Nasal and Exhaled Nitric Oxide Is Reduced in Adult Patients With Cystic Fibrosis and Does Not Correlate With Cystic Fibrosis Genotype*

Stephen R. Thomas, MD; Sergei A. Kharitonov, MD, PhD; Sandra F. Scott; Margaret E. Hodson, MD, MSc, DM; and Peter J. Barnes, MA, DM, DSc

Study objectives: Inducible nitric oxide synthase (iNOS) is upregulated in a number of inflammatory lung conditions, and exhaled nitric oxide (NO) concentration is increased. However, previous studies in children with cystic fibrosis (CF) have shown that exhaled NO is reduced. The purpose of this investigation was to study exhaled NO concentration in adults with CF, and to investigate the effect of CF genotype and respiratory tract infection on this measurement.

Design: Exhaled and nasal NO levels were measured in 54 adult CF subjects and 37 healthy nonsmoking age-matched subjects using a chemiluminescence analyzer. Spirometry (FEV₁ and FVC), CF genotype, and bacterial colonization were also recorded.

Setting: This study was conducted at a national CF center.

Results: The mean age of patients was 26.9 years, and the mean FEV₁ was 50.5% predicted (range, 17 to 104%). Nasal NO in the CF patients (mean, 520 parts per billion [ppb]; confidence interval [CI], 452 to 588) was significantly lower (p < 0.001) than in control subjects (987 ppb; CI, 959 to 1,015). Exhaled NO was significantly lower (p < 0.001) in CF patients (5.0 ppb; CI, 4.1 to 6.1) than in control subjects (7.3 ppb; CI, 6.8 to 7.8). FEV₁ did not correlate with nasal or exhaled NO. No association was observed between genotype and NO values or colonization with Pseudomonas aeruginosa.

Conclusions: Despite the airway inflammation that is characteristic of CF, both nasal and exhaled NO were reduced. There was no association with genotype or infection status. As NO has bacteriostatic effects and may augment mucociliary clearance, this observation may be of clinical importance.

Key words: airway inflammation; cystic fibrosis; nitric oxide; nitric oxide synthase

Abbreviations: CF = cystic fibrosis; CI = confidence interval; IL = interleukin; iNOS = inducible NOS; NO = nitric oxide; NOS = NO synthase; ppb = parts per billion; TNF = tumor necrosis factor

Cystic fibrosis (CF) is characterized by a widespread abnormality of epithelial surfaces, and in terms of both morbidity and mortality, the most important pathology is pulmonary.1 CF is caused by a variety of mutations of the CF transmembrane regulator gene on chromosome 72; although the pathogenesis of the disease is not fully understood, patients experience recurrent respiratory infections, and the airways are usually colonized initially by Staphylococcus aureus and later by Pseudomonas aeruginosa. Infection is associated with neutrophilic inflammation, and there are elevated concentrations of several cytokines, such as interleukin (IL)-8, IL-1β, and tumor necrosis factor (TNF)-α in sputum and BAL.3–6

Nitric oxide (NO) has increasingly been recognized as having an important signaling role in the regulation of a variety of physiologic functions,7 and NO is believed to participate in the pathophysiology of a variety of inflammatory disorders. NO can be detected in exhaled air in humans and animals.8 NO is synthesized by isoforms of NO synthase (NOS) from L-arginine in a wide variety of cell types.9 Neuronal NOS and endothelial cell NOS are consti-
tutively expressed by neuronal and endothelial cells. Inducible NOS (iNOS) is expressed by a variety of cell types including macrophages, neutrophils, and bronchial epithelial cells. Upregulation of iNOS occurs at the level of transcription by proinflammatory cytokines, such as TNF-α, interferon-γ, and IL-1β. iNOS is expressed in the bronchial epithelium in asthma patients, and this correlates with elevated concentrations of NO in exhaled air.

In patients with bronchiectasis that is not associated with either CF or disorders of ciliary motility, elevated levels of exhaled NO have been related to the extent of bronchiectasis. In view of the gross airway inflammation and bronchiectasis that occurs in CF, there has been an interest in the measurement of exhaled and nasal NO in this disorder. Earlier studies, mainly in pediatric CF patients, have shown that exhaled NO is not increased in this disorder and nasal NO is even reduced. These results would appear to be consistent with the observed absence of iNOS expression in the airway epithelium of the CF mouse and in airway epithelial cells from CF patients, suggesting that the CF gene defect in some way results in a failure of iNOS expression. If this is the case, then differences in NO production between different genotypes might be anticipated. The aim of this study was to establish what the true effect of CF was on exhaled NO in adult patients, and investigate the effect of genotype and infection status on exhaled NO.

