Reduction of the Single Breath CO Diffusing Capacity in Cystic Fibrosis*

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The single breath diffusing capacity of the lung for carbon monoxide (Dsb) was measured using three equations to describe CO uptake separately during inhalation, breath holding, and exhalation in 24 patients with cystic fibrosis and 30 control subjects with similar age and height distributions. Using the control group, we developed two prediction equations for Dsb: one based on height, age, and sex; and another based on alveolar lung volume (VA) to the 2/3 power. We also developed a prediction for Dsb/VA (Kcv) based on height. The Dsb as percent predicted (% pred) using either prediction equation decreased with increasing age and height as well as with decreasing % pred maximal inspiratory flow rate (FEF25-75) in cystic fibrosis patients but not in controls. The Kcv (% pred) also decreased in cystic fibrosis with increasing age and decreasing percent pred FEF25-75. We conclude that in patients with cystic fibrosis, Dsb decreases with variables that relate to increasing disease severity (age, height, and increasing airflow obstruction).

In cystic fibrosis, pathologic studies indicate the presence of panacinar emphysema,1 bronchiolar dilatation,2 and a relative reduction in capillary number and increase in pulmonary arterial wall thickness with advancing right ventricular hypertrophy.3 Despite evidence of pulmonary vascular hypoplasia4,5 and loss of elastic recoil6 in this disease, the single breath diffusing capacity of the lung for carbon monoxide (Dsb) has been reported to be either increased,4 decreased,6 or unchanged.7,8 Since recent pathologic studies suggest involvement of the pulmonary microcirculation4,5 in this disease, and since it has been recognized that conventional methods1,2,12 of measuring Dsb may be affected by alterations in exhaled flow rates,13,14 as well as the size and timing of the alveolar gas sample,14,15 we have re-examined the changes in Dsb in cystic fibrosis using a new method15,17 that minimizes these effects. We have found that Dsb decreases in cystic fibrosis with increasing age, height, and airflow obstruction.

Methods

We studied 24 patients with cystic fibrosis and 30 control subjects. After obtaining informed consent, all filled out a standard technician-administered questionnaire.9 The diagnosis of cystic fibrosis was made by standard clinical criteria10 and by the presence of an elevated sweat chloride (Table I). The patients had not experienced any acute changes in symptoms in the previous six weeks. The control subjects were recruited through the cooperation of hospital employees. All control subjects were free of acute respiratory symptoms in the previous six weeks, denied any previous history of respiratory symptoms by questionnaire, were lifetime nonsmokers, and denied a history of any other chronic medical condition.

Subjects and patients performed maximal expiratory flow-volume maneuvers, and patients also performed single breath nitrogen tests using equipment previously described.11 To measure the Dsb, we recorded flow and volume with a pneumotach mounted on the wall of a bag-in-box system and measured instantaneous changes in carbon monoxide (CO) and helium (He) concentration during single breath maneuvers using rapid responding analyzers for CO and He.12,13

Both patients and control subjects were studied seated at rest with a nose clip in place. Each subject and patient performed two acceptable trials of a single breath maneuver which consisted of slow inhalation and exhalation (10 percent) of the vital capacity/second) and either five or 10 seconds (s) of breath holding at TLC. We calculated

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Table I—Anthropomorphic and Pulmonary Function Data (Mean ± 1 SD)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>TLC % pred</th>
<th>FVC % pred</th>
<th>FEV1 % pred</th>
<th>FEF25-75 % pred</th>
<th>Dsb ml/min/mm Hg 10 sec breath hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>17/13</td>
<td>13.3 ± 4.0</td>
<td>156 ± 16</td>
<td>46.7 ± 14.3</td>
<td>103.2 ± 12.2</td>
<td>95.7 ± 12.7</td>
<td>97.1 ± 11.1</td>
<td>95.0 ± 20.8</td>
<td>20.3 ± 6.2</td>
</tr>
<tr>
<td>Cystic</td>
<td>24</td>
<td>14/10</td>
<td>14.2 ± 3.6</td>
<td>154 ± 15</td>
<td>41.5 ± 12.2</td>
<td>94.9* ± 11.9</td>
<td>84.5* ± 18.4</td>
<td>66.5* ± 21.8</td>
<td>38.3* ± 25.9</td>
<td>18.3 ± 4.9</td>
</tr>
</tbody>
</table>

*p<0.05.  
†p<0.001.
Dsb using three separate equations to describe CO uptake during the single breath maneuver\textsuperscript{9,17} and obtained alveolar volume from analysis of the mean He concentration in the exhaled vital capacity.\textsuperscript{7} We have previously found\textsuperscript{17} that this method provides a better estimate of lung volume in patients with airflow obstruction than previously employed single breath dilution methods.

