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# New Insights Into the Pathogenesis of Cystic Fibrosis

## Pivotal Role of Glutathione System Dysfunction and Implications for Therapy

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## Abstract

The Cystic Fibrosis Transmembrane Regulator (CFTR) should no longer be viewed primarily as a 'chloride channel' but recognized as a channel that also controls the efflux of other physiologically important anions, such as glutathione (GSH) and bicarbonate. More effective approaches to cystic fibrosis treatment may result from this reconceptualization of the CFTR by researchers and clinicians. For example, oxidant damage in cystic fibrosis has been assumed to be a significant part of the pathophysiology of the disease. Generally speaking, antioxidant status in cystic fibrosis is compromised. However, until recently this was seen as secondary to the excessive chemoattraction of neutrophils in this disease caused by mutation of the CFTR protein, leading to a high oxidant burden. New findings suggest that the cystic fibrosis mutations in fact cause a primary dysfunction in the system of one of the body's most important antioxidant and immune-signaling substances: the reduced GSH system. Cystic fibrosis mutations significantly decrease GSH efflux from cells without redundant channels to the CFTR; this leads to deficiency of GSH in the epithelial lining fluid of the cystic fibrosis lung, as well as in other compartments, including immune system cells and the gastrointestinal tract. This deficiency is exaggerated over time as the higher-than-normal oxidant burden of cystic fibrosis leads to successively larger decrements in GSH without the normal opportunity to fully recover physiologic levels. This GSH system dysfunction not only may be the trigger for initial depletion of other antioxidants but also may play a role in initiating the over-inflammation characteristic of cystic fibrosis. Proper GSH system functioning also affects immune system competence and mucus viscosity, both of relevance to cystic fibrosis pathophysiology. In a way, cystic fibrosis may be thought of as the first identified disease with GSH system dysfunction.

This overview provides a review of the most pertinent recent research findings in this area. Exogenous augmentation of GSH in the lung epithelial lining fluid is possible, and therapeutic approaches include administration of aerosolized buffered GSH, intravenous GSH, and oral GSH. However, it is important to remember that cystic fibrosis pathophysiology is multifactorial, and rectification of GSH system dysfunction in cystic fibrosis patients will not eliminate all harmful effects of the disease. The promising results of two clinical trials of aerosolized buffered GSH in cystic fibrosis patients have been published or accepted for publication at the time of this writing. GSH depletion in lung epithelial lining fluid has also been noted in other respiratory diseases such as COPD, idiopathic pulmonary fibrosis, and adult respiratory distress syndrome, and therapies to augment GSH may be contemplated in these diseases as well.



Cystic fibrosis disease is the result of a mutation of the cystic fibrosis transmembrane regulator (CFTR) protein, resulting in missing or defective cellular anion efflux channels in epithelial cells. Most cystic fibrosis patients ultimately die of respiratory failure, as a result of deterioration in pulmonary function. There are several causes for deterioration in lung function, including colonization of the lungs by bacteria and fungi and auto-destruction of lung tissue by excessive inflammation even in the absence of pathogen challenge (and accelerated by pathogen challenge when it occurs). The excessive inflammation has been linked to the cystic fibrosis mutation itself and is associated with greater than normal chemoattraction for neutrophils as a result of higher

constitutive levels of, for example, interleukin (IL)-8.[1-8] The oxidant burden caused by such excessive inflammation appears to overwhelm natural antioxidant defenses. The resulting damage to lung tissue allows for greater adhesion of pathogens,[9] in addition to reducing lung function directly.[10]

New research findings, however, suggest that the traditional view of disease pathogenesis needs some modification. It now appears that cystic fibrosis mutations cause a primary dysfunction in one of the most important antioxidant and immune-signaling substances: the reduced glutathione (GSH) system. Dysfunction in the GSH system may be the catalyst for initial depletion of other antioxidants and may play a role in priming and perpetuating excessive inflammation characteristic of cystic fibrosis. Nevertheless, it is important to remember that cystic fibrosis pathophysiology is multifactorial and that not all cystic fibrosis disease manifestations can be linked to GSH system dysfunction.

