision, while one treated subject withdrew due to flatulence/abdominal pain. The per-protocol population excluded 19 patients: 2 had a major protocol violation, 16 had study drug compliance <80%, 8 had sputum MPO missing at baseline or week 16, and 6 had more than one of these criteria.

Compliance and Study Drug Discontinuation

Mean study drug compliance for the treated and control groups were 86% and 87% respectively ($p=0.82$; Table E2). Eighty-one percent of the treated group had compliance $\geq 80\%$ as compared to 76% of the control group. Two subjects (5.6%) in the treated group permanently discontinued study drug as compared to 4 (10.8%) in the control group.

Primary Endpoint

The change in sputum MPO over the 16 week treatment period was not significantly different between groups. The adjusted mean difference between groups was $-0.17 \log_{10}(\text{ng/mL})$ (95% CI= $-0.51, 0.17$; $p\text{-value}=0.32$) (Figure 2).

Clinical Efficacy Endpoints

Pulmonary exacerbations: The time to first pulmonary exacerbation and rate of pulmonary exacerbations were more favorable in the antioxidant treated group. The antioxidant treated group had a significantly lower risk of first pulmonary exacerbation requiring antibiotics than the control group (covariate-adjusted hazard ratio = 0.50, 95% CI = 0.25, 0.98, $p\text{-value} = 0.04$) (Figure 3). To account for the possibility that a higher number of older subjects (> 30 years) in the control group might be contributing to the difference in time to first exacerbation between the two groups, we included this age category in our adjusted model and found similar results

(adjusted hazard ratio = 0.47, 95% CI = 0.24, 0.91, p-value = 0.03). Nineteen out of 36 antioxidant treated subjects (53%) experienced 28 protocol defined exacerbations as compared to 25 out of 37 control subjects (68%) who experienced 39 exacerbations (difference = -15%; 95% CI = -35%, 7%; p-value = 0.24; rate ratio 0.72, p=0.17). While the hospitalization event rate was lower in the treated group, it was not significantly different compared to the control group (0.60 hospitalizations/participant-year in the treated group versus 1.08 in the control group; rate ratio = 0.54: 95% CI = 0.20, 1.31: p-value = 0.17).

Lung function: At week 4, the absolute change in mean FEV₁ % predicted in the treated group was -0.31% and -2.60% in the control group (difference = 2.29%; 95% CI = -0.66, 5.25; p-value=0.12). The 16 week absolute change in mean FEV₁ % predicted in the treated group was -0.76% compared to -2.20% in the control group (difference = 1.43%; 95% CI = -2.30, 5.16; p-value=0.44) (Figure 4). Similarly, no significant differences between groups were observed for absolute or relative changes of FVC and FEF₂₅₋₇₅%.

Weight: No significant differences between groups were observed for 16 week change in weight or BMI. The 16 week difference in unadjusted weight z-score was 0.07 (95% CI = -0.10, 0.25; p-value = 0.41) (Figure E2).

Circulating Antioxidant and Vitamin Concentrations

At baseline, circulating antioxidant and vitamin concentrations were similar between the two groups. At week 16, the following antioxidants were significantly higher in the antioxidant treated group compared to the control group (adjusted mean difference; p<0.001 for each): β-carotene (0.04 μg/mL), CoQ10 (0.11 log₁₀(μg/mL)), γ-tocopherol (0.38 log₁₀(μg/mL)) and lutein (0.37 log₁₀(μg/mL)) (Figure 5). Pre-post changes in lycopene and glutathione peroxidase activity did not differ between groups. During the treatment period, the circulating vitamin levels (retinol, 25-hydroxy vitamin D, α-tocopherol, PIVKA-II) were not different between groups (Table E3).

Biomarkers of Inflammation and Oxidative Stress

Several markers of inflammation and oxidative stress were assessed in blood, sputum and urine specimens. None of these measures were different between groups at baseline (Tables E4 and E5). At week 4, there were significant reductions in circulating calprotectin (mean difference -0.13 log₁₀(μg/mL), p=0.03) and MPO (mean difference -0.13 log₁₀(ng/mL), p=0.04) in the antioxidant treated group relative to the control group (Table E4). At week 16, however, changes in systemic (Table E4) and sputum (Table E5) biomarkers were not significantly different between the two groups.

