After bronchoconstriction has been experimentally induced, healthy subjects can partially improve lung function by performing deep inspirations. This physiologic function of the lungs has been broadly termed deep inspiration-induced bronchodilation.1 We and others have previously demonstrated that the bronchodilatory effect of deep inspiration, albeit decreased, also exists in asthmatics.2-5 Our preliminary findings indicate that the bronchodilatory effect of deep inspiration is progressively reduced with increasing severity of asthma.6 On the basis of these observations, we have hypothesized that the impaired ability of deep inspiration to distend narrowed airways may contribute to the occurrence of asthmatic symptoms.

It is reasonable to further hypothesize that deep inspirations exert their beneficial effects through radial traction that is applied on the airway wall by virtue of the interdependence between intraparenchymal airways and the surrounding parenchyma.
Radial traction is sustained by a network of alveolar attachments to the airway walls. Structural alterations of the lung parenchyma (eg, destruction of alveolar attachments) or of the airway wall, which are prominent in COPD, could impair the effectiveness of the distending forces, so that a deep inspiration would not be capable of generating enough force to stretch narrowed airways and/or reopen closed airways.

Based on the above, we hypothesize that in patients with COPD, the ability of deep inspiration to dilate the airways is impaired, thus contributing to the occurrence and/or severity of chronic respiratory symptoms. The aim of the study was to test this hypothesis.

**Materials and Methods**

**Subjects**

We studied 19 subjects affected by COPD (mean age, 67.8 ± 7.1 years; range, 53 to 80 years [± SEM]) and 17 healthy individuals (mean age, 62.5 ± 9.3 years; range, 48 to 82 years). The patients with COPD fulfilled the diagnostic criteria of the Global Initiative for Chronic Obstructive Lung Disease guidelines. The healthy subjects had no history of respiratory symptoms consistent with the diagnosis of COPD or other lung disease, nor had they ever received a diagnosis of COPD or other lung disease from a physician. Obviously, none of the healthy subjects were receiving any respiratory medication regimen. Although airways hyperresponsiveness is not a functional criterion in the diagnosis of COPD, we assessed the response to methacholine under a conventional bronchoprovocation challenge for the purposes of this study. All patients with COPD were smokers (mean, 60 pack-years; range, 22.5 to 125 pack-years), and all but five healthy subjects were nonsmokers (mean, 29 pack-years for the five smokers; range, 1.2 to 60 pack-years). The patients with COPD were recruited from the outpatient clinic for respiratory diseases of the University Hospital in Palermo, Italy. None of the subjects had an upper respiratory infection for at least 4 weeks before evaluation. At the time of the study, all subjects were in clinically stable condition, and had withheld inhaled short-acting β-agonist and/or anticholinergic agents for at least 8 h, and long-acting β-agonist agents for at least 24 h before the study. No subjects were receiving leukotriene-modifier agents. Five patients with COPD were receiving inhaled corticosteroids, and one of these five patients was also receiving an oral methylxanthine at the time of the study; the latter was withheld 24 h before the study. Subjects were ineligible for the study if they had a history of myocardial infarction, congestive heart failure, cor pulmonale, or arrhythmia. Subjects were also excluded if they had a history of asthma or other symptoms suggestive of asthma. The study protocol conformed to guidelines issued by the local ethics committee (University of Palermo, Palermo, Italy), and all subjects gave written informed consent prior to participating in the study.