This modification of the traditional view is in line with cutting-edge cystic fibrosis research, which suggests that the sole focus on the CFTR channel as a chloride efflux channel has obscured other significant functions associated with it. Current research now views the CFTR channel as, at the very least, a chloride, bicarbonate, and GSH efflux channel. Undoubtedly this list will be expanded in the future. This new and fundamental shift in the way the CFTR channel is viewed may allow for the development of innovative and effective therapies for cystic fibrosis. In this article, we will focus on the CFTR as a GSH efflux channel and discuss its significance for the respiratory system. It is worth noting that preliminary studies of the role of bicarbonate secretion in the lung have shown that the lack of functional CFTR may result in a decrease in the pH of the cystic fibrosis lung, which may also have pathologic consequences;[11,12] however, this finding is disputed elsewhere.[13-15] The newly understood role of CFTR as a GSH and bicarbonate efflux channel also has important implications for gastrointestinal complications associated with cystic fibrosis. In this article, discussion will be confined to the effects of CFTR mutation on the respiratory system only.



## 1. Understanding the Role of the Glutathione (GSH) System in Normal Lung Health

Virtually all cells of the body produce thiol-reduced GSH from the three amino acids glutamine, glycine, and cysteine. Cysteine serves as the rate-limiting amino acid for GSH production. It is estimated that an adult male produces approximately 10g of GSH per day. Not only is GSH present in the cells of the body, it also bathes the extracellular spaces of the body, with high extracellular levels in organ systems that come in contact with the oxidant-rich atmosphere, such as the cornea and the lung. One of the most important roles of GSH, then, is to act as a water-soluble antioxidant. Not only can it neutralize oxidants through an enzymatic pathway utilizing GSH peroxidase, it is also capable of neutralizing oxidants directly without the use of an enzymatic pathway.[16] In cystic fibrosis, however, epithelial cells still produce GSH normally, but one result is significant impairment of the ability of cells that do not possess a channel redundant to the CFTR to efflux GSH to fulfill its functions in the extracellular milieu.

Each antioxidant system – fat-soluble, water-soluble, and enzymatic – protects the cell within its own sphere of action. Some systems operate within the cytosol, which others operate at the cell membrane or are active in the extracellular milieu. GSH operates both within the cytosol and in the extracellular milieu. In these compartments, it is capable of directly reducing oxidants and it also reacts with GSH peroxidase, located in the cell membrane, to neutralize oxidants. GSH is replenished in two ways: by interaction of GSH disulfide (GSSG) with the enzyme GSH reductase and by synthesis of GSH within the cell (i.e. *de novo* or after cleavage of extracellular GSH and transport of component amino acids back into the cell). Circulation of GSH effluxed from cells throughout the body may allow for higher levels of GSH particularly in the extracellular compartments, such as the lung.

The various antioxidant systems are interdependent, to one degree or another, for proper function. The crippling of one system leads to decreased protection by other antioxidant systems. Without GSH, as we have seen, GSH peroxidase is unable to function as an antioxidant.[17] Antioxidant systems such as GSH, ascorbic acid, tocopherol, and ubiquinol-10[18,19] are interdependent, and normal levels of each in reduced form are required to maintain normal levels of the others in reduced form.[20] In addition, GSH deficiency is linked to decreased activity of catalase and superoxide dismutase.[20] GSH deficiency also taxes the fat-soluble antioxidant systems by permitting greater levels of lipid peroxidation, yielding damaging metabolites.[20-27] This genetic chink in the antioxidant armor of cystic fibrosis patients predisposes them to have successively larger decrements in antioxidant protection over time, as other antioxidants are consumed in greater quantities or left unused as a result of impaired GSH efflux.

Furthermore, recent studies have demonstrated the importance of S-glutathiolation of proteins under conditions of oxidative or nitrosative stress.[28,29] To prevent irreversible loss of intracellular and extracellular protein function under such stress, mixed disulfides are formed between protein cysteines and GSH. These S-glutathiolated proteins are more stable and can be dethiolated by either non-enzymatic reduction or enzymatic cleavage of the disulfide bond. Thus, S-glutathiolation allows for reversible regulation, and therefore generalized protection, of sensitive proteins.

