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Antioxidants in cystic fibrosis Conclusions from the CF Antioxidant Workshop, Bethesda, Maryland, November 11-12, 2003☆

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Abstract

Although great strides are being made in the care of individuals with cystic fibrosis (CF), this condition remains the most common fatal hereditary disease in North America. Numerous links exist between progression of CF lung disease and oxidative stress. The defect in CF is the loss of function of the transmembrane conductance regulator (CFTR) protein; recent evidence that CFTR expression and function are modulated by oxidative stress suggests that the loss may result in a poor adaptive response to oxidants. Pancreatic insufficiency in CF also increases susceptibility to deficiencies in lipophilic antioxidants. Finally the airway infection and inflammatory processes in the CF lung are potential sources of oxidants that can affect normal airway physiology and contribute to the mechanisms causing characteristic changes associated with bronchiectasis and loss of lung function. These multiple abnormalities in the oxidant/antioxidant balance raise several possibilities for therapeutic interventions that must be carefully assessed.

Introduction

Cystic fibrosis (CF) is the most common fatal disease caused by a single gene defect in Europe and North America [1,2]. The defective gene was identified in 1989 [3-5] and shown to encode the cystic fibrosis trans-membrane conductance regulator protein (CFTR). CFTR is a cyclic AMP-regulated anion channel with greatest selectivity for chloride, and bicarbonate [6-8]. Furthermore, CFTR regulates sodium transport across epithelial membranes through interactions with the epithelial sodium channel ENaC [9], and facilitates the trans-membrane export of the small organic anionic peptide glutathione [10]. The expression of CFTR is located in the apical membrane of epithelial cells that line mucous membranes and submucosal glands

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[11,12]. The tissues most affected by CFTR deficiency include the vas deferens, exocrine pancreas, liver, large intestine, sinuses, and the lower airways. The loss of water from secretions at the surface of these epithelial tissues is thought to be a key feature leading to mucus impaction and obstruction. Many CF infants develop signs of maldigestion manifested by failure to thrive. Obstruction of ducts in the exocrine pancreas traps proteins such as proteases and lipases in the pancreatic tissue, leading to failure of the exocrine pancreatic function and poor absorption of lipids [13]. Pancreatic insufficiency is present in 85% of patients with CF.

Oxidative stress in the lung

Oxidative stress may play a significant role in the pathophysiology of CF. The lung, the main organ responsible for morbidity and mortality in this disease, is particularly vulnerable to high levels of oxidative stress. It is exposed to 8000 L of oxygen-rich air per day as well as toxic particles, nitrogen dioxide, ozone, and other oxidants [14,15]. Further, there are large internal sources of oxidants including mitochondrial metabolic processes, peroxisomal fatty acid metabolism, cytochrome P450 reactions, phagocyte activation, and the nitric oxide synthase system (Fig. 1).

Oxidants are crucial for host defense. The respiratory tract is protected from infection by reactive oxygen species (ROS) generated by phagocytic and epithelial cells, and decreases in ROS can result in chronic bacterial infections [16]. In addition to killing bacteria, ROS stimulate wound repair, assist in protein folding, modify apoptosis, and promote signal transduction [17-19].

Although oxidants in the airway provide many benefits, an overabundance of oxidants can cause biomolecular damage that may play an important role in a number of lung diseases, including CF [20]. The reducing environment found in healthy lung cells prevents generation of an exaggerated inflammatory response to inhaled stresses. An oxidative environment, however, influences multiple intracellular signaling events, leading to apoptosis, increased synthesis and secretion of mucin [21], and alterations in ion transport including chloride secretion [22-26]. The result is a cascade of excessive inflammation, mediator release, and tissue injury-events that characterize CF lung disease. Beginning in infancy, individuals with CF are plagued by constant airway inflammation. Large numbers of activated neutrophils migrate into the airways to attack invading bacteria, and have the potential to release into their microenvironment vast amounts of oxidants, including superoxide anion, hydrogen peroxide (H_2O_2), and hypochlorous acid. Bacteria such as *Pseudomonas aeruginosa* that chronically infect the CF airways also generate oxygen radicals through the release of pyocyanin and other phenazine pigments [27]. Thus, the CF airway is exposed not only to the normal environmental oxidant burden, but also to oxidants derived from inflammatory and infectious processes, causing a net balance of excess oxidation, as demonstrated by protein and lipid oxidative markers [28-34].

Normal airways are able to cope with oxidative stress through a variety of mechanisms, including the absorption and/or biosynthesis of both nonenzymatic and enzymatic antioxidants. Higher levels of dietary or plasma antioxidants have been associated with better pulmonary function, as demonstrated by a higher level and lower rate of decline of the forced expiratory volume in 1 s (FEV_1), and a decreased risk of chronic obstructive pulmonary disease (COPD) [35,36]. Major nonenzymatic antioxidants present at respiratory tract surfaces include ascorbate, urate, α-tocopherol, reduced glutathione (GSH), mucins, and albumin. Metal-binding proteins such as lactoferrin, transferrin, and ceruloplasmin also presumably function as intralumenal airway antioxidants. Although most major enzymatic antioxidants are primarily intracellular, superoxide dismutase and small amounts of catalase and glutathione peroxidase (GSHPx) have also been described in airway fluids [37,38].

Concentrations of the antioxidants that are available extra-cellularly vary widely by location (Table 1). In the normal lung, GSH and ascorbate, for example, are present in fairly high concentrations in bronchoalveolar lavage fluid (BALF), but in the upper airways, where CF disease would be prominent, concentrations are lower and mucin may represent the major scavenging antioxidant [39,40]. On the other hand, α-tocopherol is present in low concentrations throughout the airway surface fluid (ASF) compared to rather high levels in plasma, where its concentration is related to overall lipid levels [39].

The antioxidants that are present in normal ASF are also found in CF ASF, although levels are sometimes altered. In order to understand how these numerous antioxidant systems can be overwhelmed in CF, it is important to examine the major antioxidant host defenses deployed in the CF airway.

Antioxidants in the airway lumen

Mucin

One of the most abundant proteins in sputum, mucin is both an antioxidant and an important determinant of mucus viscosity. The antioxidant properties of mucin are due to its large number of highly reactive cystine and cysteine residues and to the antioxidant properties of its carbohydrates [41]. Oxidative stress increases mucin gene expression [21] and secretion [42], resulting in increased antioxidant levels at respiratory tract surfaces.

Mucin viscosity is regulated by the movement of water, bicarbonate, and glutathione, all of which are influenced by CFTR [43]. A decrease in any of these factors, which follows from loss of CFTR function, increases the viscosity of the mucin. The thicker, more viscous mucus at the airway epithelial surface provides a stronger antioxidant barrier, although airway mucociliary clearance function is simultaneously compromised.

Mucin levels in CF sputum can be as concentrated as 40 mg/g sputum and may therefore represent much of the antioxidant potential in the CF airway. Too much mucin, however, can be counterproductive for the health of the airways; respiratory mucin actually protects pathogenic bacteria such as *Pseudomonas aeruginosa* from neutrophil-mediated killing [44]. Inhibition of mucin synthesis has been proposed as a potential therapeutic strategy in cystic fibrosis [45]. If this approach is developed, careful consideration will need to be given to the possibility that a decrease in mucin synthesis may also weaken the airway epithelial antioxidant barrier.

