Deep inspiration-induced changes in lung volume decrease with severity of asthma

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Summary
We have previously reported that the magnitude of deep inspiration (DI)-induced bronchodilation is only slightly reduced in mild asthmatics, compared to healthy subjects. The aim of this study was to evaluate whether increased severity of asthma is associated with impairment in the ability of DI to induce changes in lung volume. Thirty-six consecutive asthmatics recruited from the Pulmonary and the Allergy Outpatient Clinics of the Institute of Respiratory Diseases of the University of Palermo were divided into 3 groups: Intermittent (I), Mild Persistent (MP) and Moderate–Severe (MS), based on GINA guidelines. Single dose methacholine (Mch) bronchoprovocations were performed in the absence of DI, to induce at least 15% reduction in inspiratory vital capacity (IVC) from baseline. The post-Mch IVC was followed by 4 consecutive DI and by another IVC, to determine the bronchodilatory effect of DI. The bronchodilatory effect of DI was found to significantly decrease with increasing severity of asthma (I: 68 ± 5.4%, MP: 45 ± 7.2%, MS: 4 ± 15.6%; ANOVA: P < 0.0001). Bronchodilation by DI, but not FEV1 or FEV1/FVC, was also inversely correlated to symptom scores (r = −0.42, P = 0.01) and to weekly salbutamol usage (r = −0.47, P = 0.004). These observations provide support to the hypothesis that the attenuation of the bronchodilatory effect of DI contributes to the severity of the clinical manifestations of asthma.

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Introduction
The fundamental abnormality of asthma is excessive baseline airway tone and narrowing in response to bronchoconstrictive stimuli. Deep inspirations have been demonstrated...
to counteract airway narrowing, in that, they are able to reverse bronchoconstriction that has been experimentally induced in healthy individuals.\(^1,6\) In a previous study, we reported similar levels of deep inspiration-induced bronchodilation in healthy and in asthmatic subjects.\(^7\) However, bronchodilation by deep inspirations was exclusively tested in asthmatics with mild disease. In 1980, Orehek and colleagues suggested that there are two populations of asthmatics: those in whom deep inspirations taken after the inhalation of carbachol caused bronchodilation, as measured by airway resistance, and those in whom deep inspirations had no effect.\(^8\) The former group had milder disease than the latter, based on the frequency of dyspneic paroxysms. Recently, the bronchodilatory effect of deep inspirations was also investigated in children with different severity of asthma by using the ratio of maximal over partial forced expiratory flows\(^9\): in that study, the ability of a deep inspiration to improve expiratory airflows decreased with increasing severity of asthma, as assessed by international guidelines.

Our experimental design to test the effect of deep inspiration involves a single dose methacholine challenge model in which, after the induction of targeted bronchoconstriction, subjects are asked to take 5 consecutive deep inspirations with subsequent repetition of lung function measurement.\(^5,7,10-12\) We have more recently introduced a spirometric inspiratory vital capacity (IVC) maneuver to provide us with the primary outcome in these studies. As will be discussed later, the measurement of IVC has the advantage of avoiding the inherent effect of a deep inspiration in the spirometric maneuver. However, IVC is a volume outcome and the question has been raised whether the findings reported in the above-cited work using airway resistance or flow characteristics can be confirmed with a volume measurement. We, therefore, designed the current study to test the hypothesis that the bronchodilatory effect of deep inspiration is impaired in adults with more severe asthma using IVC as the primary outcome. We used an unselected and consecutively recruited group of asthmatics and examined whether the bronchodilatory ability of deep inspiration was related to the severity of their disease, as assessed by the GINA guidelines,\(^13\) as well as to the control of the disease, as assessed by the Asthma Control Questionnaire (ACQ),\(^14\) which takes into account symptom scores and short-acting \(\beta\) agonist (reliever medication) usage.

