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Flow-independent Nitric Oxide Exchange Parameters in Cystic Fibrosis

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Exhaled nitric oxide (NO) remains a promising noninvasive index for monitoring inflammatory lung diseases; however, the plateau concentration (CNO,plat) is nonspecific and requires a constant exhalation flow rate. We utilized a new technique that employs a variable flow rate to estimate key flow-independent parameters characteristic of NO exchange in a group (n = 9) of 10 to 14 yr-old healthy children and children with cystic fibrosis (CF); maximum flux of NO from the airways (JNO,max pl s⁻¹), diffusing capacity of NO in the airways (DNO,air pl s⁻¹ ppb⁻¹), steady-state alveolar concentration (Ctiss,air ppb), and mean tissue concentration of NO in the airways (Ctiss,air ppb). We determined the following mean (± SD) values in the healthy children and patients with CF for JNO,max,max, DNO,air, Ctiss,air, and Ctiss,air respectively: 784 ± 465 and 607 ± 648 pl s⁻¹; 4.82 ± 3.07 and 17.6 ± 12.1 pl s⁻¹ ppb⁻¹; 4.63 ± 3.59 and 1.96 ± 1.18 ppb; and 198 ± 131 and 38 ± 25 ppb. DNO,air for healthy control subjects and patients with CF are not statistically different at both exhalation flow rates of 50 ml/s (17.5 ± 11.5 and 11.5 ± 8.97) and at 250 ml/s (7.11 ± 5.36 and 4.28 ± 3.43). We conclude that DNO,air, Ctiss,air and CNO,air may be useful noninvasive markers of CF.

Keywords: nitric oxide; parameter estimation; airways; inflammation; healthy children

Nitric oxide (NO) is an important endogenous mediator that arises from the airways and alveoli and can be detected in the exhaled breath (1, 2). Several inflammatory disorders, such as asthma, are associated with elevated exhaled NO concentrations (3–7). Although plateau NO concentration (CNO,plat) in patients with cystic fibrosis (CF), a congenital lung disease characteristic of NO exchange in a group (n = 9) of 10 to 14 yr-old healthy children and children with cystic fibrosis (CF); maximum flux of NO from the airways (JNO,max pl s⁻¹), diffusing capacity of NO in the airways (DNO,air pl s⁻¹ ppb⁻¹), steady-state alveolar concentration (Ctiss,air ppb), and mean (over radial position) concentration of NO within the tissue phase (Ctiss,air ppb, equal to the ratio JNO,max/DNO,air). These provide potentially useful and reproducible information regarding net production and diffusion of NO in the airways and alveolar regions of the lungs. Thus, by providing a more detailed description of NO exchange dynamics throughout the lung, we hypothesize that this new technique will distinguish NO exchange dynamics in patients with CF from healthy children.

METHODS

Subjects

Nine healthy children and nine children with previously diagnosed CF (ages 10–14) participated in this study. Subjects were categorized as healthy on the basis of standard spirometry (FEV₁ > 75% of predicted on the basis of their race, age, and height) and no clinical history of chronic lung disease. Subject characteristics are presented in Table 1 including details of their clinical history. The Institutional Review Board at the University of California, Irvine approved the protocol, and informed consent was obtained from subjects and parents.

Experimental Protocol

Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) were measured in all subjects (Vmax229; Sensormedics, Yorba Linda, CA) by using the best performance (see Table 1) from three consecutive maneuvers before measuring the indices of NO exchange dynamics.

Each subject performed two types of exhalation maneuvers: (1) vital capacity maneuvers in triplicate at a constant exhalation flow of ~ 50 ml/s and ~ 250 ml/s according to the ATS (American Thoracic Society) and the ERS (European Respiratory Society) (17, 18) to determine CNO,plat without breathhold; (2) five repetitions of a 20-s preexpiratory breathhold followed by a decreasing flow rate maneuver (16). During the breathhold, a positive pressure of > 5 cm H₂O was maintained to prevent nasal contamination (17). A schematic of the experimental apparatus has been previously presented (16). After the breathhold, the exhalation valve was opened allowing the patient to expire. The expiratory flow rate progressively decreased during the exhalation from 6% to 1% of vital capacity per second using a Starling resistor (Hans Rudolph Inc., Kansas City, MO) with a variable resistance.