**Study Design and Specific Methods**

The study consisted of two phases. In the first phase, clinical and functional assessments were performed. The clinical evaluation included a questionnaire that derives from the International Union Against Lung and Tuberculosis Disease bronchial symptom questionnaire and a physical examination. Functional assessment included plethysmography, single-breath carbon monoxide transfer, and methacholine bronchoprovocation. Total lung capacity (TLC) was determined by measuring thoracic gas volume, followed immediately by a slow vital capacity maneuver (V6290 AntoBox; SensorMedics; Yorba Linda, CA). TLC was expressed as percentage of predicted based on the prediction formula of Goldman and Becklake. Single-breath diffusing capacity for carbon monoxide was determined using a fully computerized, water-sealed Stead-Wells spirometer (Baires System; Biomedin; Padua, Italy). The transfer factor of the lung for carbon monoxide (TLCO) and the transfer coefficient for carbon monoxide were measured to evaluate the magnitude of lung parenchymal destruction. At least two determinations of TLCO that were within 5% of each other were obtained, and the highest value was retained for analysis. Conventional methacholine bronchoprovocation was performed with the method of Chai et al. First, baseline spirometry was recorded as the best of three acceptable forced expiratory maneuvers. Sterile diluent (phosphate-buffered saline solution) followed by increasing concentrations of methacholine (0.025, 0.075, 0.25, 0.75, 2.5, 7.5, and 25 mg/mL) were administered with five slow breaths, from functional residual capacity to TLC, through an ampul-dosimeter (Mefar Elettromedicali; Bovezzo, Italy), which was activated by an inspiratory effort for 0.5 s at a time. Spirometric measurements in triplicate were repeated 3 min after saline solution inhalation and after each dose of methacholine; the best FEV₁ among the three maneuvers was recorded. The methacholine bronchoprovocation was terminated when a 20% decrease in FEV₁ from the postdiluent value was obtained or the highest concentration of methacholine (25 mg/mL) was delivered. The provocative concentration of methacholine causing a 20% fall in FEV₁ was calculated by interpolation of the dose-response curve.

In the second phase of the study, the bronchodilatory effect of deep inspiration was determined (Fig 1). This phase consisted of several visits in which the dose of methacholine inducing at least a 15% reduction in inspiratory vital capacity (IVC), in the absence of deep breaths, was established through a series of single-dose methacholine bronchoprovocations. To obtain IVC, the subject slowly expired from end-tidal volume to residual volume (RV) and immediately inhaled to TLC (IVC = TLC − RV). The protocol is depicted in Figure 1, left, a. To calculate the single methacholine dose for each subject, increasing doubling doses of spasmogen were delivered (a single dose per challenge) until the targeted level of reduction in IVC was reached. At the baseline of each single-dose challenge, three IVC measurements were obtained and the best value was used for analysis. The subject was thereafter instructed to abstain from deep inspirations for 20 min, at which point the methacholine was delivered. Three minutes later, a single IVC maneuver was performed. To assess the bronchodilatory effect of deep inspiration, the single-dose methacholine challenge in which ≥ 15% reduction in IVC from baseline was obtained was extended by inviting the subject to perform four consecutive deep breaths. Immediately after, a single spirometry for IVC was again performed (Fig 1, right, b). The ability of deep inspiration to reverse the induced bronchoconstriction was calculated in two ways: (1) by using the ratio of the difference between post-deep inspiration (post-DI) IVC and post-methacholine (post-Mch) IVC over the post-methacholine IVC. This was termed *bronchodilatory index* 1:

\[
\text{post-DI IVC } - \text{ post-Mch IVC} \times 100
\]

and (2) by using the ratio of the difference between the percentage reduction in IVC from baseline after methacholine...
and the percentage reduction in IVC from baseline after deep inspiration over the percentage reduction in IVC after methacholine. This was termed bronchodilatory index 2:

$$\frac{\% \text{ reduction post-Mch IVC}}{\% \text{ reduction post-DI IVC}} \times 100$$

**Data Analysis**

Unpaired t tests were used to compare the two study groups in terms of the following: (1) baseline characteristics; (2) the percentage reduction in IVC, in the absence of deep inspirations, during the single-dose methacholine challenges; and (3) the bronchodilatory effect of deep inspiration. The Mann-Whitney U test (a nonparametric test) was applied to assess the difference between the two groups in the single doses of methacholine required to induce the targeted reductions in IVC in the absence of deep inspirations (methacholine doses are not a continuous variable). In the COPD group, we constructed simple regression models to evaluate the relationship between the bronchodilatory effect of deep inspiration and FEV1 percentage of predicted, FEV1/FVC, Tlco percentage of predicted, and age. In all analyses, values of p ≤ 0.05 were considered statistically significant.