Glutathione

GSH, a tripeptide, is the major low molecular weight thiol compound in animals and has many different roles (Table 2). In the lung cell, GSH functions as an antioxidant in combination with GSHPx or peroxiredoxin. The resulting glutathione disulfide (GSSG) is either rapidly reduced by glutathione reductase and NADPH or utilized in the protein folding process in the endoplasmic reticulum. There, GSSG is recycled by protein disulfide isomerase to form GSH. Because of these recycling mechanisms, GSH is an extremely efficient intracellular buffer for oxidative stress. GSH can reach a concentration of 10 mM inside the cell and, coupled with GSSG, defines the redox state of the cell, although considerations must be given to the intracellular distribution of these species as the various subcellular compartments contain different ratios of this redox pair (e.g., cytosol compared to endoplasmic reticulum compartments).

GSH can also be found in the fluid surrounding the lung cell, either free or conjugated to xenobiotics, hydroxynonenal, and other nonforeign metabolites and oxidative products. Conjugation with GSH is a requirement for the externalization of many organic compounds.

The extracellular GSH moiety of the secreted conjugates can be broken down by γ-glutamyl transpeptidase (GGT) and other enzymes on the cell surface to form components that can be reabsorbed. Most glutathione in the lung lining fluid, however, appears in the reduced state and acts as a mucolytic due to its ability to cleave disulfide bonds. The method by which GSH is maintained in the extracellular space is unknown, but rapid secretion and degradation are likely to be involved since neither reduction outside the cell nor uptake of GSSG are likely.

Increasing GSH synthesis rates is an important adaptive response to oxidative stress throughout the body [46-49]. Synthesis of GSH can be increased by raising levels of glutamate cysteine ligase (GCL) [48-50], the rate-limiting enzyme in GSH synthesis. GCL activity depends on the intracellular availability of cysteine, so increasing cysteine levels can partially overcome the normally tight regulatory feedback inhibition of GSH synthesis [42,47-49]. In addition, intracellular GSH levels can be raised by augmenting GGT activity, thereby increasing recruitment of cystine and cysteine from outside the cell through the scavenger pathway. This pathway is especially important in lung cells, which along with kidney cells are the only known net importers of GSH. This is useful because both cell types are exposed to high levels of oxidative stress.

Although GSH is not the most abundant antioxidant in human epithelial lining fluid (ELF)-both urate and mucin are present in larger amounts-it appears to play an important role there. In healthy subjects, ELF contains much higher levels of GSH (100-400 μM) than plasma (3 μM) [51]. In fact, GSH is the only antioxidant which is selectively elevated in ELF compared to plasma [40]. Chronic oxidative stress such as that caused by smoking can adaptively induce enzymes that result in a doubling of GSH levels in BALF over those in healthy nonsmokers [51]. The great majority of this is in the reduced state: 96% of the GSH in ELF of nonsmokers and 98% of that in smokers are reduced. In human epithelial cells, the GSH increase induced by oxidants and cigarette smoke is associated with a simultaneous decrease in CFTR function [26,52]. CFTR function is also decreased in healthy smokers as determined by nasal potential difference measurements [52], a response that may represent an antioxidant defense aimed at transiently increasing mucus viscosity and decreasing GSH loss from epithelial cells.

In the individual with CF, however, GSH levels are decreased to about 5-10% of normal in the ELF and 50% of normal in plasma [53]. A number of lung diseases are associated with low ELF GSH, such as idiopathic pulmonary fibrosis [54] and the acute respiratory distress syndrome [55], suggesting that chronic inflammation could be partly responsible [56]. Surprisingly, high levels of GSH have been observed in CF sputum, possibly reflecting the large number of airway inflammatory cells present [54,57]. These contrasting observations suggest that ELF and sputum represent different compartments. Infants and young children with CF-related pulmonary inflammation display less than half the normal amount of GSH in ELF even before there is extensive pulmonary disease [58]. However, GSH levels were shown to be increased in the peripheral blood lymphocytes of some individuals with decreasing lung function due to CF [59], demonstrating the complexity of the story.

Poor nutritional status, which affects the ability to maintain cellular and tissue GSH levels [60], may contribute to low GSH levels in CF [59]. Albumin, which can provide the cysteine needed for GSH production, can be low in individuals with CF due to poor nutrition and activation of inflammatory-immune processes [60]. However, it is likely that decreased GSH levels in the lower respiratory tract lining fluid of the person with CF are at least partially due to faulty CFTR. Healthy carriers of the ΔF508 CFTR mutation exhibit decreased ELF GSH compared to ELF GSH from healthy subjects without the CF mutation (Griese, personal communication). Likewise, the uninfected CFTR knock/out (k/o) mouse displays a 50% decrease in both reduced and disulfide forms of glutathione in lavage fluid [61]. When exposed

to *P. aeruginosa*, the k/o mouse is unable to express a normal increase in ELF GSH [62] and suffers increased lung inflammation and mortality over wild type [63].

In addition to a reduction in extracellular GSH levels caused by loss of GSH transport through CFTR, CF lung cells apparently may reclaim spent extracellular glutathione faster, as evidenced by the increased GGT activity found in children with pulmonary inflammation due to CF [58]. GGT activity is typically increased by oxidative stress [64]: very young children with CF but without inflammation can have normal GSH levels, perhaps due to alternative, developmentally regulated mechanisms for GSH transport that are lost with age [58].

Regardless of the cause, depletion of extracellular lower respiratory tract GSH could contribute to the wide range of consequences of oxidative damage present in CF [65], or, alternatively, it could be a marker of this damage. A direct link between decreased extracellular GSH and these consequences, however, has not been established.

It is unclear whether CF causes changes in the intracellular GSH content of airway epithelial cells. The decreased ability of CF cells to transport GSH into the extracellular space could affect internal concentrations, and a resulting overabundance of intracellular GSH could account for the delay of apoptosis seen in CF lung epithelial cells [66]. On the other hand, decreased intracellular GSH would account for the augmentation of proinflammatory processes seen in CF, including those dependent on NFκB activation and arachidonic acid pathways. In fact, if GSH levels could be increased inside the cell, some NFκB activation by proinflammatory cytokines (such as TNFα) might be ameliorated [60]. Another potential benefit of augmenting intracellular GSH could be a reduction in Th2 response [67], resulting in a decrease in inflammation, as achieved in mice with *P. aeruginosa* lung infections [68, 69]. Furthermore, increased GSH levels could augment levels of GSNO, a molecule that might improve maturation of CFTR and stimulate other chloride channels to increase chloride and GSH flux [70]. Unfortunately, there could also be dangers in altering the levels of such a pluripotent molecule. Increasing GSH could cause conformational changes in a variety of proteins, for example, resulting in alterations to vital cell responses such as immune modulation [71].