Airstream Analysis

A chemiluminescence NO analyzer (NOA280; Sievers, Inc., Boulder, CO) was used to measure the exhaled NO concentration. The instrument was calibrated on a daily basis using a certified NO gas (45 ppm in N₂;Sievers, Inc.). The zero point calibration was performed with an NO filter (Sievers, Inc.) and done immediately before the collection of a profile. The flow rate and pressure signals were measured using a pneumotachometer (RSS100; Hans Rudolph Inc.); The pneumotachometer
TABLE 1. PHYSICAL CHARACTERISTICS OF SUBJECTS

| Subject | Sex | Age (yr) | Hgt (in) | Wgt (lb) | lwgt (lb) | $V_{air}$ (ml) | FVC (l) | % pred | FEV$_1$ (l) | % pred | FEV$_1$/FVC |
|---------|-----|----------|----------|----------|-----------|---------------|--------|--------|-------------|--------|-------------|
| (A) Healthy Children | | | | | | | | | | | |
| 1 | F | 13 | 63 | 134 | 123 | 136 | 2.88 | 94 | 2.55 | 94 | 95 |
| 2 | F | 11 | 58 | 97 | 108 | 119 | 3.05 | 111 | 2.55 | 106 | 84 |
| 3 | M | 11 | 59 | 115 | 119 | 130 | 2.93 | 112 | 2.54 | 115 | 94 |
| 4 | M | 12 | 60 | 88 | 122 | 134 | 3.78 | 121 | 3.02 | 115 | 81 |
| 5 | F | 11 | 63 | 129 | 124 | 135 | 2.79 | 98 | 2.22 | 90 | 81 |
| 6 | M | 12 | 65 | 161 | 141 | 153 | 4.11 | 122 | 3.43 | 117 | 83 |
| 7 | F | 10 | 55 | 75 | 98 | 108 | 3.10 | 103 | 1.96 | 100 | 87 |
| 8 | F | 14 | 63 | 106 | 103 | 137 | 3.35 | 107 | 2.70 | 97 | 83 |
| 9 | M | 12 | 61 | 119 | 126 | 138 | 3.06 | 103 | 2.44 | 94 | 80 |
| Mean | | 12 | 61 | 114 | 120 | 132 | 3.14 | 108 | 2.6 | 103 | 85 |
| (B) Patients with Cystic Fibrosis | | | | | | | | | | | |
| 1 | M | 12 | 61 | 119 | 126 | 138 | 3.06 | 103 | 2.44 | 94 | 80 |
| 2 | F | 14 | 63 | 106 | 123 | 137 | 3.35 | 107 | 2.70 | 97 | 83 |
| 3 | F | 10 | 55 | 75 | 98 | 108 | 2.34 | 103 | 1.96 | 100 | 87 |
| 4 | M | 12 | 65 | 161 | 141 | 153 | 4.11 | 122 | 3.43 | 117 | 83 |
| 5 | F | 11 | 63 | 129 | 124 | 135 | 3.78 | 121 | 3.02 | 115 | 81 |
| 6 | M | 12 | 60 | 88 | 122 | 134 | 2.79 | 98 | 2.22 | 90 | 81 |
| 7 | F | 10 | 55 | 75 | 98 | 108 | 3.10 | 103 | 1.96 | 100 | 87 |
| 8 | F | 14 | 63 | 106 | 103 | 137 | 3.35 | 107 | 2.70 | 97 | 83 |
| 9 | M | 12 | 61 | 119 | 126 | 138 | 3.06 | 103 | 2.44 | 94 | 80 |
| Mean | | 12 | 61 | 114 | 120 | 132 | 3.14 | 108 | 2.6 | 103 | 85 |
| (C) Clinical History of the Patients with Cystic Fibrosis | | | | | | | | | | | |
| Subject | Genotype | Diagnosis | Penetration | Organisms | Atopy | RAD | Therapies |
|----------|-----------|-----------|-------------|-----------|-------|-----|----------|
| 1 | ^f508 2789 + 56-A | Mild lung disease | SA | Yes | Yes | Albuterol, intal, enzymes, DNase, motrin, inhaled str |
| 2 | ^f508 | Fetus | Sinus | SA, PSA | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 3 | ^f508 | 1989 | Sinus | PSA | Yes | — | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 4 | ^f508 | 1990 | Sinus | SA, PSA | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 5 | ^f508 | 1990 | Sinus | SA, PSA | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 6 | ^f508 | 1993 | Sinus | PSA, MRSA | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 7 | g542x | 1996 | Hyperplenia, FTT, Dm, sinus, hemoptysis, thrombocytopenia | SA, STENO | Yes | — | Albuterol, intal, enzymes, DNase, nasal str, insulin, actigal |
| 8 | ^f508 | 1996 | Moderate lung disease | SA, PSA | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |
| 9 | — | Severe lung disease | SA, STENO | Yes | Yes | Albuterol, intal, enzymes, DNase, tobi, nasal str, inhaled str |