A disrupted systemic antioxidant shield leads to predictable damage to lung tissue by oxidants. Oxidants directly harm sensitive lung epithelia. In addition, they are able to inactivate antiproteases, which then leads to increased elastase damage, increased mucus secretion, and deranged immune signaling.[30-34] Oxidants also adversely affect ciliary beat function in the lung, and lung surfactant levels are diminished by the oxidant burden.[33] There is an increased production of chloramines, which further decreases epithelial integrity.[35-37] A higher oxidant burden also creates cell structure abnormalities, which may lead to impaired cell function or even premature cell death.[38-53] In the lung, generalized bronchoconstriction can be another consequence of decreased antioxidant functioning.[54] Damage to the epithelial tissue of the lung also permits greater adhesion of pathogens.[55] Oxidants can also inactivate other parts of the GSH system, such as GSH reductase and γ-glutamylcysteine transferase, both necessary for cellular protection and proper redox functionality.[56-58] Protein S-glutathiolation will decrease, resulting in irreversible loss of sensitive protein function.[29]

A second consequence of impaired GSH efflux is increased viscosity of mucus. GSH plays an important role in mucolysis of disulfide bonds in mucus, in much the same manner as the more well known cysteine donors such as N-acetylcysteine (NAC).[59] Increased viscosity of mucus has important consequences to the lung environment.[59-67]

Finally, the redox system of GSH, as indicated by the GSH **:** GSSG ratio manifesting redox potential, is an important immune system signal. The GSH **:** GSSG ratio is usually greater than 9 **:** 1, sometimes reaching over 100–200 **:** 1, depending on the compartment. When that ratio is substantially decreased or there is a decrease in total GSH (GSH + GSSG), the body appears to interpret such events as a call for assistance from the immune system to cope with some threatening challenge that is resulting in pathologic oxidative reactions that are outpacing GSH replenishment. For example, Day and colleagues[68,69] have found that when normal mouse lung tissue is challenged with *Pseudomonas aeruginosa*, there is a 3-fold induction in epithelial lining fluid (ELF) GSH levels and a 2-fold induction in CFTR levels, presumably to offset increased oxidative reactions. The inability to effect this large increase in ELF GSH because of ineffectual transport due to CFTR mutation will substantially alter both the redox ratio and the level of total GSH.

It is important to understand how the body reads the effects of CFTR absence or malfunction on the GSH system. Cells without channels redundant to CFTR, such as lung epithelia (whose channels redundant to the CFTR are at the basolateral, not the apical, surface), will not be able to export GSH to the extracellular milieu, and the extracellular deficit may become quite severe. Levels of total GSH within such cells may remain normal, but the GSH **:** GSSG ratio may become substantially decreased. However, immune system cells are among the class of cells that have channels redundant to the CFTR. With a growing extracellular deficit, immune system cells may actually attempt to efflux GSH to rectify that deficit. Furthermore, with the increasing oxidant burden, immune system cells may use up their stores of GSH in self-protection. Immune cells then become GSH deficient. All in all, what the body senses in cystic fibrosis is that there is some threat that is using up all of the GSH in oxidative reactions, even though that threat is nonexistent. What is really occurring in cystic fibrosis is defective GSH efflux, but the body has no way of telling the difference.

The body responds by mobilizing itself to meet the nonexistent threat. In short, it inflames. GSH deficiency in leukocytes causes increased release of oxidants such as hydrogen peroxide.[70] Cellular GSH deficiency causes increased transcription of nuclear factor-κB, which then codes for greater levels of inflammatory cytokines, such as tumor necrosis factor-α, activator protein-1, monocyte chemoattractant protein-1, IL-8, and IL-1a.[71-85] Such a cytokine profile creates inflammation and recruitment of neutrophils and macrophages even in the absence of a threat, which is precisely what occurs in cystic fibrosis. (Of course, when a pathogen threat does present itself, the inflammation becomes even more excessive.) As long as full GSH replenishment cannot occur (because of defective GSH efflux from most of the cells of the body), the inflammation will continue and become chronic, as it is in cystic fibrosis.