Human studies of GSH supplementation

Trials have been conducted in attempts to increase intracellular GSH by oral supplementation. Oral supplementation of healthy volunteers with up to 3 g GSH did not alter plasma levels of cysteine or GSH [72], perhaps because the intestinal mucosal epithelia and liver have very high levels of GGT and the supplemented GSH was rapidly metabolized. The half-life of GSH in human plasma is less than 3 min [73]. Attempts to increase GSH levels by increasing cysteine stores using lipoic acid [74] or whey protein [75,76] have met with some success. Lymphocyte GSH levels in healthy young adults rose 35% in one study using a cysteine donor [75] and in a report of one patient with COPD, increases in whole blood GSH levels correlated with clinical improvements in lung function (FVC and FEV_1) [77]. In young adults with CF, 3 months of supplementation with a commercially available whey protein resulted in a 40% increase in lymphocyte GSH [78]. However, no improvements in muscle performance, sputum neutrophils, or lung function were seen, perhaps due to well-established disease, inadequate dose, or the short period of the study. Patients with COPD, for example, had decreased exhaled hydrogen peroxide levels only after 6 months of supplementation with a cysteine donor [79].

Attempts to normalize GSH in the ELF of individuals with CF have been made using inhaled GSH. This approach could carry more risk than an oral route. For example, inhaled GSH has been shown to induce bronchospasm in individuals with mild asthma [80]. Also, although BALF glutathione from healthy subjects is over 90% reduced, GSH that is aerosolized into the airway of CF patients and recovered by lavage is almost all oxidized, possibly by trace amounts

of iron present in CF airway mucus [81]. GSH inhalation could therefore be associated with undesired effects: oxidized glutathione (GSSG) could affect cellular receptor and signaling activities by inducing changes in protein structure and function via effects on redox-active protein thiol groups [82]. Furthermore, the inflammatory cells recovered in the BALF in this study produced less superoxide anions than before therapy, suggesting the GSH treatment could potentially compromise airway antimicrobial host defenses [83]. Preliminary trials of inhaled GSH have shown no significant adverse clinical events in either healthy subjects or individuals with CF [84,85], although the number of subjects in these studies was small.

Therapeutic use of inhaled GSH may be complicated by its rapid clearance from the lung; in one study of sheep, the alveolar lining fluid GSH returned to baseline levels within 2 h of a 600 mg aerosol dose [86]. Another important consideration is that inhaled GSH must reach the proper compartment which is the area between epithelial cells where neutrophils migrating into the lung are releasing myeloperoxidase to synthesize toxic hypochlorous acid [87].

To explore the potential usefulness of GSH aerosolization therapy, a trial was conducted using an over-the-counter preparation of GSH (pH 5.2; 66 mg/kg body weight divided into 4 inhalation sessions per day) for 8 weeks in 19 volunteers with CF, aged 6 to 19 [88]. The volunteers were assigned to either placebo or GSH, with 10 people in the GSH group. No significant differences occurred between treatment and placebo groups in FVC, FEV_1, or FEF25-75. Peak flow and subjective feelings of wellness were significantly improved in the GSH treatment group, but because of the strong sulfur smell of GSH (quinine was added to the placebo), the effectiveness of the blinding can be questioned.

More rigorous testing was performed in a trial of inhaled GSH that examined the intrapulmonary deposition of the drug and assessed the tolerance and short-term effects of an optimized inhalation system (AKITA inhalation device, connected to a Pari LC Star nebulizer) [84]. Tests using inhaled ^{99m}Tc-labeled Fe_3O_4 aerosol particles in 6 subjects aged 35±2 years, with an average FEV_1 of 69.6±7% (range 50-99%) demonstrated that the majority of material was deposited in the peripheral airways with a small fraction in the central airways. GSH tolerance and effects were assessed in 17 volunteers with CF, age 15-36 years with FEV_1 of 61% predicted (range=43-104% predicted). Either 300 or 450 mg GSH (pH 7.0) was inhaled three times daily for 2 weeks. Bronchoalveolar lavage demonstrated increased levels of GSH and GSSG following inhalation. FEV_1 and FVC both showed significant improvement following 2 weeks of GSH inhalation. No significant changes were seen in the numbers of neutrophils, lymphocytes, eosinophils, or macrophages in the lavage fluid, but CD4- and CD8-positive T cells with cytokine receptor 5 (CCR5) surface markers were significantly increased in the lavage fluid taken at the end of the 2-week treatment period. There was no apparent change in oxidative stress as measured by carbonyls, thiols, or lipid peroxides in the BAL fluid, but there was a very high level of oxidative stress in these subjects (at least 20-fold higher than controls). The levels of tested cytokines and chemokines (IL-1B, IL-2, IL-4, IL-5, IL-6, IL-10, TNFα, INFγ, G-CSF, MCP-1, and MIP-1β) did not change significantly following GSH inhalation.

Pulmonary function improvements following inhaled GSH treatments suggest that the therapy may be useful. However, any clinical trial investigating the effectiveness of inhaled GSH must consider half-life issues as well as appropriate concerns about the overall safety, optimal dose, duration of treatment, and efficacy.

Oral N-acetylcysteine

N-Acetylcysteine (2-acetamido-3-sulfanyl-propanoic acid, NAC) is absorbed by the intestine more readily than cysteine. Once taken up by cells NAC is deacylated to form cysteine, an essential precursor for GSH synthesis. Oral NAC added to a regimen of prednisone and

azathioprine in patients with idiopathic pulmonary fibrosis, a chronic respiratory disease characterized by lung inflammation, high lung oxidant stress, and low ELF GSH, was associated with a slower deterioration in lung function over a 1-year treatment period [89]. Recently, a high-dose oral NAC trial in 18 CF subjects treated for 1 month demonstrated a statistically significant decrease in sputum elastase activity, IL-8, and neutrophil burden [90]. The number of subjects enrolled into the trial and the duration of the study were too small to allow the detection of any changes in clinical parameters such as lung function. The therapy was well tolerated and no adverse reactions were reported. While these results should encourage further studies, the authors caution against the uncontrolled use of oral NAC by CF patients since the nutraceutical status of commercial sources of NAC available over the counter prevents the proper quality control of most formulations.

Nacystelyn

Trials are also being conducted using cysteine-rich Nacystelyn (NAL), a mucolytic with antioxidant and anti-inflammatory activity [91-94]. NAL differs from *N*-acetylcysteine (Mucomyst), which has a long history of use in CF, by the addition of lysine, raising the pH from 2.2 to 6.0 and increasing the water solubility. The mucolytic effect of NAL is about the same as that of Pulmozyme and the effects are synergistic, as determined by an in vitro test of CF mucus transport [95]. In addition, NAL may have the potential to decrease oxidantinduced IL-8 production [93], decrease the oxidative burst from neutrophils, and increase intracellular GSH levels [91].

NAL may be administered in a dry powder inhaler, with 23.5% of the drug deposited in the lungs of adults and 16.5% in the lungs of children with CF [96]. Clinical trials have demonstrated the safety of NAL in healthy adults, smokers, and people with CF [96,97]. In a multicenter phase 2 trial, a placebo-controlled, double-blinded, randomized trial lasting 24 weeks demonstrated an improvement in mucus viscoelasticity in expectorated sputum and decreased self-reported respiratory infections with NAL aerosol administration (8 or 16 mg bid). Lung function did not improve, but the dose may have been suboptimal. A phase 1a double-blind and placebo-controlled trial in 12 patients receiving up to 80 mg NAL over 3 days showed that all doses were tolerated with no serious adverse events. Further clinical trials are now being planned.