**Methods**

**Subjects**

We recruited individuals attending for the first time the Pulmonary and the Allergy Outpatient Clinics of the Institute of Respiratory Diseases of the University of Palermo, Palermo, Italy and who received the diagnosis of asthma by a pulmonologist. The severity of asthma was determined in accordance with the GINA guidelines.\(^13\) Because some individuals were already on asthma treatment, categorization by severity took into account their current therapeutic regimen, as required by the GINA guidelines.\(^13\) All subjects were skin test positive to at least one Aeroallergen that could explain the pattern of their lower airway symptomatology, and none was a current smoker (two subjects were former smokers). If an upper or lower respiratory infection was present, assessment was postponed for at least 4 weeks. All subjects were in stable condition and subjects allergic to pollen were studied out of season. Short-acting agonists were withheld for at least 12 h and long-acting agonists for 24 h prior to each evaluation. None of the subjects was receiving theophylline at the time of the study. Corticosteroid therapy and leukotriene modifiers were maintained throughout the study. Coffee or tea were not allowed before the bronchoprovocations. The study was approved by the Ethics Committee of the University of Palermo and all subjects gave written, informed consent prior to participation.

**Study design**

The study was designed to assess the association between the degree of deep inspiration-induced bronchodilation and the severity of asthma, and included both clinical and functional evaluations. Clinical evaluation consisted of the assessment of the frequency of daily and nocturnal symptoms, as well as recording of salbutamol usage. These assessments led to the categorization of asthma severity. Asthma control was measured by using a self-administered Asthma Control Questionnaire (ACQ). The ACQ is a validated 7-item instrument, each item (symptom) rated on a six-point scale, ranging from "none" to "extremely severe".\(^14\) The questions addressed symptoms occurring within the week preceding the evaluation. The questionnaire was completed by each subject on the day of the evaluation.

Functional assessment included conventional spirometry and measurement of the bronchodilatory effect of deep inspiration. The bronchodilatory effect of deep inspiration was assessed as described before\(^5,7\) by a series of single dose methacholine bronchoprovocations. First, the single dose of methacholine that, in a protocol completely devoid of deep inspirations, induces an at least 15% reduction in inspiratory vital capacity (IVC) from baseline, was determined. The protocol of the single dose methacholine bronchoprovocation (Fig. 1) consists of a 20-min period of deep-breath prohibition that begins after baseline spirometry. During this period, subjects are observed by a study staff member and are repetitively reminded of the need to abstain from any deep inspirations. At the end of this period, a single concentration of methacholine is administered through five tidal breaths; 3 min later, spirometry is repeated. The spirometric measurement is a combined maneuver (a partial

![Figure 1 Depiction of the single dose-bronchoprovocation protocol that was employed to measure the bronchodilatory effect of deep inspiration. For details, see Section Methods. S = Spirometry; Mch = single dose Methacholine; DI = Deep inspiration.](image-url)
The bronchodilatory effect of deep inspiration in asthma

followed by a maximal forced expiratory maneuver), which allows for the IVC to be determined. IVC is the volume of air inhaled from the residual volume reached at the end of the partial forced expiratory maneuver to the total lung capacity reached with the immediately ensuing deep inspiration. IVC is used as the primary outcome in these studies because it is devoid of any effects of deep inspiration as it is dependent on the residual volume reached at the end of the partial expiratory maneuver. This is an advantage of IVC over FEV1 or FVC, in that those outcomes may be influenced by the deep inspiration that precedes their recording. On the other hand, the combined maneuver allows for simultaneous FEV1 and FVC determinations. If the reduction in IVC from baseline is less than 15%, the subject is invited to repeat this procedure on a separate occasion, with an increased single dose of methacholine (approximately half log increment). When the expected level of reduction in IVC is attained (15% or more), subjects are asked to take 4 consecutive deep inspirations followed by another combination maneuver, to determine the bronchodilatory effect of deep inspirations. The starting concentration of methacholine used in these single dose challenges was 25 mg/ml.

For the determination of the magnitude of deep inspiration-induced bronchodilation, we calculated the difference in the % post-methacholine reduction in IVC from baseline between the pre- and post-deep inspiration maneuvers, divided by the % reduction in IVC from baseline obtained immediately after the methacholine administration (pre-deep inspiration). For comparison with IVC, the same outcome was also calculated using FEV1.