In addition to chronic inflammation, the continuing inability to replenish GSH, especially in immune cells, creates a situation of immune incompetence. GSH deficiency in leukocytes causes, in general, impaired release of lysosomal enzymes, decreased phagocytosis, and premature apoptosis.[70,86-100] GSH deficiency also creates a situation of incomplete immune system signaling, because GSH reduction of disulfide bonds is necessary for such signaling. For example, antigen-presenting cells use the reductive power of GSH to present antigens to T cells.[101-104] B cells appear similarly affected,[105,106] and activation of T and B cells appears related to GSH levels.[107-112] Interferon-γ signaling is also dependent on the presence of GSH.[113] Such interruptions of appropriate immune signaling begin to shift the organism to a more T helper-2 type of response, which is less effective in pathogen clearing.[101,107,114-118] In addition, GSH is necessary to create a reservoir of nitric oxide (NO) [via creation of s-nitrosoglutathione (GSNO)], and the lack of such a reservoir leads to a generalized lack of NO itself in the lung environment.[119-130] NO not only has important bactericidal properties but also is necessary in cell signaling and smooth muscle relaxation and helps regulate ciliary

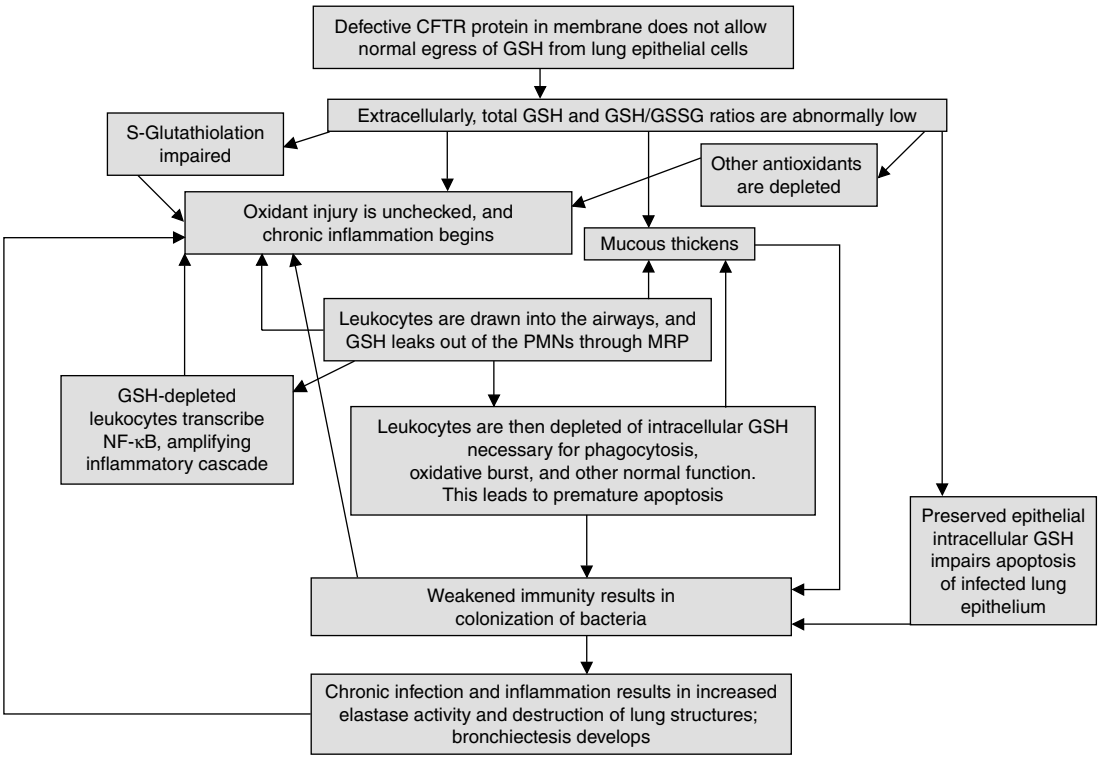


**Fig. 1.** The influence of diminished glutathione transport on the pathophysiology of cystic fibrosis. **GSH** = glutathione; **GSSG** = glutathione disulfide; **MRP** = multidrug resistance-associated protein; **NF-κB** = nucleus transcription factor κB; **PMN** = polymorphonuclear leukocyte.



beat function.[131-137] When antioxidant defenses are compromised, superoxide anion will scavenge NO almost instantaneously, the reaction limited only by the extent of diffusion. The depletion of NO has important consequences for both lung function and immune function.