Vitamin E (α-tocopherol)

Vitamin E, or α-tocopherol, is a powerful membrane-localized anti-lipid peroxidation antioxidant. As with other antioxidants, vitamin E has multiple roles in the body, including indirect stimulation of the transcription of certain genes encoding drug-metabolizing cytochrome P450 enzymes [98]. Consequently, high doses of vitamin E could potentially change the metabolism of many drugs.

Vitamin E deficiency has produced broad effects in animal models including modulation of innate and acquired immune functions, such as T- and B-cell mitogenesis, T-helper cell function, macrophage function, neutrophil chemotaxis, phagocytosis, and H_2O_2 production [99,100]. Prolonged deficiency of vitamin E in people can result in neuromuscular degeneration including cerebellar ataxia, neuropathy, or myopathy, particularly in children with cholestatic liver disease or intestinal resection [101-103]. Hemolytic anemia, retinal degeneration, and cognitive defects have also been observed [104,105].

Vitamin E, like other fat-soluble vitamins, is frequently deficient in CF. Because α-tocopherol is carried almost exclusively in circulating lipoproteins, serum levels depend on the level of circulating lipids, which are often low in individuals with CF. Consequently, vitamin E levels in the person with CF should be expressed as a ratio of vitamin E to total serum lipids. In

infants, a ratio of at least 0.6 mg to tocopherol/g total lipids reflects adequate vitamin E; for older children and adults, the ratio should be at least 0.8 mg/g lipids. Using this method, 22.8% of infants identified with CF at 4-6 weeks of age through a newborn screening program demonstrated vitamin E deficiency [106]. In the only randomized study of newborn screening versus conventional diagnosis, there was no difference in the prevalence of vitamin E deficiency at diagnosis; vitamin E deficiency was seen in 49% of all patients. However, the duration of deficiency of this important antioxidant was 15 months longer in the patients diagnosed by the presence of symptoms than that in the newborn screened infants. Only 8 and 3% of patients had low α-tocopherol levels at 3 and 6 months after treatment, respectively [105]. In another report on vitamin E status in infants diagnosed by newborn screening, of the initial patients who were vitamin E sufficient, 12% subsequently became deficient despite supplementation, and 10% remained persistently deficient [107]. Numerous studies have shown that the great majority of children and adults with CF who do not receive vitamin E supplementation are deficient in vitamin E, even if they receive standard multiple vitamins and are on pancreatic replacement enzymes [107,108]. Unfortunately, no lung tissue or airway secretion determinations of vitamin E levels have been made in people with CF.

The recommended dietary intake of vitamin E is 6-10 IU/day in healthy children from birth to age 7 years and 15-25 IU/day in healthy individuals age 8 years to adult. High amounts of polyunsaturated fatty acids in the diet may increase the need for vitamin E to prevent lipid peroxidation. The current CF Foundation consensus conference on nutrition (Table 3) [109] recommends 40-400 IU/day (in addition to a multiple vitamin) to normalize plasma levels of vitamin E. Almost all individuals with CF are able to attain normal serum levels of vitamin E when given 5-10 IU/kg/day (approximately 400-800 IU/day for adults) of almost any form of vitamin E when given with food and pancreatic enzymes [110]. Low levels despite supplementation [106] may be due to lack of adherence, malabsorption despite pancreatic enzyme supplementation, intestinal resection, or liver disease. Individuals with cholestatic liver disease should be supplemented with D-α-tocopheryl- polyethylene glycol 1000 succinate (TPGS), the only truly water-soluble form available in the United States [111].

Vitamin E supplementation has proven beneficial in certain populations: healthy elderly volunteers receiving 800 IU daily for 1 month significantly increased T-cell proliferation, IL-2 production, and response to DTH skin tests, and decreased PGE2 production [112-114]. Vitamin E supplementation also increased GSH levels in liver cells, possibly due to a “sparing” effect, with resultant benefits [115,116]. However, supplementation is not without potential risk. A recent metaanalysis of 19 clinical trials demonstrated an increased risk of mortality in trials using more than 150 IU/day in patients with a variety of diseases but with normal fat absorption [117], and the recent heart outcomes evaluation (HOPE) trial suggests that vitamin E supplementation may increase the risk for heart failure [118]. Adults who receive above 1500 IU/day and who are on coumadin or who are vitamin K deficient may have increased coagulopathy with a potential for decreased neutrophil function. This is especially significant in CF, as a number of patients are at least borderline deficient in vitamin K. Toxicity in children has not been sufficiently studied; some trials using large doses of iv vitamin E in premature infants resulted in increased risk of bacterial and fungal sepsis and necrotizing enterocolitis [119]. However, numerous trials of large oral doses of vitamin E in adults have not demonstrated any increased risk of infection.

The clinical results of vitamin E trials may be influenced by the nature and dose of the administered vitamin E supplement. The all-*racemic* form, or D,L-α-tocopherol is actually a mixture of eight stereoisomers and is the one commonly found in vitamin supplements because it is the least expensive form to manufacture. However, D-α (or RRR-α) tocopherol is more bioavailable. TPGS-vitamin E, which contains D-α tocopherol, is water soluble and highly bioavailable, but is found only in ADEKS drops and two liquid preparations (Liqui-E,

Twinlabs, and Aqua-E, Yasoo Health) and is not present in the ADEKS or Vitamax tablets that are commonly used by the CF population.

No prospective trials have demonstrated an effect on pulmonary outcomes of vitamin E supplementation in individuals with CF. The level of lipid peroxidation as measured by 8-isoprostane-$PGF_2\alpha$ decreases significantly with vitamin E supplementation in CF [120,121], but the clinical relevance of this is unknown. Nevertheless, preventing and correcting vitamin E deficiency in CF are essential in order to prevent neuromuscular degeneration that has been reported.

Carotenoids

Carotenoids found in the body are classified by their ability to be converted into vitamin A (retinol). Provitamin A carotenoids include β-carotene, the most prominent carotenoid in human nutrition, α-carotene, and β-cryptoxanthin. Nonprovitamin A carotenoids include lutein, zeaxanthin, and lycopene.

Carotenoids are present in carrots, tomatoes, watermelon, spinach, and many other foods. The preparation of the food significantly affects absorption and bioavailability of the carotenoids [122]. Raw fruits and vegetables such as carrots and tomatoes generally contain carotenoids with poor bioavailability because their crystalline structures are hard to break down. Mildly cooking the food or processing it with oil can increase the bioavailability significantly. The highest bioavailability is obtained from oil solution or water-dispersable beadlets of carotenoids [123].

Adverse clinical manifestations of carotenoid deficiency in man are unknown as long as vitamin A levels are normalized. Although higher levels of lutein and zeaxanthin in the diet were related to lower incidence of retinal pigmentary abnormalities [124], increasing the intake of these carotenoids has not been demonstrated to be protective against the development of age-related macular degeneration [125].