**Materials and Methods**

Patients were recruited from the Adult Cystic Fibrosis Clinic at Royal Brompton Hospital. The patients who were selected were not using either oral or inhaled corticosteroids, as corticosteroids may reduce exhaled NO levels. Patients colonized by *P aeruginosa* were excluded from the study to avoid concerns about cross-infection from shared equipment. Fifty-four CF patients (mean age, 26.9 years; range, 17 to 46 years) and 37 age-matched healthy control subjects (mean age, 30.0 years) were recruited. The patients who were selected were approved by the Royal Brompton Hospital Medical Ethics Committee, and informed consent was obtained from each subject. NO was measured using a chemiluminescence analyzer (Model LR2000; Logan Research, Rochester, UK) sensitive to NO from 1 to 5,000 parts per billion (ppb) by volume, and with a resolution of 0.3 ppb. Measurements were all made in the afternoon. Average ambient levels of NO were from 1 to 10 ppb.

The analyzer was designed for on-line recording of exhaled NO concentration. The response time of this analyzer was <0.5 s and achieved a high reproducibility. The analyzer was equipped with a feedback control unit that maintained a constant pressure and flow. The sampling rate was 250 mL/min. The analyzer was calibrated using certified NO mixtures (90 ppb and 500 ppb) in nitrogen and certified 5% CO₂ (BOC Special Gasses; Surrey Research Park, Guildford, UK). Measurements were performed in triplicate, and mean values were calculated. Data on airway bacterial colonization and CF genotype was obtained from the patients' case notes. As the data for exhaled NO were normally distributed only after log transformation, the geometric mean for each group was calculated. Student's t tests were used to make comparisons between groups. A p value <0.05 was considered statistically significant.

**Results**

Both exhaled and nasal NO were reduced in the CF subjects (mean age, 26.9 years; range, 17 to 46 years; FEV₁, 51 ± 3% predicted; FVC 75 ± 3% predicted) compared with control subjects (mean age, 30 years; range, 27 to 46 years; FEV₁, 96 ± 4% predicted; FVC, 96 ± 4% predicted). Mean nasal NO in the CF subjects was significantly lower (520 ppb; 95% confidence interval [CI], 452 to 588 ppb) than in control subjects (987 ppb; CI, 959 to 1015; p < 0.0001; Fig 1). Geometric mean exhaled NO was also significantly lower in CF subjects (5.0 ppb; CI, 4.1 to 6.1) than in control subjects (7.3 ppb; CI, 7.1 to 7.5; p < 0.001; Fig 2).

In patients not colonized by *P aeruginosa* (n = 9), exhaled NO was 6.6 ppb (CI, 4.2 to 10.5); in

![Graph showing nasal NO in CF patients and healthy control subjects. Control subjects had higher NO levels than CF patients.](http://example.com/graph.png)

**Figure 1.** Nasal NO in CF patients and healthy control subjects. Results are expressed as mean with 95% CIs (p < 0.001).
Colonized patients (n = 43), mean exhaled NO was 4.8 ppb (CI, 4.5 to 5.6; p = 0.20). In colonized patients, mean nasal NO was 556 ppb (CI, 343 to 770); in noncolonized patients, mean nasal NO was 513 ppb (CI, 437 to 588). Nasal and exhaled NO did not correlate with FEV1 percent predicted. There was a trend toward both exhaled and nasal NO being higher in patients who were not homozygous for the ΔF508 CF transmembrane regulator mutation. Geometric mean exhaled NO in ΔF508 homozygotes (n = 20) was 4.3 ppb (CI, 3.3 to 5.7); for other subjects (n = 23), it was 5.3 ppb (CI, 4.0 to 6.9; not significant). Mean nasal NO in ΔF 508 homozygotes was 487 ppb (CI, 373 to 600); for other subjects, it was 529 ppb (CI, 431 to 626; not significant).