In short, then, a generalized GSH deficiency will cause inflammation coupled, paradoxically, with decreased immune system competence to clear pathogens. These effects are in addition to the loss of antioxidant protection and mucolytic activity noted above in connection with GSH deficiency. The CFTR mutations that cause cystic fibrosis produce these consequences as a result of severely impaired efflux of GSH from most cells of the body. Figure 1 represents a summary of how diminished GSH transport influences the pathophysiology of cystic fibrosis.

## 2. GSH System Dysfunction in Cystic Fibrosis

The evidence for a primary GSH system dysfunction in cystic fibrosis is steadily growing.

### 2.1 Effect of CFTR Mutation on Efflux of GSH

In retrospect, it was the work of Linsdell and Hanrahan[138] that first identified that the CFTR channel played a role in the efflux of GSH. Afer clamping the CFTR channels of Chinese hamster ovary cells, Linsdell and Hanrahan compiled a list of substances that were subsequently not effluxed. GSH was on the list. Since that study published in 1998, however, it was the work of Gao and colleagues[139] that arguably pushed that insight further. Using cell lines of cystic fibrosis lung epithelia, this team was able to demonstrate markedly decreased GSH efflux. Velsor and colleagues[140] also found a 50% reduction in GSH levels in the ELF of the lung in uninfected CFTR knockout mice and a lack of normal induction of GSH in the ELF when challenged by *P. aeruginosa*.[69] Kogan and colleagues[141,142] found this same diminished GSH efflux with a variety of CFTR mutants, including G551D, R347D, K464A, and K1250A, and through the use of sophisticated tests were able to confirm that purified CFTR protein alone directly mediated nucleotide-dependent GSH flux and not via other associated chloride transport proteins.[142] Finder et al.[143] also document a 49% reduction of GSH in bronchoalveolar lavage fluid from cystic patients with fibrosis. The findings from these five research teams constitute the 'smoking gun': cystic fibrosis directly causes significantly impaired GSH efflux.

### 2.2 GSH Deficiency in the Cystic Fibrosis Lung Environment

If we conceive of three distinct compartments in the cystic fibrosis lung environment, we find empirical evidence that GSH deficiency arises in the two compartments where the deficiency would be expected if a transport abnormality were at fault. The three compartments to visualize are (i) cells that do not have a channel redundant to the CFTR (at least at the apical surface), such as the epithelium of the lung; (ii) the extracellular milieu, composed primarily of the lung ELF; and (iii) cells that do have channels redundant to the CFTR (such as leukocytes or erythrocytes).

Gao and colleagues[139] found normal levels of total GSH in the first compartment, which included cystic fibrosis lung epithelial cells that did not have a channel redundant to the CFTR (at least at the apical surface). This should be expected, as there is no GSH synthesis defect in cystic fibrosis as there is, say, in AIDS. The GSH **:** GSSG ratio in these cells was not ascertained and remains to be analyzed. Note that the lack of ability to be depleted of GSH may lead to decreased levels of appropriate apoptosis when these cells are infiltrated by pathogens.

In the second compartment, consisting of ELF, several studies have found progressive GSH deficiency arising and persisting over time in patients with cystic fibrosis. Hull et al.[144] found that non-infected cystic fibrosis do not appear to have a GSH deficiency in their ELF, though only the total GSH, and not the GSH **:** GSSG ratio, was analyzed. An altered GSH **:** GSSG ratio in the ELF of infants with cystic fibrosis would be evidence that the impaired GSH efflux has begun to have an impact in the respiratory system of these patients.[145,146] Hull et al. did find that infected infants with cystic fibrosis infants had slightly lower total levels of GSH in their ELF. Brown et al.[147] determined that, beyond infancy, plasma sulfhydryls decreased significantly with age in patients with cystic fibrosis. Roum et al.[148] found a profound deficiency of GSH in the ELF in adult patients with cystic fibrosis, with levels of 5–10% of normal when oxidant burden was factored in. This team also found plasma GSH levels of about 50% of normal in these adult patients with cystic fibrosis; in both the ELF and plasma, they also found an extremely decreased GSH **:** GSSG ratio. Finder et al.[143] found a 49% reduction of GSH in bronchoalveolar lavage fluid from patients with cystic fibrosis.