Definition of abbreviations: ABPA = allergic bronchopulmonary aspergillosis; Dm = diabetes mellitus; FEV$_1$ = forced expiratory volume in 1 s; FVC = forced vital capacity; FTT = failure to thrive; GTUBE = gastrostomy tube; Hgt = height; lwgt = ideal body weight; MRSA = methicillin-resistant Staphylococcus aureus; PSA = Pseudomonas aeruginosa species; PSAM = Pseudomonas aeruginosa mucoid strain; PSANM = Pseudomonas aeruginosa nonmucoid strain; RAD = reactive airways disease; SA = Staphylococcus aureus; STENO = Stenotrophomonas maltophilia; str = steroid; lwgt = body weight.

* Statistically different from healthy control subjects (t test with $\alpha < 0.05$).
Thus, airway compartment is not well mixed, but the concentration depends on the axial or longitudinal direction and is characterized by two parameters: $J_{\text{NO, max}}$ and $D_{\text{NO, air}}$. $J_{\text{NO, max}}$ is the maximum flux of NO from the airway tissue, which is equal to the airway compartment flux if the gas phase concentration, concentration (ppb) of NO in the airway compartment ($C_{\text{air}}$), were zero.

An alternative presentation of the three flow-independent parameters includes the use of the mean (over radial position) tissue concentration in the airways, $C_{\text{tiss, air}}$, instead of $J_{\text{NO, max}}$. $C_{\text{tiss, air}}$ is simply the ratio $J_{\text{NO, max}}/D_{\text{NO, air}}$. This is more easily demonstrated by expressing $J_{\text{NO}}$ in an alternate but equivalent form to Equation 1 (16, 21, 22):

$$J_{\text{NO}} = J_{\text{NO, max}} - D_{\text{NO, air}} C_{\text{air}}$$  \hspace{1cm} (I)

Note that $J_{\text{NO, max}}$ is simply the product $D_{\text{NO, air}}/H_{\text{tiss, air}}$. In Equation 2, $J_{\text{NO}}$ is expressed as proportional to the appropriate concentration difference between the airway tissue ($C_{\text{tiss, air}}$) and the gas stream ($C_{\text{air}}$).

The coefficient of proportionality is $D_{\text{NO, air}}$. Thus, $D_{\text{NO, air}}$ can be interpreted as a conductance for mass transfer of NO between the airway tissue and the gas phase.

Figures 2A (healthy) and 2C (CF) are representative experimental exhalation profiles as well as the model simulation. Figures 2B (healthy) and 2D (CF) are the corresponding exhaled volume, flow, and pressure tracings. The model does not predict phase I and II of the exhalation profile, where the accumulated NO during breathholding in the conducting airways and transition region of the lungs exits the mouth. This discrepancy is attributed to axial diffusion that our model neglects. Although the precise shape of phase I cannot be accurately simulated with the model, the absolute amount of NO in phase I and II can be predicted. Thus, our technique utilizes the information from the airway tissue, which is equal to the airway compartment flux if the gas phase concentration, concentration (ppb) of NO in the airway compartment ($C_{\text{air}}$), were zero.

Figure 1. Schematic of two-compartment model used to describe NO exchange dynamics. Exhaled NO concentration, $C_{\text{exh}}$, is the sum of two contributions—the alveolar region and the airway region—which depends on three flow-independent parameters: maximum total volumetric flux of NO from the airway wall ($J_{\text{NO, max}}$, pl s$^{-1}$), diffusing capacity of NO in the airways ($D_{\text{NO, air}}$, pl s$^{-1}$ ppb$^{-1}$), and steady-state alveolar concentration ($C_{\text{alveo}}$, ppb). $J_{\text{NO}}$ is the total flux (pl/s) of NO between the tissue and gas phase in the airway, and inversely depends on the residence time of a gas bolus and thus the exhalation flow rate, $V_e$. The expression presented for $J_{\text{NO}}$ is valid for exhalation flows larger than approximately 1% of the vital capacity per second.