As with other lipophilic antioxidants, carotenoid and retinol levels are commonly decreased in CF. Children with CF display 9-24% of normal values [126-133]. The plasma β-carotene/cholesterol ratio is also very low, with a value of about 18% of normal [134]. Poor absorption, cholestatic liver disease, or rapid turnover of carotenoids due to active inflammation could all contribute to low carotenoid levels [135,136]. However, despite severe reduction of lutein and zeaxanthin in both plasma and retina of people with CF, no related abnormalities of visual function under daylight conditions have been found [137], though poor nocturnal vision in some individuals has been corrected by retinol supplementation [138].

The oxidant burden imposed by active CF respiratory tract disease could increase the importance of carotenoids. Several clinical features in CF may correlate with carotenoid concentrations including increased inflammation and decreased infection control [126]. Further, inflammatory markers in plasma correlate negatively with β-carotene, lycopene, and retinol [139].

Because of this close association, a number of trials have examined β-carotene supplementation in CF (Table 4) [128-130,132,140,141]. Plasma levels before supplementation were consistently very low. Plasma β-carotene levels achieved after supplementation ranged from 0.30 to 3.99 μmol/L, depending on dose and formula; water-miscible β-carotene is more effectively absorbed into the blood.

Carotenoid supplementation trials have generally demonstrated favorable results on biomarkers of oxidative stress. Malondialdehyde, a marker of lipid peroxidation which is

elevated in children with CF [128], decreased in 5 out of the 6 β-carotene supplementation trials where it was studied; the one trial in which it did not decrease was a low-dose supplementation trial. β-Carotene supplementation improved resistance of LDL to oxidation [128,129] and decreased plasma TNFα [132,142] and neutrophil elastase/α-1-proteinase inhibitor complexes [142], markers of inflammation. Effects on other plasma micronutrient antioxidants are not entirely clear, but plasma retinol and α-tocopherol increased in at least one trial [132]. The increased plasma α-tocopherol could be due to increased adherence to vitamin supplementation due to the trial or a sparing effect of α-tocopherol by β-carotene.

Clinical outcomes have generally also been favorable. In an 8-year longitudinal study of 1194 French subjects from the general population, β-carotene was found to protect against the decline of FEV_1 [143]. Furthermore, in a study of CF subjects, the number of days on antibiotics decreased significantly in a high-dose regimen study, although it did not change in the low-dose arm of that same study [144]. FEV_1 increased significantly in some studies and FVC increased in those people with CF who experienced an increase in plasma carotenoid levels [132,144]. Unfortunately, the use of a combination of antioxidants in the last trial confounded interpretation of the results. Finally, weight and height *z* scores increased significantly in the two studies in which they were measured [142,145].

Side effects of β-carotene supplementation have been limited. In a 16-month trial of β-carotene supplementation in volunteers with CF there was a significant decrease in ALT and AST liver functions, but a significant increase in GGT (from 23.1±31 to 36.1±63.5) [146]. In addition, detrimental effects of β-carotene supplementation have been documented in heavy cigarette smokers in non-CF populations [147].

However, in individuals with CF, β-carotene supplementation has generally been associated with favorable results such as decreased levels of lipid peroxidation markers, circulating markers of inflammation, and number or severity of pulmonary exacerbations (Table 5). Increases in ex vivo resistance to LDL oxidation and improvements in lung function have also been demonstrated.

Coenzyme Q-10

Coenzyme Q-10 (CoQ) or ubiquinone is a lipid-soluble component of the mitochondrial electron transport chain. CoQ also helps reduce vitamin E, maintaining it as a viable antioxidant [148], and is an antioxidant in its own right. Diet provides more than 25% of the circulating CoQ pool; the remainder is synthesized through the cholesterol pathway. Because CoQ is primarily found in tissues containing high percentages of mitochondria, meats, poultry, fish, and liver are good sources of this antioxidant. The typical daily intake of CoQ is 3-5 mg.

Malabsorption or oxidation of CoQ may result in decreased circulating levels in people with CF, possibly resulting in decreased ATP production or protection against oxidative stress or both. In a study of 274 patients with CF, over 50% of subjects had below normal levels of total (oxidized and reduced) CoQ (0.4 μg/ml in children under 8 years; 0.3 μg/ml in individuals over 8 years of age) (R. Sokol, personal communication). As expected, pancreatic insufficient patients demonstrated much lower levels of CoQ (mean=0.31 μg/ml) than pancreatic sufficient patients (mean=0.49 μg/ml, $P<0.0001$). Because decreased CoQ levels may reflect decreased lipid levels in the serum, CoQ levels should be interpreted in the context of cholesterol levels and, if possible, triglyceride and phospholipid levels as well [149-151].

Despite a deficiency in CoQ levels in individuals with CF, in a retrospective study there did not appear to be a relationship between CoQ and pulmonary function (as expressed by days hospitalized, number of admissions, FVC % predicted and FEV_1% predicted, R. Sokol, pers.

commun.) [152]. Also, CoQ levels did not appear to correlate with *Pseudomonas* infection and weight and height *z* scores.

Enzymatic antioxidants

The nonenzymatic antioxidants described thus far have low rate constants (10^5) and are only moderately efficient. In contrast, the enzymatic antioxidant extracellular superoxide dismutase (EC-SOD), which is found at high concentrations in the normal lung [153], has a much higher rate constant (10^9) and is very efficient. EC-SOD is essential for protecting NO from oxidation and therefore is essential for protecting the lung from inflammation. The concentration of EC-SOD in the CF lung is unknown.

Recombinant human Cu^{2+}/Zn^{2+} superoxide dismutase has been delivered to the sheep airway epithelial surface by aerosol and shown to retain its activity against superoxide for several hours [154]. However, clinical studies of this approach have not been reported.

A mimetic of EC-SOD with a long half-life and broad antioxidant properties has been developed. This new molecule was successful in reducing neutrophil migration into the lungs of rats exposed to 8 weeks of oxidative stress in the form of tobacco smoke [155]. The mimetic also reduced eosinophilia and inflammatory mediators in a mouse model of asthma [156]. Its usefulness in CF has not been explored.

Zinc

Zinc (Zn) is an essential trace element that functions as a cofactor for more than 200 intracellular and extracellular enzymes. As such, it is involved in many cell processes including growth, differentiation, and signaling. Zn is important in antioxidant defenses due to its association with EC-SOD and a cytosolic copper-zinc-SOD. Further, there is evidence that Zn stabilizes lipid membranes and prevents peroxidation. Some alternative chloride channels that are Zn sensitive might even be targets for CF therapy [157].

Severe Zn deficiency causes loss of appetite and impaired growth. In mouse models of asthma, Zn deficiency correlated very strongly with high levels of eosinophilia and increased Th2 responses [158,159]. That may be especially relevant to the CF population, where there is often a large Th2 response. There is no robust marker, however, for Zn deficiency. Plasma levels of Zn can be apparently normal and the individual may still be clinically deficient such that Zn supplementation improves growth and resistance to infection. Dozens of trials of oral Zn supplementation have demonstrated decreases in infectious diarrhea or lower respiratory tract infections without large changes in circulating Zn levels. Zn levels in red blood cells provide a more accurate picture, but this test is not routinely performed, and Zn content in hair or nails is too variable to be of use.