Analyses of variance and co-variance were used to test for equality of slopes, stepwise regression analysis to determine prediction equations and linear regression analysis to assess correlations between Dsb and anthropomorphic variables as well as other tests of lung function. Unpaired two-tailed Student's t-test was used for comparison of control subjects vs cystic fibrosis patients.

Values (Table 1) obtained from control subjects and cystic fibrosis patients were compared using the following previously published normal regressions: for the forced expired volume in one second (FEV\textsubscript{i}), the forced vital capacity (FVC), and the maximum midexpiratory flow rate (FEF\textsubscript{25-75}) from Knudson et al.\textsuperscript{18} for total lung capacity (TLC) from Weng and Levison;\textsuperscript{19} and for the phase 3 slope of the single breath nitrogen test (\textDelta N\textsubscript{2}/L) from Cooper et al.\textsuperscript{20} This study had been approved by the University of Saskatchewan President's Advisory Committee on Ethics in Human Experimentation.

**RESULTS**

The mean age, height, weight, and male to female ratio were similar between control subjects and cystic fibrosis patients (Table 1) and the distributions of age and height were also similar. The FVC, FEV\textsubscript{i}, MMFR, and FEV\textsubscript{i}/FVC \times 100 (p<0.001) and TLC (p<0.05) were all lower in patients than in control subjects (Table 1). For the patient group, the hemoglobin value was 14.0±1.5 g/dl (mean±1 SD) and the sweat chloride level was 112±25 mEq/L (n=23).

In normal subjects, Dsb for the 10 s breath holding maneuver correlated with height (r = 0.88; p<0.0001), age (r = 0.76; p<0.005) and alveolar volume (r = .93; p<0.0001) and alveolar volume to the 2/3 power (r = .93; p<0.0001). Using an analysis of co-variance, the increase in Dsb with age and height was smaller in cystic fibrosis patients than in control subjects (p<0.05). Using stepwise linear regression analysis, we obtained the following regression equations for the three-equation method in control subjects for the 10 s breath holding maneuver:

**Equation 1:**

Females: \(Dsb = -22.3 + .404A + .227H\)

Males: \(Dsb = -18.85 + .404A + .227H\)

**Equation 2:**

\(Dsb = -4.75 + 9.28 (VA^{0.21})\)

where A is the age in yrs, H is the height in cm, and VA is the breath holding absolute BTPS lung volume in liters.

A regression equation was developed for FEF\textsubscript{25-75} using our control group (FEF\textsubscript{25-75} = \(-5.509 + .5658H; r = .79\)) and we expressed the FEF\textsubscript{25-75} values for the patients as a percentage of the values predicted (% pred) by this equation. Dsb (% pred) was correlated with FEF\textsubscript{25-75} % (% pred) as well as age and height. In cystic fibrosis patients but not in control subjects, a significant correlation was noted between Dsb (% pred) and FEF\textsubscript{25-75} (% pred) when Dsb was predicted from age, height, and sex (Equation 1, Fig 1). A similar correlation using equation 1 was found for Dsb (% pred) vs % pred\textsuperscript{9} FEV\textsubscript{i}/FVC \times 100 (r = .56; p<0.005), in cystic fibrosis patients but not in control subjects. The Dsb (% pred, equation 1) also declined with increasing % pred \textDelta N\textsubscript{2}/L (r = -.67, p<0.001) in cystic fibrosis patients. There was also a significant correlation between Dsb expressed as percent predicted on the basis of VA\textsuperscript{0.21} (equation 2) and FEF\textsubscript{25-75} (% pred, Fig 2) in cystic fibrosis patients but not in

**FIGURE 1.** Correlation between % pred Dsb from equation 1 (upper graph), equation 2 (middle graph), and % pred \textDelta N\textsubscript{2}/L (lower graph) vs % pred FEF\textsubscript{25-75} (regression obtained from control subjects). Solid circles are individual data and the solid line is the linear regression for cystic fibrosis patients. For comparison, the linear regressions for the data from the control subjects (dashed line) (p = 0.5 or greater) is shown in each graph.
In patients with cystic fibrosis (but not in control subjects), the $K_{CO}$ (% pred) declined with decreasing % pred FEF25-75 (Fig 1).

We found that while there was no decline in Dsb (% pred, equation 1) with age or height in control subjects, Dsb declined 2.8 percent of expected per year of age (Fig 2) and 0.6 percent of expected per centimeter increase in height ($r = -0.54; p<0.005$) in cystic fibrosis patients. The Dsb expressed as percent predicted using $VA^{1/y}$ (Equation 2) declined 2.3 percent of expected per year of age (Fig 2) and declined .47 percent of expected per centimeter increase in height ($r = -0.50; p = 0.01$) in cystic fibrosis patients but not control subjects. The $K_{CO}$ declined 2.2 percent of expected per year of age (Fig 2) and declined .46 percent of expected per centimeter increase in height ($r = -0.49; p<0.05$).