Figure 2. Representative exhaled NO concentration profiles (dashed lines) and model fit (dark line) utilizing the 20-s preexpiratory breathhold followed by a decreasing flow rate maneuver are shown as a function of exhaled volume for healthy child (A) and a patient with CF (C). In addition, the corresponding exhalation flow rate, exhaled volume, and pressure tracings during the exhalation are also presented as a function of time for the healthy child (B) and the patient with CF (D). Flow rates are controlled at 6–1% of the each subject’s vital capacity per second, and > 5 cm H$_2$O of pressure is maintained through the breathing maneuver. Detailed description of parameters and regression techniques are described in the text and Appendix.
from phase I and II by forcing the model to simulate the total amount of NO eliminated in phase I and II (area under the curve as depicted in Figures 2A and 2C) of the exhalation in addition to simulating the precise exhaled concentration (ppb) \( C_{\text{exh}} \) over phase III. To ensure complete emptying of the airway compartment after breathhold, we define the transition from phase II and III as the point in the exhalation for which the slope \( dC_{\text{exh}}/dV \) of the exhalation profile is zero.

We assessed the ability of the model to simulate the profile by comparing the area under the curve in phase I and II in the experimental and theoretical data. Figure 3 presents the percentage of the experimental area under the curve predicted by the model for all 45 breathing maneuvers (nine subjects times five repeated maneuvers). It is evident that there are a few outliers (solid circles, < 80% of the experimental area under the curve) in each population, which represent profiles that the two-compartment model cannot adequately simulate. These profiles were subsequently discarded. There were no more than two discarded profiles from any individual subject. Thus, the population mean values for each of the parameters for the healthy children and patients with CF are based on exhalation profiles in which the model-predicted area under the curve for phase I and II is > 80% of the experimental area under the curve.

The intramanuever (superscript m), inrasubject (superscript s), and intrapopulation (superscript p) variability have been described previously (16), and will be characterized by the 95% confidence interval expressed as a percentage of the estimated parameter value, intramanuever 100(1−α)% confidence interval (CFm), inrasubject 100(1−α)% confidence interval (CFs), and intrapopulation 100(1−α)% confidence interval (CFp), respectively. CNO,plat and the flow-independent parameters are expressed as a mean and 95% confidence interval (CI). To determine the relation between pulmonary function (FVC and FEV1) and the estimated three flow-independent parameters, linear regression and correlation were performed. A p value of < 0.05 is considered significant.

RESULTS

FVC, FEV1, FEV1/FVC, and the clinical history of the CF subjects are presented in Table 1. Seven of the nine subjects with CF had concurrent reactive airways diseases, and all of these subjects were currently taking inhaled corticosteroids. The two subjects who did not have reactive airways disease were taking only nasal corticosteroids. FVC and FEV1 were significantly lower in CF than in healthy children (p < 0.05). Means for each of the nine subjects in each category and the population mean are presented in Figure 4. In the nine children with CF, only six were able to complete the 20-s breathhold. In the three children who were not able to hold their breath for 20 s, one held their breath for 15 s, and two for 10 s. Estimated JNO,max is not significantly different between healthy control subjects and patients with CF (p = 0.52); however, DNO,air is significantly elevated (p = 0.007) and CNO,air and CNO,tiss are significantly reduced (p = 0.05 and 0.002, respectively) in CF.

Mean values for CF for healthy children and CF are 46% and 82%, 49% and 53%, 60% and 46%, and 51% and 51% for JNO,max, DNO,air, Calv,ss, and CNO,tiss,air respectively. Thus, the variation across the population of healthy children and patients with CF is approximately the same for each of the parameters.

The population means of CNO plat and CNO for each parameter are presented in Figure 5 for the healthy control subjects and patients with CF. JNO,max and CNO,air for healthy controls are estimated with high accuracy (population mean CNO plat = 38% and 97%, respectively) relative to CNO,air (population mean CNO = 128%). For CF, the population mean for CNO for the three flow-independent parameters are 118%, 167%, and 284% for JNO,max, DNO,air, and CNO,air respectively, which are much larger than the healthy children. In contrast to CNO, population mean values for CNO for each of the parameters are not statistically different between healthy control subjects and patients with CF, and range from 26% to 65% (Figure 5).

None of the flow-independent parameters had any significant correlation with standard indices of lung volume (FVC) or airway obstruction (FEV1/FVC) for both healthy children and patients with CF.