**Results**

Demographics and baseline lung function characteristics for all subjects participating in the study are depicted in Table 1. The two groups were not statistically different in terms of age. As expected, both FEV1 percentage of predicted and FEV1/FVC values differed between COPD and healthy subjects. Four of the 19 patients with COPD showed airways hyperresponsiveness under the conventional bronchoprovocation methacholine challenge (provocative concentration of methacholine causing a 20% fall in

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**Table 1—Demographic and Functional Characteristics of the Subjects Participating in the Study**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>COPD Patients</th>
<th>Healthy Subjects</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>19</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>67.8 ± 7.1</td>
<td>62.5 ± 9.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Male/female gender, No.</td>
<td>17/2</td>
<td>13/4</td>
<td></td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>79.0 ± 17.2</td>
<td>106.3 ± 13.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.64 ± 0.11</td>
<td>0.78 ± 0.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tlco, % predicted</td>
<td>71.5 ± 11</td>
<td>85.7 ± 13.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Single-dose methacholine, mg/mL</td>
<td>20 (0.025–75)</td>
<td>25 (10–75)</td>
<td>0.19</td>
</tr>
<tr>
<td>Reduction in IVC from baseline, %</td>
<td>20.1 ± 7.3</td>
<td>22.7 ± 10.0</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD unless otherwise indicated.†Presented as median (range).
FEV1, 2.5 ± 1.8 mg/mL [mean ± SEM]). No healthy subjects were hyperresponsive to methacholine. TLCO percentage of predicted was significantly lower in patients with COPD than in healthy subjects. The median single methacholine dose inducing the targeted reduction in IVC in the absence of deep inspiration was similar in the two groups (Table 1).

The IVC reduction from baseline prior to any deep inspirations in the single-dose bronchoprovocation protocol was also of similar magnitude in both groups, allowing for the bronchodilatory effect of deep inspiration to be compared. In the patients with COPD, the reduction in IVC after methacholine inhalation and the remaining reduction in IVC after the series of deep inspirations were not significantly different (20.1 ± 1.6% vs 17.4 ± 1.9%, respectively; p = 0.18) [Fig 2]. In the healthy subjects, the postdeep inspiration reduction in IVC from baseline was significantly lower than the reduction in IVC that was obtained immediately after methacholine inhalation (22.7 ± 2.4% vs 13 ± 1.8%, respectively; p < 0.0001) [Fig 2]. In other words, the series of deep inspirations that all subjects were asked to take after reaching the targeted reduction in lung function by methacholine partially reversed bronchoconstriction in the healthy subjects but not in the subjects with COPD. This was further confirmed by bronchodilatory index 1, which focuses on the changes in spirometry induced by the deep inspirations, irrespective of the level of bronchoconstriction. This analysis was possible on the basis of the similar levels of bronchoconstriction that were obtained in both study groups. The bronchodilatory index was statistically lower in the COPD group than in the healthy group (3.9 ± 2.6% and 13.7 ± 3.0%, respectively; p = 0.02) [Fig 3, top]. Also, the bronchodilatory index in the COPD group was not different from zero by one-sample t test. Interestingly, among the COPD group, five subjects had negative bronchodilatory index values (range, −1 to −23%); in these subjects, the series of deep inspirations following methacholine further enhanced bronchoconstriction. We also calculated a second bronchodilatory index (bronchodilatory index 2) using the methacholine-induced percentage reductions in IVC from baseline, before and after the series of deep inspirations, as evaluated in our early studies (Fig 3, bottom). Again, this index was significantly lower in the COPD group (11.6 ± 10.0%) than in the healthy subject group (40 ± 5.8%; p = 0.02) [Fig 3, bottom]. Also, the second bronchodilatory index in the COPD group was not different from zero (p = 0.26, one-sample t test).