In 11 patients with a low FEV1 (48.4±10.0 % pred) compared to the 13 cystic fibrosis patients with a higher FEV1 (81.2±17.1 % pred), we found that Dsb (% pred) using either equation 1 or 2 and $K_{CO}$ (% pred) were all significantly lower ($p<0.05$) in the most vs the least obstructed group (Table 2).

Although we have compared Dsb in control subjects vs cystic fibrosis patients using data from a 10 s breath holding maneuver, we have also measured Dsb following 5 s of breath holding. For 5 s of breath holding, Dsb was significantly lower ($p<0.05$) in cystic fibrosis patients (17.3±4.8 ml/min *mm Hg$^{-1}$) compared to control subjects (20.9±6.6 ml/min *mm Hg$^{-1}$). The Dsb following 5 vs 10 s of breath holding was similar in control subjects but was decreased in cystic fibrosis patients ($p<0.05$). Furthermore, Dsb was lower in cystic fibrosis patients vs control subjects following 5 s of breath holding using analysis of co-variance with either age alone ($p<0.05$) or height alone ($p<0.05$) as the independent variable.

Although TLC was lower in cystic fibrosis patients than in control subjects (Table 1), we found no difference in the measured TLC for the 5 s breath hold (3.96±1.36 L) vs the 10 s breath hold maneuvers (4.03±1.37). When we examined the changes in % pred TLC in cystic fibrosis patients, we also found no significant correlations with age, height, FEF25-75 (% pred), $K_{CO}$ (% pred), or Dsb (% pred, equation 2), but Dsb (% pred, equation 1) decreased significantly with decreasing TLC ($p<0.05$).

**DISCUSSION**

In this study, we have found that Dsb decreases significantly with increasing age, height, and airways obstruction in patients with cystic fibrosis compared to a group of normal children of similar age, height, and sex distribution. This finding supports previous observations on changes in the pulmonary vasculature that occur in this disease.$^{13}$ At autopsy,$^{13}$ cystic fibrosis

**Figure 2.** Correlation between % pred Dsb from equation 1 (upper graph) and from equation 2 (middle graph) and % pred $K_{CO}$ (lower graph) vs age. Format is the same as Figure 1.

control subjects.

When Dsb was expressed as a ratio of the simultaneously measured volume, this ratio ($K_{CO}$) declined with height ($r = -0.4; p<0.05$) in control subjects:

$$K_{CO} = 6.8 - 0.014 \times \text{Ht (cm)}$$
Table 2—Dsb in Cystic Fibrosis Patients with Lower vs Higher FEV₁ (% Pred) (Mean ± 1 SD)

<table>
<thead>
<tr>
<th>FEV₁</th>
<th>Dsb % Pred (Equation 1)</th>
<th>Dsb % Pred (Equation 2)</th>
<th>Kco % Pred (Equation 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>FEV₁ (% Pred, 21)</td>
<td>TLC (% Pred, 22)</td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60% pred</td>
<td>13</td>
<td>81.2</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>± 17.1</td>
<td>± 7.4</td>
<td>± 14.7</td>
</tr>
<tr>
<td>≤60% pred</td>
<td>11</td>
<td>48.4†</td>
<td>90.1</td>
</tr>
<tr>
<td></td>
<td>± 10.00</td>
<td>± 14.6</td>
<td>± 14.1</td>
</tr>
</tbody>
</table>

*p<0.05.
†p<0.01.
‡p<0.001.

patients with right ventricular hypertrophy (RVH) had reduced background haze by arteriography, fewer arteries per unit area of lung, and increased intra-acinar arterial wall thickness proportional to the degree of RVH. Based on the response of the pulmonary vasculature to generalized hypoxia in rats,²³ Reid et al. postulated that hypoxemia in cystic fibrosis might cause premature muscle growth of intra-acinar arteries and lead to a reduction in intra-acinar artery number. Since small airways obstruction²⁴ and pulmonary hypertension²⁵ may develop early in cystic fibrosis, the normal growth of the pulmonary vasculature may be uniquely affected because normal alveolar multiplication continues up to age eight and alveoli continue to increase in diameter and develop new intra-acinar arteries³ until skeletal growth ceases. The progressive reduction in Dsb with advancing disease could be caused not only by failure of alveolar multiplication and/or enlargement, but also by failure of normal development of the pulmonary capillary bed.