Mean CNO,plat (± CI) from the experimental maneuvers were 20.2 ± 10.1 ppb and 10.2 ± 3.97 ppb for healthy children and 8.91 ± 5.38 ppb and 4.79 ± 2.48 ppb for patients with CF at the target flow rates of ~ 50 ml/s and ~ 250 ml/s, respectively. The experimental values for each of the individuals are presented in Table 2 with model-predicted CNO,plat in parenthesis. CNO,plat at the target flow rate of 50 ml/s and 250 ml/s are lower for patients with CF when compared with healthy control subjects; however, the mean exhalation flow rates for patients with CF were larger in both cases (Table 2). Because a larger flow rate will decrease CNO,plat, one must attempt to control for this confounding variable. We used the estimated flow-independent parameters for each of the subjects to estimate CNO,plat using the two-compartment model (see governing equation in the Appendix) at precisely an exhalation flow rate of 50 and 250 ml/s (Table 2). Mean CNO,plat (± CI) from the model were 17.5 ± 8.97 ppb and 7.11 ± 4.03 ppb for healthy children and 11.5 ± 6.89 ppb and 4.28 ± 2.64 ppb for patients with CF at 50 ml/s and 250 ml/s, respectively. The model-predicted CNO,plat are still lower for children with CF when compared with healthy children, but the difference is no longer significant (p > 0.05).

DISCUSSION

This study has estimated several flow-independent parameters characteristic of NO exchange dynamics in the lungs, which have not been measured in CF before, and compared these to the plateau concentration at a constant exhalation flow rate. When compared with healthy control subjects, we found differences in CNO,plat that were not significant if one controlled for differences in the exhalation flow rate. In contrast, the flow-independent parameters differed substantially between patients with CF and healthy control subjects. Thus, our new method provides greater sensitivity in distinguishing NO exchange dynamics between healthy control subjects and patients with CF.

The difference between CNO,plat and the flow-independent parameters is highlighted by examining the composite experimental and model-predicted exhalation profiles from the population of patients with CF and healthy control subjects (Figure 6). Figure 6A depicts the composite experimental profile for each population by taking the mean exhaled concentration at equivalent exhaled volumes. Figure 6B presents the composite model predicted profiles by using the average values for the flow-independent parameters in the model equation (see Appendix). It is evident that there are small changes in the exhaled NO concentration during phase III due to small changes.
in $C_{\text{NO,plat}}$ and $J_{\text{NO}}$. However, there are very large differences in the concentration of NO in phase I and II in which the differences in NO exchange dynamics between healthy control subjects and patients with CF are magnified during a breathhold. The magnification of the airway compartment allows one to estimate $D_{\text{NO,air}}$, $J_{\text{NO}, \text{max}}$, and $t\text{iss}_{\text{air}}$, achieve more detailed information regarding NO exchange dynamics, and distinguish healthy children from patients with CF.

Interpreting the significant increase in $D_{\text{NO,air}}$ observed in patients with CF requires understanding the factors that affect its value. Thicker and more viscous mucus present in patients with CF would tend to increase the diffusion distance for NO as well as the “ease” at which NO can diffuse. Both of these observations would decrease $D_{\text{NO,air}}$ and contrast with our experimental observation of an elevated $D_{\text{NO,air}}$.

![Figure 4](image4.png)

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![Figure 5](image5.png)

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**TABLE 2. MODEL PREDICTED AND EXPERIMENTALLY OBTAINED $C_{\text{NO,plat}}$**

| Subject | $C_{\text{NO,plat}}$ (Experimental Data [Model-Predicted]) | $C_{\text{NO,plat}}$ (Model-Predicted) |
|---------|------------------------------------------------------------|----------------------------------------|
|         | $\text{ppb}$ | $\text{ml/s}$ | $\text{ppb}$ | $\text{ml/s}$ | $\text{ml/s}$ | $\text{ml/s}$ |
| (A) Healthy Children | | | | | | |
| 1 | 14.4 (8.12)* | 64.7 | 10.8 (6.19) | 95.8 | 9.86 | 3.66 |
| 2 | 41.0 (36.2) | 47.6 | 17.7 (18.1) | 159 | 35.0 | 15.2 |
| 3 | 20.6 (19.5) | 61.6 | 13.3 (12.7) | 142 | 22.1 | 10.3 |
| 4 | 9.60 (5.83) | 44.5 | 3.23 (2.74) | 233 | 7.78 | 2.64 |
| 5 | 33.0 (30.0) | 61.1 | 15.8 (15.3) | 213 | 34.5 | 14.4 |
| 6 | 7.21 (5.63) | 90.3 | 4.75 (3.68) | 170 | 8.93 | 2.96 |
| 7 | 10.7 (9.52) | 59.7 | 6.73 (6.33) | 101 | 11.0 | 3.56 |
| 8 | 9.34 (8.61) | 68.9 | 6.36 (5.72) | 129 | 10.9 | 4.07 |
| 9 | 36.0 (33.1) | 45.6 | 13.5 (12.3) | 227 | 31.0 | 11.8 |
| Mean | 20.2 (17.7) | 60.4 | 10.2 (9.23) | 163 | 17.5 | 7.11 |
| CI | 10.1 (12.3) | 10.9 | 3.97 (5.48) | 39.9 | 8.97 | 4.03 |
| CI* | 10.1 (12.3) | 10.9 | 3.97 (5.48) | 39.9 | 8.97 | 4.03 |
| CI† | 10.1 (12.3) | 10.9 | 3.97 (5.48) | 39.9 | 8.97 | 4.03 |