![Figure 2. Reductions in IVC from baseline in the protocol employed to assess the bronchodilatory effect of deep inspiration in the two study groups. Bars represent mean ± SEM. Closed bars represent percentage reductions in IVC in the bronchoprovocation methacholine challenge immediately before the deep inspirations. Open bars represent the percentage residual reductions in IVC obtained immediately after the deep inspirations. See Figure 1 legend for expansion of abbreviation.](image)

![Figure 3. Mean values for deep inspiration-induced bronchodilation in patients with COPD and healthy subjects. Bronchodilation by deep inspiration is calculated by using the two formula described in the “Study Design,” and termed bronchodilatory index 1 (top), and bronchodilatory index 2 (bottom), respectively.](image)
Using simple regressions, we evaluated whether in subjects with COPD, the impairment in deep inspiration-induced bronchodilation is associated with the degree of parenchymal alterations and/or the level of baseline airway obstruction. Bronchodilation was found to inversely correlate with TLCO percentage of predicted \((r = 0.53, p = 0.05)\) [Fig 4, top], but not with airway obstruction, as assessed by FEV\(_1\) \((r = 0.17, p = 0.33)\) or FEV\(_1\)/FVC \((r = 0.06, p = 0.80)\) [Fig 4, middle and bottom].

Because of our previous findings that aging per se affects the bronchodilatory effect of lung inflation, and since our study population consists of subjects > 50 years old, we also regressed bronchodilation against age. The bronchodilatory effect of deep inspiration was found to significantly decrease with aging \((r = 0.46, p = 0.005)\) when subjects from the two groups were pooled together; however, it was not related to aging if only the patients with COPD were included in the regression analysis \((r = 0.17, p = 0.49)\). This indicates that, in this group, the disease status is the strongest predictor of bronchodilation induced by deep inspirations.

**Discussion**

The ability of deep inspirations to relieve airway obstruction that has been experimentally induced in healthy subjects is a well-known phenomenon. Some attenuation of this effect has been previously demonstrated in mild asthmatics. The primary finding of our study is that the bronchodilatory effect of deep inspiration is profoundly impaired in individuals with mild COPD. The reduction of the bronchodilatory effect of deep inspiration was associated with the reduction in TLCO. This suggests that the underlying pathology of the lung parenchyma may be responsible for the impaired bronchodilation by deep inspiration.

Changes in lung volume are associated with changes in transmural pressure that are transmitted to the airway wall by virtue of the forces of interdependence between airway walls and surrounding parenchyma. Therefore, as long as airway-parenchyma interdependence is intact, a deep inspiration can apply radial traction on the airway wall. As a result, airway caliber can increase. The stretch on the airway wall is also imposed on the smooth muscle and may be responsible for causing relaxation, albeit through mechanisms that are not understood. Inability of lung inflation to improve airway patency could stem from various factors, including reduced forces of airway-parenchyma interdependence, increased airway stiffness, or reduced amplitude of stretch because of preexisting hyperinflation.

Two main pathologies are described in COPD: emphysema and peripheral airway disease. Emphysema is accompanied by destructive changes of alveolar walls and consequent reduction in the number of alveolar attachments on the airways. These changes lead to the well-described reduction of the elastic recoil of the lung. This not only reduces the effective driving pressure during a forced expiration.

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**Figure 4.** Correlations between the bronchodilatory effect of deep inspiration and the degree of parenchymal alterations (top), or the magnitude of bronchial obstruction (middle and bottom).
but also allows the airway to collapse because of loss of radial traction. In addition to the parenchymal changes, small airways (<2 mm in diameter) are narrowed in patients with COPD and show inflammatory changes such as cellular infiltration, edema, enlargement of mucous glands, and increase in bronchial smooth-muscle mass.9 However, in patients with COPD, the most important factor that increases the airway resistance and RV (air trapping) is the loss of radial traction, not the intrinsic narrowing of the airways.