Adverse Event Profile

The incidence of adverse and serious adverse events was similar between the two groups (Table E6). All serious adverse events were deemed unrelated to study treatment.

DISCUSSION

In this multicenter, randomized, controlled trial in subjects with CF, antioxidant supplementation was safe and well tolerated. While changes in lung function and weight did not differ significantly between groups, the antioxidant treated group had a significantly lower risk of first pulmonary exacerbation requiring antibiotics. Adherence to study medication was high (approaching 90%) and circulating antioxidant concentrations (β-carotene, CoQ10, γ-tocopherol, lutein) increased in the treated subjects, demonstrating absorption of this antioxidant-enriched multivitamin supplement. Significant reductions in circulating calprotectin and MPO were observed in the antioxidant treated group relative to the control group at week 4, though no significant reductions in sputum MPO (primary outcome measure) or any of the secondary systemic and sputum markers of inflammation and oxidative stress were found at week 16.

This specific formulation, which utilizes micelle-like particles to enhance absorption of fat-soluble nutrients¹⁵, is a “cocktail” containing several vitamins and micronutrients with

antioxidant properties including carotenoids (particularly β -carotene), CoQ10, mixed tocopherols (particularly γ -tocopherol), and selenium. Numerous studies have shown that children and adults with CF are deficient in vitamin E^{4;16}, β -carotene and other carotenoids^{2;3;17;18}, CoQ10^{6;19}, and selenium^{7;20}. The improvements in systemic antioxidant levels we found in this trial were analogous to previous pilot studies^{12;13}. We can only conclude that our findings are due to using the exact formulation investigated in this trial and cannot make presumptions about other combinations and formulations of antioxidants and vitamins. Improving antioxidant status alone may be sufficient justification to add antioxidants to commercially available vitamin or nutritional supplements. However, a key question we were trying to determine in this trial was whether improving antioxidant status in turn leads to reduction in inflammation and oxidative stress and positively affects health outcomes in CF.

There is strong rationale to normalize antioxidant concentrations in CF. Epidemiologic surveys among the general population have found that higher dietary antioxidant vitamin consumption (including β -carotene and vitamin E) and higher circulating antioxidant levels are associated with better pulmonary function and lower rates of lung function decline²¹⁻²⁴. Similarly, in CF, correlations between antioxidant concentrations and lung function have been reported^{20;25}. Low circulating concentrations of vitamins A and E have also been associated with an increased number of pulmonary exacerbations in CF²⁶. Circulating antioxidant levels have been reported to decrease during acute exacerbations and increase after antibiotic treatment²⁷.

The effect of this antioxidant formulation on time to first pulmonary exacerbation was significant and clinically meaningful. Consistent with our findings, previous studies have reported that β -carotene supplementation in CF normalizes β -carotene concentrations, leading to a reduced need for antibiotic therapy^{18;28}. The 50% reduced risk of time to first exacerbation observed in our trial compares to that observed in previous trials of recombinant human deoxyribonuclease (rhDNase)²⁹, azithromycin³⁰, and lumacaftor–ivacaftor³¹. While the time to first pulmonary exacerbation is a more feasible endpoint compared with frequency (total

number) of exacerbations, these two exacerbation endpoints are linked³². Shorter time intervals between exacerbations are associated with more frequent exacerbations and both are risk factors for lung function decline³². Additionally, when designing a study to detect a difference in pulmonary exacerbation frequency or treatment, it is important to account for the varying risk of exacerbations across the CF population as lower lung function, female gender, prior exacerbation history, and *Pseudomonas aeruginosa* infection are associated with increased pulmonary exacerbations requiring treatment³³.