Methacholine was delivered through a ampul-dosimeter (Mefar Elettromedicali; Bovezzo, Italy), which was activated by an inspiratory effort for 0.5 s at a time. All spirometric measurements were obtained from a computerized water-sealed spirometer (Biomedin; Padua, Italy), which allowed compliance with ATS criteria to be confirmed on-line.

**Data analysis**

ANOVA was employed to compare baseline FEV1 and FEV1/FVC among the three groups. ANOVA was also used to compare the mean single doses of methacholine required to induce the targeted reductions in IVC in the absence of deep inspirations, the % reductions in IVC attained by the single dose challenges and the % bronchodilation. Post hoc analysis was performed with the Fisher’s PLSD test. Linear regressions were constructed to assess the relationships between changes in lung volume induced by deep inspiration, as well as baseline FEV1 and FEV1/FVC with symptom score and weekly salbutamol usage. In all analyses, two-tailed P-values ≤0.05 were considered statistically significant.

**Results**

Based on the frequency of daily and nocturnal symptoms and on the use of rescue medication, 14 subjects were categorized as having intermittent asthma (I group), 12 mild persistent asthma (MP group), and 7 and 3 individuals moderate and severe asthma, respectively. The latter two groups were collapsed into the moderate-severe group (MS group). One subject with moderate asthma reported a history of emergency department visits for acute respiratory distress. Two subjects with severe asthma also reported previous admissions to the hospital for severe respiratory complaints, one of which required intubation. The demographic and clinical characteristics of the study participants are depicted in Table 1. FEV1% predicted did not differ among groups (for the I group: 99±3.6%; for the MP group: 101±2.8%; for the MS group: 96±5.7%, mean ± SEM; ANOVA: P = 0.67). Similarly, FEV1/FVC was not significantly different (for the I group: 0.78±0.03; for the MP group: 0.81±0.02; for the MS group: 0.72±0.03, ANOVA: P = 0.06). Symptom score within the week preceding the study was 8±1.6 in the I group, 12±2.1 in the MP group and 15±3.6 in the MS group (P = 0.12). Similarly, salbutamol usage per week was 3±0.9 in the I group, 6±1.5 in the MP group and 8±3.2 in the MS group (P = 0.17). The single dose of methacholine required to induce ≥15% reduction in IVC in the absence of deep inspirations was 8.3±1.8 mg/ml in the I group, 8.2±3.0 mg/ml in the MP group and 3.4±0.9 mg/ml in the MS group (P = 0.24). The reduction in IVC attained in the no deep inspiration-single dose challenge was similar among the 3 groups (% induced bronchoconstriction in IVC, for I: 25±2.9%; for MP: 27±4.5%; for MS: 28±3.9%; P = 0.82).

The bronchodilatory effect of deep inspiration is shown in Fig. 2. The % bronchodilation was 68±5.4% in the I group, 45±7.2% in the MP group and 4±15.6% in the MS group (ANOVA: P < 0.0001). The deep inspirations performed after methacholine-induced bronchoconstriction were able to increase IVC by 719±0.12 ml in the I group, 461±0.11 ml in the MP group, and 170±0.08 ml in the MS group (mean ± SEM; ANOVA: P = 0.005). Reduction in deep inspiration-induced bronchodilation with increasing severity of asthma could also be demonstrated when FEV1, instead of IVC, was used (51±6.5% in the I group, 19±8.9% in the MP group and 2±10.8% in the MS group; P = 0.0008). Interestingly, two out of the three severe asthmatics showed negative values for bronchodilation, indicating that the series of deep inspirations further worsened lung function (bronchoconstrictor effect of deep inspiration). This is in agreement to the work of Orehek and colleagues. Post-hoc analysis for the IVC-based deep inspiration-induced bronchodilation showed significantly lower % bronchodilation in the MS group as compared to the I and MP groups (P < 0.0001 and 0.005, respectively). The difference among the study groups remained significant even when the three severe asthmatics were removed from the analysis (ANOVA: P = 0.0002).