Individuals with CF can experience a wide range of Zn deficiencies. Twenty to 30% of infants with CF identified by newborn screening have insufficient plasma levels of Zn by 2 months of age [160], and some infants with CF can have severe Zn deficiency by that time [161,162], resulting in rash, diarrhea, and a potentially fatal decrease in resistance to infection. Because Zn pools in the body depend on fat absorption [160], pancreatic enzyme administration greatly increases Zn levels [163], but supplementation is still needed for normalizing plasma levels in many individuals with CF.

Zn supplementation could produce multiple benefits in individuals with CF. In individuals without CF in developing countries, Zn supplementation can decrease mortality due to infection and increase linear growth in children who are more than 2 standard deviations (SD) below the mean [164]. Such a response in linear growth would be helpful in the CF population,

since stature correlates with survival and the average adult with CF is 5 inches below predicted height.

Because clinical deficiencies can exist in the presence of normal plasma levels, Zn supplementation should be tried especially in individuals who are 2 SD below the norm in height or weight. Most vitamins used in the CF population, except for Vitamax, already contain Zn, although at doses lower than those used in the Zn trials in developing countries. As with other nutrients, higher levels may be needed in the individual with CF due to absorption difficulties. It is not known whether supersupplementation of Zn, beyond that required to normalize plasma levels, would improve health.

Clinical trials should be conducted before Zn supplementation is mandated for all individuals with CF. Because one study has shown an association between Zn supplementation and a very mild developmental delay [165], these trials must be conducted extremely carefully. Furthermore, a biomarker (for Zn levels or oxidative stress) may not be readily available for a proof of concept trial.

Selenium

Selenium (Se) is an essential component of a number of enzymes including GSHPxs, thioredoxin reductases, thyroid hormones, and circulating selenoprotein-P. In addition to its antioxidant functions, Se is needed for a variety of other functions including neutrophil responses to pathogens [166], cell cycling [167], and metabolism of carcinogens [168].

Se levels in the body reflect the varying content of Se in foods, depending in part on where the food is grown. The average intake of Se from food in the United States is 108 μg/day, resulting in an average Se serum level of 1.57 nmol/L in adults and 1.52-1.54 nmol/L in children 9-18 years of age [169]. In the United Kingdom, the average plasma Se level is considerably lower (0.86-0.88 nmol/L in 4-18 year olds) [170]. Factors other than dietary intake may affect Se plasma levels; Se levels in the UK study increased with age and were lower in lower social classes and in children of parents who smoked.

Se deficiency results in reduced GSHPx activity, which can be restored by Se supplementation. In fact, the Recommended Daily Intake (RDI) for Se (70 μg/day for men and 55 μg/day for women) is based on the amount needed to maintain GSHPx activity, plus a 30% safety factor inflation. Plasma levels of selenium respond quickly to supplementation. GSHPx activity can be used to measure Se status, but it saturates at low levels and is only an approximate characterization of suboptimal intake.

Increasing plasma levels of Se may have important health implications. Higher plasma levels of Se are associated with decreased risk of asthma [171] and with higher FEV_1, particularly in the face of chronic oxidative stress caused by active or passive exposure to cigarette smoke [172]. Selenium supplementation has been associated with a reduction in cancer risk [173]. In individuals with CF, plasma levels of Se are generally 1 SD below normal (0.65 μm/L Se vs 0.85 μm/L Se [174]). The reasons for low levels are unclear, but may include differences in dietary intake, absorption, distribution, or excretion. Although these levels do not indicate Se deficiency, they are low enough that supplementation would be expected to increase GSHPx activity. However, the consequence of that response is not clear.

Se supplementation studies in individuals with CF suggest benefit. Use of a Se-containing pancreatic enzyme supplement increased plasma Se levels and GSHPx activity [174]. Also, a randomized controlled trial in 46 children with CF, 10-12 years of age, compared a supplement containing 10 mg vitamin E and 500 μg vitamin A with an antioxidant cocktail containing 90 μg Se (delivered as Se-methionine), 200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene,

and 500 μg vitamin A over 8 weeks [133]. Although the baseline level of plasma Se in this study (1.0 μm/L) was already higher than in many of the earlier studies, plasma Se increased 0.60 μm/L ($P<0.001$). No change occurred in oxidative stress as a result of treatment, as measured by F_2-isoprostanes, but the increase in plasma Se levels correlated with an increase in percentage predicted FEV_1. Thus, this short-term study showed both statistical and biological significance of supplementation.

The heterogeneity of Se status in CF in relation to disease progression is not known, and should be examined to determine the kinds of patients who are most likely to benefit from supplementation. In a cancer prevention trial in a non-CF population, individuals with lower initial Se plasma levels received greater benefit from supplementation [173], suggesting many individuals with CF may benefit, since plasma selenium levels in these individuals are often low. However, caution is needed in designing a supplementation trial due to possible toxic effects. The upper limit of Se intake is set at 400 μg/day, and Se content in the pancreatic enzyme preparations in use must be considered.

Iron

Iron is a metal ion essential for the synthesis of numerous heme-centered enzymes and other proteins involved in oxygen transport, oxidative metabolism, and host defenses. However, iron that is not bound to proteins can undergo reduction to the ferrous (Fe^{2+}) state, a process catalyzed by superoxide anions. The Fe^{2+} ion greatly accelerates autoxidation of catechols, conversion of hydrogen peroxide to hydroxyl radicals, and lipid peroxidation. Most iron in biological compartments is bound in the ferric (Fe(III)) state to proteins such as ferritin, transferrin, and lactoferrin. In this form it is not readily available to catalyze ROS formation [175]. Iron-catalyzed ROS synthesis is also decreased by the presence of ceruloplasmin, a copper-rich protein synthesized in the liver and secreted in plasma. The ferroxidase activity of ceruloplasmin oxidizes Fe^{2+} to the Fe (III), thus preventing hydroxyl radical formation [176]. Transferrin and to a lesser extent ceruloplasmin in the ELF of healthy individuals can prevent iron-mediated lipid peroxidation [177]. Unfortunately individuals with CF whose lungs are colonized by *Pseudomonas aeruginosa* are at an increased risk of iron-mediated ROS formation because elastase from the bacteria cleaves transferrin and lactoferin [81]. Indeed, lactoferrin and transferin cleavage products appear in the ELF of most individuals with CF and *Pseudomonas aeruginosa* [178]. The iron released from these cleaved proteins can catalyze hydroxyl radical formation. Although no proven effective therapy is currently available to prevent iron-catalyzed ROS formation in the lung, these data point to the importance of preventing and treating airway *Pseudomonas aeruginosa* infection in the CF lung as an antioxidant strategy.

History of clinical trials of antioxidants in other diseases

Before designing clinical trials of antioxidant supplements in CF, it is useful to examine data from animal models and human trials in other diseases. Studies of high efficiency antioxidants in more than 20 animal models of inflammatory diseases demonstrate that severe inflammatory stress is diminished by high antioxidant levels, and accelerated by low antioxidant levels. Powerful antioxidants have not been available for human trial, but supplementation with weaker antioxidants has been tested for prevention of a variety of diseases. Results have been mixed. In the National Health and Nutrition Examination Survey iv trial, levels of high-sensitivity C-reactive protein, an inflammatory marker, were inversely related to the levels of serum antioxidant micronutrients including Se [179]. However, results of antioxidant supplementation trials have largely been disappointing, demonstrating little effect of Se and vitamins E and C on gastric cancer [180] and no effect of vitamin E and β-carotene on the prevention of heart disease [181]. Some randomized, controlled trials have actually displayed adverse effects of supplementation with antioxidants such as β-carotene and vitamins E or A

on lung cancer and heart disease [118,182,183], demonstrating a need for caution. In other trials, the results have been mixed: Se supplementation correlated with an increased risk of some cancers and a reduced risk of others [173].