While subjects in the control arm experienced an acute decline in lung function at 28 days relative to participants in the treatment arm, there were not obvious differences between the groups at baseline. Per study inclusion criteria, participants had to be clinically stable with no significant changes in health status within 2 weeks prior to randomization. The groups were very well matched in terms of clinical characteristics at the time of randomization, including the comparable use of chronic inhaled antibiotics and azithromycin therapy. These data do not indicate that the control group were at more risk for experiencing decline in lung function and more frequent pulmonary exacerbations. One potential explanation for the decrease in FEV₁ observed in the control group was that the four-week run-in/wash-out period was an insufficient length of time to allow for β -carotene and other antioxidants to be depleted from the body of control subjects. Most of the commercially available CF multivitamins that study subjects were taking at the time of enrollment had added antioxidants and control subjects may not have reached their antioxidant nadir at the end of the run-in period but rather during the first 4 weeks of the active treatment period. We also cannot exclude the possibilities that the respiratory health of control participants was more optimally controlled relative to the antioxidant treatment subjects at the time of study entry or that control subjects were more adherent to treatments leading to short term improvements in lung function during the screening and run-in period. These reasons might explain an acute decline in lung function observed in the control group as they regressed to their mean values. Furthermore, since pulmonary exacerbations are

associated with lung function decline^{32;34}, this could also account for the difference in time to first exacerbation between the groups.

It is ~~also~~ important to recognize the effect of this antioxidant supplementation as an “add on” therapy in intensely treated CF participants, approximately half of whom were prescribed inhaled antipseudomonal antibiotics and chronic azithromycin therapy. This trial was performed prior to the approval of lumacaftor–ivacaftor and treatment with these CFTR modulator therapies was an exclusion criterion for participation in this study. It is unknown whether the benefits of antioxidant supplementation would be observed on top of CFTR modulator treatment or whether modulator therapies might actually enhance the efficacy of antioxidants. But in contrast to these mutation specific therapies, antioxidant therapy holds the promise of benefiting all CF patients.

Despite a significant increase in circulating antioxidant concentrations, this dietary antioxidant supplement did not appear to exert sustained anti-inflammatory or antioxidative effects, as only modest reductions in circulating calprotectin and MPO occurred at week 4. One consideration is that higher antioxidant concentrations are needed to effect a change in inflammation. The transient reductions in circulating inflammatory markers also raises the question as to whether treatment efficacy of the antioxidant-enriched multivitamin decreases over time. The lack of biologic activity in sputum may also be due in part to the relatively short study duration versus the intrinsic variability, particularly between-subject variance, of sputum biomarkers³⁵. It is possible that other standard of care CF treatments hamper an ability to detect an anti-inflammatory signal. For instance, rhDNase, chronic cycled inhaled antibiotics and azithromycin therapy have known effects on inflammatory markers³⁶⁻³⁸. Similarly, oral and intravenous antibiotics are often prescribed and these may affect biomarker measurements and treatment effects in CF clinical trials³⁹. If these antioxidants are in fact working through an anti-inflammatory mechanism, it does call into question the value of biomarkers of inflammation and oxidative stress measured in this study. Another possibility is that the antioxidants are mediating

their effects through an alternative mechanism (e.g. improved muscle function or energy metabolism) or by enhancing immune cell function, which has been observed as β -carotene concentrations increase⁴⁰.

In designing this study, we had considered whether to proceed with a larger longer term study targeting a clinical endpoint. However, recent antioxidant trials of inhaled glutathione and oral N-acetylcysteine in CF, both of six months duration, provided caution in that they did not demonstrably affect inflammation or oxidative stress or unequivocally improve clinical outcomes^{41,42}. Instead, we decided to perform a phase II study and selected sputum MPO as the primary efficacy endpoint, making sample size determinations based on this endpoint. We chose MPO rather than another marker of airway inflammation such as neutrophil elastase because MPO generates reactive oxidant species as part of its function in innate host defense mechanisms, and is considered by many a marker of oxidative stress. However, sputum MPO may not be the most appropriate marker to demonstrate the efficacy of this dietary antioxidant. While we were surprised by the lack of clear anti-inflammatory effects, the antioxidant supplement provided a benefit in an important clinical outcome. Suffice to say, developing safe and effective anti-inflammatory treatments remains a key priority to the CF community⁴³.

