Deep inspirations protect against airway closure in nonasthmatic subjects

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1Woolcock Institute of Medical Research, 2Cooperative Research Centre for Asthma, 3University of Sydney, Sydney; 4Department of Respiratory Medicine, Royal North Shore Hospital, St. Leonards; and 5Discipline of Pharmacology, Bosch Institute, University of Sydney, Sydney, Australia

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Chapman DG, Berend N, King GG, McParland BE, Salome CM. Deep inspirations protect against airway closure in nonasthmatic subjects. J Appl Physiol 107: 564–569, 2009. First published May 14, 2009; doi:10.1152/japplphysiol.00202.2009.—The mechanism by which deep inspirations protect against increased airway responsiveness in nonasthmatic subjects is not known. The aim was to investigate the role of airway closure and airway narrowing in deep inspiration bronchoprotection. Twelve nonasthmatic and nine asthmatic subjects avoided deep inspirations (DI) for 20 min, then took five DIs expired to functional residual capacity (DI-FRC) or, on a separate day, no DI (no DI) before inhaling a single dose of methacholine. On another day, eight nonasthmatic subjects took five DIs expired to residual volume (DI-RV). Peripheral airway function was measured by respiratory system reactance (Xrs), using the forced oscillation technique, and by forced vital capacity (FVC) as an index of airway closure. Respiratory system resistance (Rrs) and forced expiratory volume in 1 s (FEV1)/FVC were measured as indexes of airway narrowing. In nonasthmatic subjects, DI-FRC reduced the response measured by FEV1 (P = 0.019), Xrs (P = 0.02), and FVC (P = 0.0005) but not by Rrs (P = 0.15) or FEV1/FVC (P = 0.52) compared with no DI. DI-RV had a less protective effect than DI-FRC on response measured by FEV1 (P = 0.04) and FVC (P = 0.016). There was no difference between all protocols when the response was measured by Xrs (P = 0.20), Rrs (P = 0.88), or FEV1/FVC (P = 0.88). DI had no effect on methacholine response in asthmatic subjects. DI protect against airway responsiveness through an effect on peripheral airways involving reduced airway closure. The protective effect of DI on FEV1 and FVC was abolished by expiration to residual volume. We speculate that the reduced airway closure is due to reduced baseline ventilation heterogeneity and/or reduced airway surface tension.

airway hyperresponsiveness; bronchoconstriction; forced expiratory volume in 1 s; methacholine

AIRWAY HYPERRESPONSIVENESS (AHR), defined as exaggerated bronchoconstriction in response to low-dose stimulation of airway smooth muscle (34), is a characteristic feature of asthma; however, its pathophysiological mechanisms are not fully understood. Prohibiting deep inspirations (DIs) before bronchial challenge increases the response to methacholine in nonasthmatic subjects (15, 24, 26). This protective effect of prechallenge DIs, known as bronchoprotection, is absent in asthmatic (7, 15, 26) and nonasthmatic subjects with AHR (15, 25). This led to the speculation that the impairment of the physiological mechanisms involved in bronchoprotection is one of the primary causes of AHR (15, 25).

The mechanisms by which DIs protect against AHR are not well understood. DI avoidance in rats leads to increased elastance after methacholine challenge, with no effect on resistance, suggesting a purely peripheral airway effect (23). Using the forced oscillation technique (FOT) to make measurements of lung resistance and elastance, Lutchen et al. (16) showed that DI avoidance produced a greater response to methacholine at oscillation frequencies between 0.1 and 2 Hz, indicating peripheral airflow changes. Computational modeling suggested this pattern of response was indicative of heterogeneous constriction involving near or complete closure of some airways (16). Although these studies suggest a peripheral effect of DIs, it is unclear whether this effect is due to airway closure or to airway narrowing. Previous studies have proposed that bronchoprotection is due to the ability of DIs to stretch unobstructed airways, leading to airways that are more resistant to airway narrowing. Some studies have argued that bronchoprotection is due to airway stretch, then the volume of expiration after DI would not be expected to alter the protection; however, the volume of expiration may affect heterogeneity and/or airway closure. The contribution of airway closure and airway narrowing to the methacholine-induced response can be determined separately by calculating changes in forced vital capacity (FVC), as a marker of airway closure, and change in forced expiratory volume in 1 s (FEV1)/FVC, as a marker of global airway narrowing, that includes both peripheral and central airway narrowing (9, 12, 28). Recent research suggests that increased airway closure, measured by the change in FVC, is a determinant of AHR independent of airway narrowing (9).