| (B) Children with Cystic Fibrosis | $C_{\text{NO,plat}}$ (Model-Predicted) |
|----------------------------------|----------------------------------------|
| $\text{ppb}$ | $\text{ml/s}$ | $\text{ml/s}$ | $\text{ml/s}$ | $\text{ml/s}$ | $\text{ml/s}$ | $\text{ml/s}$ |
| 1 | 7.70 (7.21)* | 66.5 | 4.93 (3.79) | 161 | 9.05 | 2.91 |
| 2 | 25.4 (23.3) | 88.8 | 12.4 (12.8) | 240 | 32.4 | 12.5 |
| 3 | 3.67 (4.43) | 128 | 2.68 (3.02) | 238 | 8.41 | 2.94 |
| 4 | 10.6 (10.5) | 73.1 | 5.12 (4.39) | 268 | 14.1 | 4.56 |
| 5 | 6.15 (6.35) | 106 | 3.86 (4.03) | 230 | 10.7 | 3.87 |
| 6 | 7.37 (5.30) | 57.7 | 3.55 (2.89) | 141 | 5.89 | 2.12 |
| 7 | 6.10 (6.66) | 60.6 | 3.46 (4.62) | 114 | 7.50 | 3.24 |
| 8 | 4.31 (5.61) | 57.2 | 2.56 (3.19) | 149 | 5.72 | 2.62 |
| 9 | 4.80 (5.24) | 57.2 | 2.56 (3.19) | 149 | 5.72 | 2.62 |
| Mean | 8.91† (8.29) | 75.8 | 4.79† (4.61) | 188 | 11.5 | 4.28 |
| CI | 5.38 (5.89) | 24.3 | 2.48 (3.14) | 48.2 | 6.89 | 2.64 |

**Definition of abbreviations:** CI = confidence interval; $C_{\text{NO,plat}}$ = plateau NO concentration; $V_t$ = volumetric flow rate of air during expiration.

* Model-predicted plateau NO concentrations at each constant flow rate are presented in parentheses.

† Statistically different from healthy control subjects ($t$ test with $p < 0.05$)
increase the surface area of the airways producing NO by stimulating the inducible form of nitric oxide synthase (iNOS) (34, 35). This would increase D_{NO,air} (proportional to surface area, see Appendix), which is consistent with our observation; however, iNOS expression is reduced in epithelial cells from patients with CF even though levels of inflammatory mediators such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β are increased in bronchoalveolar lavage (36–38). Furthermore, neutrophils, which increase iNOS expression in normal cells, do not enhance the iNOS expression in CF epithelial cells (37).

The impact of chemical consumption of NO, as characterized by k (a first-order rate constant), is also a possibility. In vivo, NO reacts with several substrates including oxygen, protein thiol (e.g., glutathione), and superoxide. In CF, there are increased numbers of neutrophils, which can produce toxic radical species including superoxide. Normally, glutathione (GSH) provides an effective antioxidant role in the lung; however, in CF, the concentration of GSH has been reported to be decreased (23) as a result of abnormal GSH transport related to the defective CFTR (24). The presence of neutrophils and lower levels of GSH may provide an environment in which excess superoxide can react quickly with NO to form peroxynitrite and other more stable end products such as nitrite and nitrate (25, 26).

It has been postulated that the half life, t_{1/2}, in vivo in healthy control subjects is between the range of 0.1 to 10 s, which is equivalent to between 7 and 0.07 s⁻¹ based on the simple relationship k = ln(2)/t_{1/2} (the shaded region of Figure 7). Figure 7 plots a theoretical estimate of D_{NO,air} (see Appendix for details) as a function of k. D_{NO,air} increases as k increases (t_{1/2} decreases). The relationship is highly nonlinear: D_{NO,air} is independent of k for k < 1, but becomes a strong positive function of k for k > 10. The positive relationship between D_{NO,air} and k is due to an increase in the radial gradient of NO concentration from chemical consumption and is a well-known phenomenon in chemical reaction engineering (33).