Optimizing antioxidant status in CF is an important clinical goal. In this clinical trial, we showed that an antioxidant-enriched multivitamin supplement safely increased circulating antioxidant concentrations and positively influenced a clinically relevant outcome in CF, pulmonary exacerbations. This treatment resulted in a transient decrease in systemic inflammation without having a sustained effect on oxidative, proteolytic, or inflammatory measures.

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FIGURE LEGENDS

Figure 1. CONSORT diagram of the study participants

Figure 2. Mean change in sputum myeloperoxidase (MPO) over 16 weeks by treatment

group. The change in sputum MPO over the 16 week treatment period was not significantly different between groups. The adjusted mean difference between groups was $-0.17 \log_{10}(\text{ng/mL})$ (95% CI = $-0.51, 0.17$; $p\text{-value}=0.32$).

Figure 3. Time to first pulmonary exacerbation by treatment group.

The antioxidant treated group had a significantly lower risk of first pulmonary exacerbation requiring antibiotics than the control group (covariate-adjusted hazard ratio = 0.50 , 95% CI = $0.25, 0.98$, $p\text{-value} = 0.04$).

Figure 4. Mean absolute change in FEV₁ % predicted from baseline by treatment group.

The 16 week absolute change in mean FEV₁ % predicted in the treated group was -0.76% compared to -2.20% in the control group (difference = 1.43% ; 95% CI = $-2.30, 5.16$; $p\text{-value}=0.44$).

Figure 5. Difference in 16 week change in mean circulating antioxidant levels between the

antioxidant and control groups with corresponding 95% confidence intervals. At week 16, β -carotene, CoQ10, γ -tocopherol, and lutein were significantly higher in the antioxidant treated group compared to the control group (adjusted mean difference; $p<0.001$ for each). Pre-post changes in lycopene and glutathione peroxidase activity did not differ between groups.

Table 1. Clinical characteristics of the study participants at randomization

	Antioxidant (N=36)	Control (N=37)
Gender, n (%)		
Female	20 (55.6%)	20 (54.1%)
Age (years), mean (SD)	22.3 (8.9)	22.9 (9.5)
Age distribution, n (%)		
>=10-18 yrs	13 (36.1%)	16 (43.2%)
>=18-30 yrs	17 (47.2%)	11 (29.7%)
>30 yrs	6 (16.7%)	10 (27.0%)
Race, n (%)		
Caucasian	34 (94.4%)	30 (81.1%)
Hispanic	1 (2.8%)	5 (13.5%)
African-American	1 (2.8%)	1 (2.7%)
Unknown/Other	0 (0%)	1 (2.7%)
Genotype, n (%)		
F508del Homozygous	23 (63.9%)	16 (43.2%)
F508del Heterozygous	9 (25.0%)	18 (48.6%)
Other/Unknown [1]	4 (11.1%)	3 (8.1%)
BMI (kg/m ²), mean (SD)	21.9 (4.4)	21.0 (3.2)
FEV ₁ % Predicted [2], mean (SD)	74.9% (15.4%)	76.3% (13.4%)
FEV ₁ % Predicted distribution, n (%)		
<=70%	11 (30.6 %)	10 (27.0 %)
>70-<=90%	18 (50.0 %)	22 (59.5 %)
>90%	7 (19.4 %)	5 (13.5 %)
History of Meconium Ileus, n (%)	4 (11.1 %)	4 (10.8 %)
Chronic Inhaled Antibiotics [3], n (%)	20 (55.6 %)	20 (54.1 %)
Chronic Azithromycin [3], n (%)	16 (44.4 %)	18 (48.6 %)

[1] Other refers to subjects with either two known, non-F508del CF mutations, or one known, non-F508del CF mutation and one unidentified allele which has not been classified as a CF mutation.

[2] FEV₁ % predicted is calculated using the Wang (Female < 16 years, Male < 18 years) or Hankinson equations (Female ≥16 years, Male ≥18 years).

[3] Chronic is defined as initiated ≥8 weeks prior to randomization.