Thus, despite the clear relationship between antioxidant-rich fruit and vegetable consumption and health, results from a number of large, well-designed trials of antioxidant supplements do not overtly support their use. A host of factors may cause the conflicting results, including wrong formulations or doses of the supplements and perhaps suboptimal delivery of antioxidants to the sites of inflammation-related oxidative stress. Alternatively, in these populations, which are not deficient in antioxidants, the body may simply excrete or metabolize the surplus nutrients.

Future clinical trials of antioxidants in CF

Arguments for antioxidant trials in CF appear compelling despite disappointing results in reducing risk of some other diseases associated with lower degrees of oxidative stress [183]. Levels of various antioxidants in extracellular fluids are significantly decreased in the individual with established CF lung disease. It seems possible that restoring these levels to restore redox balance could, in theory, modulate inflammatory and immune processes, reduce inflammation-related oxidative stresses, and decrease oxidative biomolecular damage with minimal detrimental effects on intracellular signaling pathways.

Recommendations

- Supplementation for normalization of serum levels of vitamins A and E should be a part of routine CF care. These lipid-soluble antioxidants, which have profound cytoprotective effects on bronchoepithelial cells, are often dramatically decreased in plasma of individuals with CF. The known extrapulmonary clinical deficiency states for these two nutrients require prevention and correction of low serum levels. The reported relationships between plasma levels and improved clinical outcomes for other lipid soluble antiox-idants are preliminary but because the evidence suggests that supplementation may be beneficial, carefully controlled trials should be a priority.
- Individuals with CF should ingest a minimum of the RDI for water-soluble antioxidants such as ascorbate, zinc, and selenium. Studies to determine optimal intakes for these and for antioxidants for which there are no RDIs, such as coenzyme Q-10, should be encouraged.
- Interventional studies using antioxidant “cocktails” or of individual antioxidants should include measurements of biomarkers of inflammation and oxidative stress, as well as scoring for clinically relevant outcomes.
- A working group should be established to study optimal levels for nutritional antioxidants in CF and to standardize the way levels are expressed, including the use of lipid correction and documentation of pancreatic function. This group should also recommend that biologic markers of antioxidant stress and inflammation to be used in future trials of antioxidants in people with CF.
- A carefully designed, proof of concept human trial of inhaled GSH prepared in a good manufacturing procedures (GMP) facility, which includes measures of safety and biologic markers of antioxidant effect, should be conducted to determine if a larger trial is warranted. Similar investigation should be conducted into the potential benefits of oral supplementation with lipid-soluble antioxidants, perhaps in combination.
- Individuals with CF should avoid exposures that can increase oxidant stress, such as environmental tobacco smoke or excessive levels of air pollutants.

- Adherence to routine CF care, including preventive care and surveillance for and treatment of pulmonary exacerbations, is important to control lung inflammation and the resultant oxidative stress.

Conclusions

Many questions must be answered to understand the potential of systemic or aerosolized antioxidant treatment in CF:

- Do oxidants at the respiratory tract surface cause injury, or are they simply markers of airway inflammatory-immune system activations?
- Can antioxidant substances at respiratory tract surfaces actually be increased and can we target antioxidants to that surface?
- If antioxidants can be augmented at the airway surface, what effects will there be on the intracellular redox state and on levels of enzymatic antioxidants in the inflammatoryimmune system cells and epithelial cells at the respiratory surfaces?
- What effects might the presence of augmented antioxidants have on antimicrobial and repair processes at airway surfaces?
- Can antioxidant therapy prevent the oxidative inactivation of key proteins involved in host defenses such as alpha-1 antiproteinase?
- Will augmentation of antioxidants at these surfaces amelio-rate the relentless and progressive respiratory tract injury in CF?

Any clinical trial must consider the pleiotropic effects of antioxidants and ensure that important oxidative host defenses are not compromised. The answers to these questions could potentially have significant effects on treatment strategies for the individual with CF.

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Abbreviations

ALT, alanine aminotransferase
AST, aspartate aminotransferase
ASF, airway surface fluid
BALF, bronchoalveolar lavage fluid
cAMP, adenosine 3,5-cyclic phosphoric acid
CF, cystic fibrosis
CFTR, cystic fibrosis transmembrane conductance regulator
COPD, chronic obstructive pulmonary disease
CoQ, coenzyme Q-10
ELF, epithelial lining fluid
ENaC, epithelial sodium channel
EC-SOD, extracellular superoxide dismutase
FEV1, forced expiratory volume in 1 s
FVC, forced vital capacity
FEF25-75, forced expiratory flow at 25-75% of forced vital capacity
GGT, gamma glutamyl transpeptidase
GSH, glutathione
GSNO, *S*-nitrosoglutathione
GSSG, glutathione disulfide
GSHPx, glutathione peroxidase
GMP, good manufacturing procedures
IL-8, interleukin-8
IL-2, interleukin-2
iv, intravenous
LDL, low-density lipoproteins
NAL, Nacystelyn
NFκB, nuclear factor kappa B
PGE2, prostaglandin E2
RDI, recommended daily intake
ROS, reactive oxygen species
SD, standard deviation
Se, selenium
SOD, superoxide dismutase
TPGS, D-α-tocopheryl-polyethylene glycol 1000 succinate