To further evaluate whether the ability of deep inspiration to reverse induced bronchoconstriction is associated with poor control of asthma, we conducted linear regression analysis between the % bronchodilation by deep inspiration and the symptom score or the salbutamol usage rate: both regressions were statistically significant (r = −0.42, P = 0.01 for symptom score and r = −0.47, P = 0.004 for salbutamol usage). These relationships were confirmed when absolute changes in IVC induced by the series of DI were regressed against the symptom score (r = −0.38, P = 0.02) and the salbutamol usage rate (r = −0.37, P = 0.03). However, it must be noted that the strength of this relationships was modest as both regressions were...
influenced by the most severe asthmatics who had negative values for bronchodilation. On the other hand, neither FEV₁% predicted nor FEV₁/FVC correlated with total symptom score or salbutamol usage (FEV₁% predicted vs. symptom score: \( r = 0.13, P = 0.44 \); FEV₁% predicted vs. salbutamol usage: \( r = 0.10, P = 0.57 \); FEV₁/FVC vs. symptom score: \( r = 0.10, P = 0.56 \); FEV₁/FVC vs. salbutamol usage: \( r = 0.12, P = 0.49 \)), indicating that, compared to spirometric parameters, the bronchodilatory effect of deep inspiration is a better tool in describing asthma control.

**Discussion**

The findings of the study confirm previous observations that changes in lung volume induced by deep inspiration worsen...
with increasing severity of asthma. In this study, the GINA guidelines were used to determine disease severity. We found that asthmatics with mild disease have a bronchodilatory ability that is close to that of healthy individuals, whereas those with moderate to severe disease have no evidence of deep inspiration-induced bronchodilation. These observations provide evidence in support of the hypothesis that the bronchodilatory effect of deep inspiration impacts the clinical manifestations of asthma. Importantly, this study demonstrates that a volume outcome, such as IVC, has the sensitivity to detect differences in the ability of deep inspiration to induce changes in lung function among groups with various severity of asthma.

Many investigators have previously shown that, in asthmatics, the effect of a deep inspiration to reverse spontaneously or experimentally induced airway narrowing is quite variable. In these studies, there was no attempt to relate the variability of the effect of deep inspiration to disease severity. In 1980, Orehek et al. reported the presence of "sensitivity" vs. "resistance" to the bronchodilatory effects of deep inspiration in asthmatics and indicated that this difference is related to disease activity, as determined by the frequency of "dyspneic paroxysms". Also, almost 20 years ago, Lim and coworkers demonstrated that, during an asthma attack, the ability of a single deep inspiratory maneuver to increase maximal expiratory airflow is severely blunted. More recently, Assefa and colleagues reported that, in asthmatic children, the difference in forced expiratory airflow during a full expiratory maneuver, from the flow during a partial maneuver is inversely correlated with the severity of asthma. Using a different methodology to measure the bronchodilatory effect of deep inspiration, our study confirms that attenuation of this effect is associated with worsening asthma in a sample of adults studied in stable condition.

It is possible that the effectiveness of the bronchodilatory function of lung inflation is determined by the interdependence between the parenchyma that surrounds and sustains the small airways and the outer wall. On this basis, increasing lung volume results in enhanced stretch applied to the outer surface of the airways, which, in turn, induces changes in airway caliber and/or opens up collapsed airways, thus relieving air trapping. As a consequence, any factor unlinking the airways and the parenchyma could cause a reduction in the distending forces that are applied on the airways leading to reduced ability of deep inspiration to bronchodilate. Increased thickness of the airway wall, such as that observed in the more severe stages of asthma, could induce greater stiffness of airways, which would oppose airway distension. Alternatively, deep inspirations could have lost their ability to reverse, or even to prevent, airway closure, due to a combination of transmural pressure. In support of a relationship between the loss of deep inspiration-induced bronchodilation and the inability of asthmatic airways to distend is the study by Pyrgos et al. in which airway distensibility, as measured by changes in airway lumenal areas from FRC to TLC assessed with high resolution computerized tomography (HRCT), was positively associated with the bronchodilatory ability of deep inspiration. In the same study, it was shown that the more severe asthmatics had the smallest changes in airway lumenal areas by HRCT. Interestingly, this phenomenon was more evident in the small airways, upon which the distending forces are, for the greater part, expected to act.