In the third compartment, including cells with channels redundant to the CFTR, in the lung environment, Tirouvanziam[149] found substantially lower levels of GSH in sputum neutrophils from patients with cystic fibrosis compared with blood neutrophils, which also correlated with cell death rates. Furthermore, in this compartment, alterations in the expression of channels redundant to the CFTR have also been noted: for example, multidrug resistance-associated protein 1 has been shown to be upregulated in cystic fibrosis erythrocytes.[150]

### 2.3 Related Phenomena

As part of this hypothesis of mutation-derived GSH efflux impairment, we should find that, other than the effects of the mutation, the rest of the GSH antioxidant system remains intact in patients with cystic fibrosis. This is in fact the case: at least normal levels of GSH peroxidase and γ-glutamylcysteine synthetase and increased levels of γ-glutamylcysteine transferase, γ-glutamyl transpeptidase, and GSH reductase have been found in patients with cystic fibrosis.[17,144,148,151-153] Therefore, the observed GSH deficiency is a primary deficiency not caused by malfunctions in other parts of the overall GSH antioxidant system.

### 2.4 Impaired GSH Efflux and Cystic Fibrosis Pathology

Many of the effects that would be expected from impaired GSH efflux in most cells have been noted as part of cystic fibrosis pathology.

Inflammation in the absence of pathogen challenge has been noted in the youngest of infants with cystic fibrosis.[1,2,154-157] Other antioxidant systems of the body have been found to be compromised in cystic fibrosis. This is in part a result of fat malabsorption due to pancreatic insufficiency in most patients with cystic fibrosis, resulting in lower levels of retinol and tocopherol, but this situation can be accelerated and aggravated by GSH deficiency. Affected systems include at least retinol, betacarotene, tocopherol, activity of GSH peroxidase, ascorbic acid, and activity of superoxide dismutase.[17,158-163] Antiproteases have been shown to be neutralized in cystic fibrosis, and surfactant levels are lower.[164,165] There is an altered cytokine profile consonant with GSH deficiency, and also exhaled NO is not elevated, as it is in other respiratory diseases with a high oxidant burden.[1-8,166-178] Interestingly, *in vitro*, the addition of S-nitrosoglutathione to delF508 cystic fibrosis cell lines has been shown to help in the maturation and functionality of the mutated protein.[179,180] Furthermore, decreased apoptosis has been noted in pathogen-infiltrated cells without redundant anion channels in cystic fibrosis.[181]

In conclusion, the view that CFTR acts as an important GSH efflux channel is gaining strength through recent empirical research findings. In addition to several 'smoking guns', the related phenomena and effects that one would expect if cystic fibrosis caused GSH efflux impairment are also empirically demonstrable (figure 1). This evidence leads us to inquire about therapeutic implications. However, it is important to remember that cystic fibrosis pathophysiology is multifactorial, and rectification of

GSH system dysfunction in patients with cystic fibrosis will not eliminate all harmful effects of the disease.

## 3. Therapeutic Implications

The usual and most direct route to augment GSH levels is to provide a cysteine donor, such as NAC, to the patient. As cysteine is the rate-limiting amino acid for GSH synthesis, this route is generally effective in otherwise healthy individuals. However, as we have seen, GSH synthesis is not impaired in cystic fibrosis; the problem is in GSH efflux from the cells in which GSH is synthesized. Nevertheless, Hosseini and colleagues[182] have used a cysteine-rich whey powder to treat C57B1/6 mice infected with *Pseudomonas* sp. and noted some improvement in mortality; therefore, precursors might usefully complement a strategy of exogenous GSH augmentation.

Direct augmentation of GSH levels in the ELF with aerosolized GSH has been carried out *in vivo*, including in patients with cystic fibrosis, AIDS, idiopathic pulmonary fibrosis (IPF), COPD, and other diseases.[183-191] Unfortunately, GSH in solution has a pH of 2.7 and is an irritant to the lung. This has hampered the usefulness of this therapy for patients with respiratory ailments. Two clinical trials, using buffered GSH with a pH of 5–6, have been carried out in patients with cystic fibrosis.