**DISCUSSION**

These observations show that exhaled and nasal NO is reduced in adult patients with CF, and there does not appear to be any correlation between exhaled and nasal NO and genotype, or with infection status.

The cellular origin of airway NO is not yet certain. Studies of perfused porcine lungs suggest that exhaled NO originates in the alveolar surface rather than from the pulmonary circulation.19 Exhaled NO peaks at the start of the CO2 plateau during exhalation.20 This would be compatible with the hypothesis that NO is predominantly formed in the respiratory and terminal bronchioles, rather than in the alveoli. A subsequent study has confirmed this observation.21 In normal individuals, exhaled NO may originate from endothelial cell NOS22 and iNOS in the bronchial epithelium.11,23 In patients with asthma, up-regulation of iNOS expression has been demonstrated on the bronchial epithelium,11 and this correlates with elevated concentrations of exhaled NO.12,24 In those with coexisting rhinitis, nasal NO is also increased.25

In CF, elevated NO levels might be anticipated. In patients with bronchiectasis, elevated levels of exhaled NO have been observed.13 In CF, there is evidence of airway inflammation early in the disease.26,27 As cytokines that are known to upregulate iNOS expression such as IL-1β4,5 and TNF-α5 are found in increased concentrations in sputum and BAL fluid from CF subjects, elevated concentrations of exhaled NO would be anticipated. Lipopolysaccharide also induces iNOS expression mainly through cytokine-dependent pathways,28 and this would be expected to potentiate iNOS expression in CF subjects colonized by *P. aeruginosa* or other Gram-negative organisms.

The low NO levels therefore seem initially to be paradoxical, but there are several possible explanations for these observations. The first possibility is that there is a defect of iNOS expression in patients with CF. An earlier study demonstrated that NO synthase activity is increased in lung homogenate,29 but the localization of NO synthesis was not investigated in this study; in view of the short half-life of NO, and as it complexes avidly to hemoglobin,7 it is possible that only NO produced by airway epithelial cells or within the airway lumen would have the potential to diffuse into the gaseous phase. Two studies have been conducted that suggest that there is reduced iNOS expression in the CF airway compared with control subjects. In the first of these,30 there was reduced iNOS expression in CF bronchial epithelium from explanted lung tissue, and cytokines increased iNOS messenger RNA in a non-CF cell line, but not in CF cells. The second study18 confirmed the absence of iNOS expression in the CF airway in both CF mice and the human trachea. The mechanism of this defect is unclear, but there may be abnormalities of other cellular proteins in CF; for example, phosphorylation of calmodulin-binding protein is defective in CF.31 Since iNOS may also be phosphorylated,32 similar mechanisms may apply.

Alternatively, the thick mucus lining the airways could result in poor diffusion into the gaseous phase. The rate of degradation of NO in the aqueous phase is very rapid,7 and an increase in the volume of secretions in the airways would be likely to reduce the concentration of NO in exhaled air. The nasal sinuses are usually affected in patients with CF,33 and obstructed sinus ostia may account for the low...
levels of nasal NO, as it is particularly elevated in the nasal sinuses,34 and obstruction will block diffusion into the nose. Finally, there may be increased degradation of NO. NO reacts with a number of substances produced by inflammatory cells; for example, neutrophil superoxides react with NO to produce peroxynitrite. The P. aeruginosa exoprotein pycocyanin inactivates NO at concentrations well below those found in CF sputum.35 Nitrite and nitrate are formed as a result of NO degradation. In view of observed raised concentrations of nitrite and nitrate in sputum obtained from CF patients,36,37 it is possible that the observed low concentrations of airway NO relate to poor diffusion or increased degradation.

There is evidence that NO has bacteriostatic effects at concentrations found in the nose38 and increases ciliary beat frequency.39 NO may also reduce the airway epithelial Na+ hyperabsorption that is characteristic of CF.18 These effects are likely to be beneficial in CF patients. The low levels of NO that are observed may therefore be clinically relevant and predispose to infection and poor clearance of inflammatory products.

REFERENCES

31 Short DK, Dormer RL, Goodchild MC, et al. Defective phosphorylation of a calmodulin-binding protein in cystic-
37 Francoeur C, Denis M. Nitric oxide and interleukin-8 as inflammatory components of cystic fibrosis. Inflammation 1995; 19:587–598