The increase in chemical consumption of NO acts to increase D_{NO,air}, or the conductance for mass transfer, but would decrease the mean tissue concentration, C_{tiss,air}. In addition, reduced iNOS expression would also tend to reduce C_{tiss,air}. Thus, the effect on D_{NO,air} and C_{tiss,air} would tend to negate each other in terms of the impact on the actual flux of NO (J_{NO} or J_{NO,max}, see Equations 1 and 2) from the airways. C_{alv,ss} is reduced in patients with CF, but this represents only 17–24% of C_{NO,plat} at an exhalation flow rate of 50 ml/s. This provides a potential explanation for why some studies on CF have reported no statistical change, or a small decrease in exhaled NO concentration, and why the flow-independent parameters are potentially more sensitive. A patient with CF could have highly elevated D_{NO,air} but greatly reduced C_{tiss,air}; thus, J_{NO} and J_{NO,max} are near normal, and a decrease in C_{alv,ss} results in a small decrease in C_{NO,plat}, which may not be detectable when compared with healthy control subjects (compare healthy subject #8 with CF subject #5). Figure 8 describes schematically the probable cascade of events in CF that leads to changes in the flow-independent parameters and little or no changes in the exhaled NO concentration as previously described.

It is important to note that asthma, or reactive airways disease, can also substantially impact NO exchange dynamics. Only two of the CF subjects (subjects #3 and #7) did not have concurrent reactive airways disease. However, these two subjects did not display remarkably different NO exchange parameters. One subject with CF had a very high J_{NO,max} and D_{NO,air} (subject #2). There was nothing remarkable in this subject’s clinical history; however, this subject had by far the best lung function of the subjects with CF (see Table 1). The clinical significance of this relationship is not known.
of exhalation flow rates is scaled to the lung volume, a smaller lung volume results in a smaller absolute range of sampled flow rates. Thus, the smaller exhaled volume results in a smaller change in the exhaled NO signal with changing flow rate thereby decreasing the accuracy of the parameter estimate (based on the nonlinear least-square regression technique). In addition, the absolute exhaled concentration of NO tends to be smaller in CF, which decreases the signal-to-noise ratio of the analytical instrument.

As previously described, only six of the nine children with CF were able to complete the 20-s breathhold. Although a 20-s breathhold is desirable, it is not required. We have previously reported (16) theoretically and experimentally that the uncertainty of \( D_{\text{NO,air}} \) decreases as the breathhold time increases up to 20 s. The estimation of \( D_{\text{NO,air}} \) depends almost exclusively on the magnitude of the peak in phase I (see Figure 6), which increases with increasing breathhold time (16).

In spite of the differences observed with CI, the intra-subject (CI') variability is not significantly different between healthy control subjects and patients with CF (see Figure 4). This finding suggests that the reproducibility of the breathing maneuver does not depend on the presence of CF despite the fact that three of the children with CF were not able to hold their breath for 20 s. Each of these three children held their breath for approximately the same length of time for each of the breathing maneuvers.

The intrapopulation (CI') variability did not differ between the healthy children and patients with CF, and is similar for all of the flow-independent parameters (range 46–82%). This variation within the population is much larger than other endogenous gases (e.g., CO₂). The mechanisms underlying the intrapopulation variability both in health and disease are not known, but might be related to the presence of subclinical inflammation in healthy control subjects or genetically determined differences in NO metabolism as characterized by the length of a repeated polymorphism in the neuronal nitric oxide synthase gene in patients with CF (39, 40).

There are permanent structural changes that occur in the CF lung, particularly those related to anatomic and physiologic deadspace. The physiological deadspace in CF is normal to slightly increased relative to healthy control subjects (41). Thus, our estimate of the airway compartment volume (volume of airway compartment \( V_{\text{air, ml}} \) Table 1), which is based on the subjects ideal body weight and age, may underestimate \( V_{\text{air}} \). We have previously shown that \( D_{\text{NO,air}} \) is the most sensitive of the three flow-independent parameters to the choice of \( V_{\text{air}} \) (16). The dependence is positive; that is, as \( V_{\text{air}} \) increases, the estimate of \( D_{\text{NO,air}} \) also increases to compensate for the dilutional effect of the larger volume. Thus, a slightly larger \( V_{\text{air}} \) for children with CF would actually serve to increase the \( D_{\text{NO,air}} \) further from the healthy control population. Nitrogen washout is a common method to estimate anatomical deadspace; however, the accuracy of this method is compromised by the presence of diseases that impact emptying patterns. The dependence of \( D_{\text{NO,air}} \) on \( V_{\text{air}} \) may explain some of the intersubject variability, and suggests that intrasubject longitudinal changes in the flow-independent parameters may have the greatest clinical utility.