The observed reduction in Dsb in cystic fibrosis might have been due to reductions in hemoglobin (Hb) with advancing disease, but no correlation was found whatsoever between Hb and FEV25-75 (% pred) in our patients (p<0.5).

While previous studies using body plethysmographic methods suggest that TLC in cystic fibrosis was normal,⁴ it was found that TLC is slightly lower in cystic fibrosis patients compared to our normal subjects. It is possible that, with increasing nonuniformity of ventilation, the single breath helium dilution method of measuring alveolar volume could have spuriously underestimated alveolar volume, and hence, could have lowered the estimation of Dsb in cystic fibrosis patients with the greatest airflow obstruction.²² When we predicted Dsb on the basis of Vₐ⁵⁻⁶,²²,²⁷,²⁸ in this study, we have confirmed previous observations²⁷ that Dsb correlates strongly with Vₐ⁵⁻⁶. When growth is complete in adults, the alveolar number is quite variable but appears to correlate best with body length.²⁹ Based on this observation, Dsb should also correlate strongly with height, a relationship found by us in the present study and previously by others.³⁰ Finally, O’Brodovich et al.³¹ have pointed out that in the sitting position, Dsb may be influenced by skeletal growth in normal children because, with increasing height, perfusion becomes reduced in a progressively increasing amount of lung in the upper lung zones. This phenomenon causes a progressive derecruitment of apical pulmonary capillaries in the sitting position with increasing height so that Dsb becomes progressively lower in the apex vs the base. In the normal adult seated subject, Dsb appears considerably lower in the apex vs the base.³¹ The difference in Dsb between the supine and sitting position, which increases with body height in normal children, reflects the increasing nonuniformity of dif-
fusion that exists in the sitting but not the supine position. While we did not study the effect of body position in this study, others have observed that Dsb fails to increase in the supine vs sitting position in patients with cystic fibrosis. These observations suggest that prediction values in control subjects for Dsb would be progressively greater in the supine vs sitting position with increasing height. The differences that we found in the sitting position between control subjects and cystic fibrosis patients might be even more marked in the supine position.

In a previous study, Dsb was increased in a group of cystic fibrosis patients and was also higher in those patients most severely obstructed. The discrepancy between the results in this study and the previous study of Keens et al. may in part be due to errors in Dsb measurements that occur using conventional methods to calculate Dsb. Conventional methods of measuring Dsb overestimate Dsb in normal subjects when the exhaled flow rate is reduced and in patients with reduced exhaled flow rates caused by airflow obstruction. For example, in normal adult subjects, we found that Dsb calculated by the method of Ogilvie et al. increased 17.5 percent when the exhaled flow rate was reduced from 2 L/s to 0.5 L/s. In contrast to conventional methods, we have used three separate equations, one each for inhalation, breath holding, and exhalation to calculate Dsb in this study. Using this method, we have previously reported that Dsb is not affected by alterations in exhaled flow rates both in a lung model and in normal adult subjects. Finally, in the same group of cystic fibrosis patients taking part in this study, we have reported that conventional methods overestimated Dsb compared to the three-equation method.

Because the three-equation method does not introduce errors in Dsb calculations with reduced inhaled and exhaled flow rates either in normal subjects or patients with airflow obstruction, we were not restricted to the "standardized" forced inhalation, 10 s breath hold, forced exhalation single breath maneuver. We chose single breath maneuvers with slow inhaled and exhaled flow rates because cystic fibrosis patients could perform the maneuver in the same manner as the control subjects and because this maneuver would reduce the possible influence of widely fluctuating pleural pressures and improve the distribution of inhaled gas in patients with airways obstruction.

We intentionally chose to analyze the 10 s breath hold maneuver in greater detail because previous investigations in COPD patients indicate that Dsb using the three-equation method decreases with decreasing breath hold time. We also found that Dsb was lower in cystic fibrosis patients for the 5 s vs the 10 s breath holding maneuver in the present study. It has been suggested that the decrease in Dsb with decreasing breath holding time in patients with ventilation maldistribution may reflect diffusion limitation within the airways due to impaired mixing of inhaled gas with resident gas. While the cause of the increase in Dsb with increasing breath hold time in the presence of airflow obstruction remains speculative, we have examined the differences between normal individuals and cystic fibrosis patients with long breath hold times where potential differences would be minimized. Although Dsb was similar in control subjects for 5 s vs 10 s breath holding (r = .95; p<.0001), the differences between control subjects vs cystic fibrosis patients are actually greater using 5 s vs 10 s breath holding maneuvers in this study.

In summary, we conclude that cystic fibrosis causes a reduction in Dsb, and that this reduction correlates with the progression of the disease as measured either by increasing age or height, or by worsening forced exhaled flow rates.

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