The airway distending forces could be ineffective because the stretch is not transmitted to the airway smooth muscle. Although a parenchymal problem cannot be excluded in asthmatics, we favor the hypothesis that airway changes due to chronic inflammation are the main cause of impairment in the bronchodilatory effect of deep inspiration in asthma. The inflammation that occurs in the airways of asthmatics is associated with factors that could potentially stiffen the airways, such as vascular engorgement and edema; airway smooth muscle stiffening has also been shown to occur as a result of exposure to several cytokines. The airways in asthma are characterized by various degrees of tissue remodeling including increased thickness of the reticular basement membrane and airway smooth muscle hypertrophy. These aspects of airway changes can also lead to reduced effectiveness of airway-parenchyma interdependence. Another possible explanation for reduced bronchodilation by deep inspiration includes increased smooth muscle tone leading to stiffer airways or to hyperinflation. The latter condition would reduce the stretch effect of deep inspiration because, since lung volume at FRC is already increased the relative expandability of the chest wall would be limited. Since baseline spirometry did not differentiate the three groups of subjects in this study, it is unlikely that smooth muscle tone lies behind the reduction in deep inspiration-induced bronchodilation.

The importance of the association between the bronchodilatory effect of deep inspiration and severity of asthma, as defined clinically by the GINA guidelines, is further highlighted by the fact that the conventional spirometric outcomes (FEV₁ and FEV₁/FVC) did not correlate with symptom scores and salbutamol usage. This finding suggests that the ability of deep inspiration to dilate constricted airways is superior than spirometry in discriminating among various degrees of clinical asthma. This concept needs further validation in a large patient pool. In addition, since two studies have shown that anti-inflammatory treatment is able to improve the bronchodilatory ability of deep inspiration, testing for this function may provide another helpful tool in characterizing and following patients with asthma. The downside of this approach is that (a) the methodology to measure deep inspiration-induced bronchodilation, as currently employed, is time-consuming and (b) individuals with significant baseline airflow limitation may not be candidates for undergoing methacholine provocation.

The innovative aspect of this study is the usage of IVC as the primary outcome to determine the magnitude of the bronchodilating effects of deep inspiration. Previously, measures of airway resistance or airflow have been utilized. Until recently, we have used simple spirometry but we believe it is important that a measure of the effect of deep inspirations does not include a deep inspiration per se. In this regard, our methodology of recording IVC (starting the vital capacity inspiratory maneuver from a residual volume that has been reached with an expiration from the end of a tidal breath) offers the advantage over conventional spirometric parameters that it is not affected by a preceding deep inspiratory maneuver.
We have extensively employed the IVC methodology in various studies but the current study is important because it demonstrates that this outcome has the ability to differentiate between various asthma severity groups. Also, validation of IVC in this study supports the notion that lung volume measures are as effective as measures of airflow and airway resistance in describing the impact of deep inspirations. This adds to our insights into the mechanism of the effect of deep inspiration.

Given that the bronchodilatory effect of deep inspiration is only minimally reduced in mild asthmatics, but completely lost in those with severe disease, we speculate that in the mild forms of asthma, the bronchodilatory effect of deep inspiration manages to adequately counteract intrinsically or extrinsically induced changes in airway tone. In more severe disease, increases of airway tone are not balanced by the dilating forces of lung inflation, thus accounting for the occurrence and/or persistence of bronchoconstriction. This may not be obvious at stable state, but may become a significant problem upon acute exposure to a bronchoconstrictive stimulus or during an asthma exacerbation. In other words, we believe that there are two possible clinical consequences from the impairment of the bronchodilatory effect of deep inspiration: on one hand, the intrinsic smooth muscle tone will not be adequately balanced leading to chronic airway narrowing and airway stiffening; on the other hand, any acute encounter with an external or internal spasmogenic stimulus would not be counteracted by the natural bronchodilating effects of deep inspiration. The latter consequence would be important in asthma because it could increase the risk for serious attacks in asthmatics. Therefore, therapeutic strategies to improve the bronchodilatory effects of deep inspiration could have central value in asthma.

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