Another possible source of error is performing the spirometric breathing maneuvers before the NO breathing maneuver. According to Silkoff and coworkers (42) and Deykin and coworkers (43, 44), spirometry can depress exhaled NO levels by 10–36% from the baseline in healthy subjects and subjects with asthma by an unknown mechanism. This may perturb one or more of the flow-independent parameters, and should be considered in any future studies.

In summary, we have quantified flow-independent parameters characteristic of NO exchange dynamics in the lungs of healthy children and age-matched patients with CF. The airway diffusion capacity and tissue concentration are significantly different between the two populations and may be a more effective technique than the plateau concentration in distinguishing NO exchange in patients with CF from healthy control subjects. In addition, the flow-independent parameters are not correlated with standard spirometry, which suggests that they may provide additional information to the clinician regarding the inflammatory status of the airways and alveolar region in patients with CF. Their precise interpretation will not be known until future studies establish the correlation of these parameters with more established markers of inflammation obtained by more invasive means.

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APPENDIX

Model Solution

Solution of the governing differential equations for the model leads to the following governing equation for the exhaled NO concentration (16):

\[ C_{exh}(t) = \left( C_{alv,ss} - \frac{J_{NO,max}}{D_{NO,air}} \right) e^{-\frac{D_{NO,air}}{V_{air}} t} + \frac{J_{NO,max}}{D_{NO,air}} \]  

where \( V_{air} \) is the volume of the airway compartment and residence time of each differential gas bolus in the airway compartment (\( \tau_{res} \)) is the residence time of a differential gas bolus in the airway compartment. \( V_{air} \) (ml) is approximated by the physiological dead space, which is derived by subject age in years plus ideal body weight in pounds (45). Equation A1 is compared with the experimental data and used to estimate the three remaining unknown parameters (\( J_{NO,max}, D_{NO,air}, \) and \( C_{alv,ss} \)) using nonlinear least-squares regression. Once values for \( J_{NO,max}, D_{NO,air}, \) and \( C_{alv,ss} \) are known, Equation A1 can be used to predict the exhalation curves presented in Figure 6 or to predict the \( C_{NO,plat} \) at a constant exhalation flow rate (Table 2).
Airway Diffusing Capacity

It has been previously shown that $D_{\text{NO,air}}$ can theoretically be approximated by the following expression:

$$D_{\text{NO,air}} = \frac{\Lambda_{\text{air}} \lambda_{\text{tiss,air}} \sqrt{D_{\text{NO,tiss}} k}}{\tanh(\xi)}$$  \hspace{1cm} (A2)

where $\lambda_{\text{tiss,air}}$ is the tissue:air partition coefficient, $k$ (s$^{-1}$) is the first-order rate constant that characterizes the rate of chemical consumption by substrates such as superoxide, airway surface area available for NO diffusion (cm$^2$) ($A_{\text{air}}$) is the surface area available for diffusion, molecular diffusivity of NO in the tissue ($D_{\text{NO,tiss}}$) (cm$^2$/s) is the molecular diffusivity of NO in the tissue, $\xi = L_{\text{tiss}} D_{\text{NO,tiss}} / k$, and thickness of the tissue layer (cm) ($L_{\text{tiss}}$) is the thickness of the tissue layer. The hyperbolic tangent ($\tanh$) is bounded between –1 and 1, and is a monotonically increasing function of its argument. From Equation A2, $D_{\text{NO,air}}$ is a positive function of $A_{\text{air}}$, $\lambda_{\text{tiss,air}}$, $D_{\text{NO,tiss}}$, and $k$, and is an inverse function of $L_{\text{tiss}}$. Values of $A_{\text{air}}$, $\lambda_{\text{tiss,air}}$, $D_{\text{NO,tiss}}$, and $L_{\text{tiss}}$ are 9,100 cm$^2$, 0.0412, $3.3 \times 10^{-5}$ cm$^2$/s, and 0.01 cm as previously reported (16, 19). Equation A2 provides units of ml/s for $D_{\text{NO,air}}$ that are equivalent to pl s$^{-1}$ ppb$^{-1}$. 

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