On the basis of the documented structural abnormalities in the lungs of patients with COPD, the loss of bronchodilation by deep inspiration was not surprising. Because of the alveolar wall destruction, the loss of airway-parenchyma interdependence (mechanical decoupling between airways and parenchyma) results in diminished airway wall and airway smooth-muscle stretch, thus impairing a primary step in the mechanism of bronchodilation by deep inspiration. This hypothesis is supported by the relationship between deep inspiration-induced bronchodilation and TLCO that we report in this article. We assume that the level of diffusing capacity for carbon monoxide reflects the degree of parenchymal alterations.20 Obviously, further studies involving direct measurement of elastic recoil are pertinent to examine the relationship between the loss of elastic recoil and the loss of the bronchodilatory effect of deep inspiration in patients with COPD.

In a subgroup of patients with COPD, we observed increased bronchoconstriction, instead of bronchodilation, following deep inspirations. This phenomenon has already been described in chronic asthma,21,22 in COPD,23 and in subjects with a history of smoking and chronic airway obstruction.24 The five patients with COPD in whom further bronchoconstriction developed by deep inspirations were also those with the lowest TLCO values, which indirectly indicate loss of parenchymal tissue. It can be speculated that in individuals with more severe emphysema, deep inspiration consistently leads to bronchoconstriction. As clearly demonstrated by Mitzner and Brown,25 the elimination of tidal stresses that occurs when the airways are decoupled from the surrounding parenchyma, converts the airway response to a deep inspiration from dilatory to constrictive.

Additional causes of reduced deep inspiration-induced bronchodilation could exist. First, increased bronchial smooth-muscle mass could render the muscle more resistant to stretch, or generate higher forces26 that could counteract bronchodilation. Second, COPD may be associated with enhancement of a bronchoconstriction reflex (myogenic reflex) that is activated by lung inflation.22,27,28 If so, the bronchodilatory effects and the myogenic reflex may counteract each other. Third, because of the existing lung hyperinflation, the IVC may be significantly reduced and a deep inspiration may not be able to exert stretch of adequate amplitude on the airway smooth muscle.

We have recently made a preliminary observation that the ability of lung inflation to reverse bronchoconstriction diminishes with aging.14 This observation was confirmed in this study. Aging is associated with structural alterations of the lungs,9 and a reduction in the connective tissue that supports and surrounds intraparenchymal airways has been documented.8 In agreement with these observations, Pernutt and Martin29 demonstrated in 1960 that lung elastic recoil pressure decreases with age in healthy individuals. Because of the significant effect that age-related alterations may exert on the bronchodilatory ability of deep inspiration, one could argue that the lack of bronchodilation that we have observed in our patients with COPD can be attributable to aging. However, our healthy control subjects were not different from the patients with COPD in terms of age. Also, in the COPD group, we found that the disease state, as expressed by TLCO, and not the age, was the major determinant of deep inspiration-induced bronchodilation.

Perhaps the lack of deep inspiration-induced bronchodilation has clinical implications for individuals with COPD. Our view of the beneficial effects of lung inflation is that it functions as a homeostatic mechanism, to control intrinsic airway smooth-muscle tone and to protect the lungs from excessive atelectasis. This is probably mediated through sighing. In the prolonged absence of lung inflation, significant atelectasis and bronchoconstriction develop in animals.25 In COPD, several patients have increased tone, as indicated by their significant responses to bronchodilators.30 It is possible that reduced or absent bronchodilation by deep inspiration contributes to the development of airway obstruction. Yet, we found no relationship between deep inspiration-induced bronchodilation and FEV1 in this study (Fig 4, bottom). This is not surprising, given that reduced elastic recoil rather than airway resistance is the major determinant of reduced FEV1 in patients with COPD.

In conclusion, we have demonstrated that the bronchodilatory effect of deep inspiration is lost in individuals with mild COPD. We speculate that the loss of the bronchodilatory effect of deep inspiration can be attributed to structural changes that occur in the lungs of patients with COPD. It is possible that the absence of deep inspiration-induced bronchodilation contributes to the development and severity of chronic respiratory symptoms in COPD.
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