In the first trial, using the AKITA® [1] inhalation device, Griese et al.[192] were able to increase the GSH level in bronchoalveolar lavage fluid in patients with cystic fibrosis through inhalation of a buffered GSH solution. One hour after inhalation, GSH levels increased 3- to 4-fold, and at 12 hours levels of GSH were still almost double those at baseline. Griese et al. found that with 14 days' use of three-times-daily buffered GSH 300–450mg, $FEV_1$ and FVC increased an average of 6–7% over baseline ($p < 0.001$).[193] No change in oxidative markers was observed, though this might be because of the short duration of therapy.

The second study carried out by Bishop et al.[194,195] was a randomized, double-blind, placebo-controlled trial. The dosage of buffered GSH was 66 mg/kg/day, divided into four inhalation sessions over a 6-week period. Results indicated that 11 of 13 clinical indicators examined favored the GSH treatment group over the placebo group, including lung function scores, and statistical significance was achieved in improvement in several of the indicators, including peak flow, and in compliance analysis and cough.

The results of these two clinical trials of aerosolized buffered GSH are very promising and warrant larger, multicenter trials of longer duration.

Oral administration of GSH is not to be overlooked. Previously, researchers could not agree on whether GSH was cleaved in the digestive tract or taken up intact in the jejunum. Newer studies seem to indicate the latter.[196-200] Furthermore, there is new and innovative research being conducted to create a novel peptide that could serve as a GSH efflux for cystic fibrosis cells.[201] Finally, intravenous GSH has been used as a treatment for radiation poisoning, as well as for other diseases such as Parkinson disease.[183,202,203] Given that it is most likely that the lung is a net importer of circulating GSH, this route might bear further investigation in the case of cystic fibrosis.

Other respiratory ailments are marked by a decrease in GSH in the ELF. Clinicians treating such illnesses may want to examine GSH augmentation in diseases such as adult respiratory distress syndrome (ARDS), COPD, idiopathic interstitial pneumonia, IPF of nonsmokers, idiopathic respiratory distress syndrome, and diffuse fibrosing alveolitis.

In summary, augmentation of GSH in the ELF is feasible and may be useful not only for cystic fibrosis, but also for several other respiratory conditions. Given the vasodilatory and anti-inflammatory properties of GSH, there may be contraindications to its use. It could be speculated that in patients with a history of hemoptysis/pneumothorax, those yielding a positive culture for *Burkholderia cepacia*, or those with an $FEV_1$ <30% predicted, the use of GSH may be contraindicated until further, more extensive trials have been conducted.

## 4. Conclusion

In conclusion, new research is beginning to alter our understanding of the CFTR channel. It is no longer possible to view it merely or even primarily as a chloride efflux channel. At this point in time, it must be viewed as a chloride/GSH/bicarbonate channel, though this list may grow in the future. As we more fully understand the nature and functions of the CFTR channel, new therapeutic approaches will begin to come into view, as we have seen with GSH. In one respect, cystic fibrosis may be viewed (at least in part) as the first identified disease with GSH transport dysfunction.

Clinicians may be able to make effective use of these new insights from cutting-edge research. However, it is important to remember that the pathophysiology of cystic fibrosis is multifactorial, and rectification of GSH system dysfunction in cystic fibrosis patients will not eliminate all harmful effects of the disease. Indeed, some clinicians report that a significant proportion of their patients with cystic fibrosis are already using GSH and/or NAC without the physician's knowledge.[204] Clinicians should inquire from their patients with cystic fibrosis if they are

**1** The use of trade names is for product identification purposes only and does not imply endorsement.

already using GSH/NAC, in order to monitor subsequent developments. In addition, patients may be unaware of possible contraindications, the importance of pH adjustment for aerosol use, or the inherent problems connected with NAC or other cysteine donor use alone. In short, given increasing patient interest in a growing body of empirical evidence demonstrating mutation-derived GSH system dysfunction in cystic fibrosis, it behooves the clinician to learn as much about this area of research as possible in order to treat cystic fibrosis patients in a more informed manner. Furthermore, clinicians may find GSH augmentation a useful intervention for other respiratory diseases, as noted in section 3, such as COPD, ARDS, and IPF.

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