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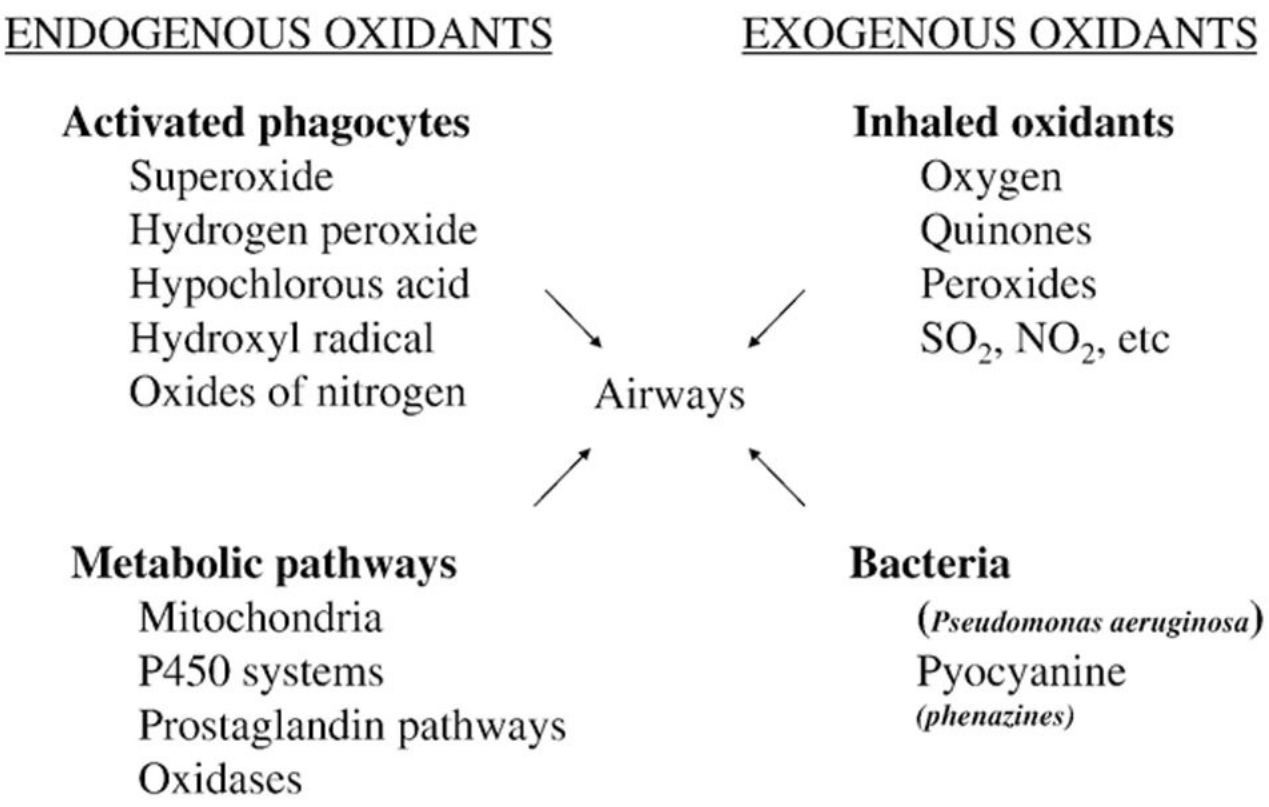

Fig. 1.
Potential sources of oxidative stress in the CF airways.

Table 1

Estimates of extracellular antioxidant concentrations in various respiratory tract lining fluids (RTLF) and plasma

	Nasal RTLF (NLF)*	Upper airways RTLF (ALF)	Lower lung RTLF (ELF)	Plasma
Ascorbate (μM)	28±19	2.4	40±18	67±25
Urate (μM)	225±105	52	207±167	387±132
GSH (μM)	<0.5	low	109±64	1.0±0.7
α-Tocopherol (μM)	low	low	0.7±0.3	16±5
Total protein (mg/ml)	low	low	9.5±6.1	74±19

*NLF, nasal lining fluid; ALF, airway lining fluid; ELF, epithelial lining fluid.

Table 2

Glutathione effects

Effects of GSH sufficiency
Antioxidant
Anti-inflammatory
Mucolytic
Facilitates phagocytosis
Helps regulate apoptosis
Increases primary antibody response
Increases B-cell and T-cell proliferation
Increases IL-2 production
Increases antibody-dependent cellular cytotoxicity
Decreases IL-4 and IgE production
Detoxifies peroxides
Plays a role in posttranslational protein processing
Modifies protein function (e.g., protein glutathionylation)
*Increased susceptibilities associated with GSH insufficiency [*65*]*
Oxidant damage to organs and tissues
Lipid peroxidation
Inhibited ciliary beat function
Decreased lung surfactant
Bronchoconstriction
Increased viscoelasticity of mucus
Increased recruitment of neutrophils
Decreased *S*-nitrosoglutathione (GSNO) and nitric oxide (NO)
Dysregulation of inflammatory-immune system mediators

Table 3

Current CF Foundation recommendations for antioxidant supplementation [109]

Supplement	How often to measure levels	Recommended supplementation dose
β-Carotene	At physician's discretion	None
Vitamin A	Annually	0 - 12 months 1500 IU
		1 - 3 years 5000 IU
		4 - 8 years 5 - 10,000 IU
		>8 years 10,000 IU
Vitamin E	Annually	0 - 12 months 40 - 50 IU
		1 - 3 years 80 - 150 IU
		4 - 8 years 100 - 200 IU
		>8 years 200 - 400 IU
Zinc	Not recommended	Supplementation is reasonable in those with suboptimal vitamin A status or with night blindness that does not respond to vitamin A supplementation. A 6-month supplementation trial of Zn should be considered for children who are not growing well.
Other non-fat-soluble vitamins and minerals		A standard, age-appropriate dose of non-fat-soluble multivitamins should be given daily.

Table 4

β-Carotene supplementation studies in cystic fibrosis

References	Study period	Dose		n	CF patients		
					Plasma β-carotene		
	Months	mg kg^{-1} day^{-1}	mg day^{-1}		Baseline μmol/1	Final μmol/1	Healthy controls μmol/1
Winklhofer-Roob et al. [128]	3	0.5	-	32	0.09±0.06	1.07±0.86	0.99±0.41
Winklhofer-Roob et al. [140]	16	0.5	-	33	0.09±0.06	1.06±1.09	0.87±0.56
Homnick et al. [141]	12	-	144(60-240)	5	0.09±0.02	0.62±0.19	-
Homnick et al. [141]	2	-	60	5	0.09±0.02	0.22±0.11[a]	-
Homnick et al. [141]	?	-	120	5	0.09±0.02	0.4±0.17[a]	-
Rust et al. [130]	3	1	Max 50	13	0.08±0.04	0.6±0.4 [b]	0.30±0.1
Rust et al. [130]	3	-	10	13	0.08±0.04	0.3±0.2 [b]	0.30±0.1
Cobanoglu et al. [132]	6	1.07±0.57	-	15	1.08[c](0.3-2.9)	2.4[c](0.38-8.8)	2.12[c](0.14-2.9)
Lepage et al. [129]	2	-	13.3	12	0.08±0.03	3.99±0.92	0.37±0.04
Wood [133]	2	-	25	24	0.00±0.02	Δ0.1±0.04	Nd

[a] Increase from baseline.

[b] Subsequent study periods.

[c] Serum concentrations (μg ml^{-1}), median (minimum-maximum).

Table 5

Reported effects of β-carotene supplementation in CF

Final plasma β-carotene (μmol/l)	Study period	Oxidative stress			Inflammation		ATB, days	Lung function, FEV_1	Weight* height† z score	Ref. No.
	(months)	MDA	LDL	8-iso-PGF2α	NE/α_1-PI	TNFα				
1.07±0.86	3	↓	↓	-	-	-	-	-	-	[104]
1.07±0.19	18	↓	-	-	↓	↓	-	-	↑*	[118]
3.99±0.92	2	↓	-	-	-	-	-	-	-	[105]
0.60±0.40	3	↓	-	-	-	-	↓	-	-	[119]
0.30±0.20	6	NS	-	-	-	-	NS	NS	↑†	[119]
0.07 to 1.63	6	↓	-	-	-	↓	-	↑	-	[108]
Δ0.1±0.04	2	-	-	NS	-	-	NS	NS	-	[109]

MDA, malondialdehyde; LDL, low-density lipoprotein oxidation; 8-iso-PGF2α, 8-iso-prostaglandin F(2)α; NE/α_1-PI, neutrophil elastase/α_1-PI; TNFα, tumor necrosis factor α; ATB, antibiotic treatment; FEV_1, forced expiratory volume in 1 s.