We hypothesize that, if DIs have a largely peripheral effect, then they will protect against methacholine-induced airway closure but have no effect on airway narrowing. The aim of the present study was to determine the effect of DIs taken before challenge on the magnitude of airway closure and airway narrowing after methacholine in asthmatic and nonasthmatic subjects. Response was measured both by spirometry, as a marker of airway closure and narrowing, and by FOT, as a marker of peripheral airway mechanics. In addition, in nonasthmatic subjects, we investigated the effect on the response to methacholine of the expiration that followed each DI by comparing expirations to functional residual capacity (FRC) with expirations to residual volume (RV).

METHODS

Subjects. Subjects were recruited from the staff and students of the University of Sydney and the Woolcock Institute of Medical Research and through the research volunteer database at the Woolcock Institute of Medical Research. Asthmatic subjects had physician-diagnosed asthma, had symptoms consistent with asthma in the preceding 12 mo, and were taking inhaled corticosteroids and/or β2-agonist medication.
Nonasthmatic subjects had no history of respiratory disease and no symptoms consistent with asthma. All subjects were nonsmokers and had no other cardiac or respiratory disease. The study was approved by the Sydney South West Area Health Service Ethics Review Committee (protocol no. X05-0285), and all subjects gave written, informed consent.

**Study design.** At the initial visit, asthmatic and nonasthmatic subjects underwent methacholine challenge to allow for calculation of the dose that caused a 20% fall in FEV\(_1\) by interpolation. This dose, or a dose of 100 \(\mu\)mol in non-responders, was given as a single dose in subsequent visits. At three subsequent visits, spirometric and FOT measurements (6, 11, and 19 Hz) were made at baseline, and then subjects were asked to avoid DIs for 20 min (Fig. 1). The effect of avoiding DIs for 20 min on airway mechanics was measured by FOT. After the FOT measurements, subjects either took no DIs (no DI) or, on another day, took five DIs through the mouth from FRC to total lung capacity and exhaled to FRC (DI-FRC). Then they inhaled the single dose of methacholine, as determined at the initial visit. On a separate study visit, nonasthmatic subjects repeated the DI protocol, but each DI was followed by exhalation to residual volume (DI-RV).

**Methacholine challenge.** Methacholine challenges (ICN Pharmaceuticals, Costa Mesa, CA) were performed in asthmatic subjects using the rapid method (35) via De Vilbiss no. 45 nebulizers (dose range: 0.1–12.2 \(\mu\)mol) and in nonasthmatic subjects by the method of Chai et al. (5, 8) via a KoKo dosimeter (PDS Instrumentation, Louisville, KY) (dose range: 0.79–200 \(\mu\)mol). Spirometry was measured according to ATS criteria (1) using a Sensormedics \(V_{\text{max}}\) spirometer (SensorMedics Corporation, Yorba Linda, CA); FVC maneuvers were sustained for a minimum of 6 s until a plateau in the expiratory volume trace was observed. Baseline measurements are reported as percent predicted (21).

Airway narrowing was assessed by the fall in FEV\(_1\)/FVC and airway closure by the percent fall in FVC. Dose response slope was calculated as the two-point slope from the fall in FEV\(_1\) at the end of challenge divided by the dose in micromoles (19, 20).

**Forced oscillation technique.** Respiratory system resistance (Rrs) and reactance (Xrs) were measured by the FOT at oscillation frequencies of 6, 11, and 19 Hz as previously described (31). Measurements were performed directly after inhalation of methacholine and before spirometry. Expiratory flow limitation was assessed as the difference in Xrs between the inspiratory and expiratory phases of tidal breathing and an Xrs inspiratory to expiratory value of \(\geq 2.8\) cmH\(_2\)O\(\cdot\)l\(^{-1}\)\(\cdot\)s\(^{-1}\) indicated expiratory flow limitation (10).

**Outcome variables and data analysis.** The data were analyzed using Analyse-It for Microsoft Excel (Analyse-It Software, Leeds, UK). Between-group comparisons of baseline characteristics were made using two-sample equal variance \(t\)-tests. Comparisons between baseline and post-20 min of DI avoidance (no DI protocol only) and between DI-FRC and no-DI protocols were made using Wilcoxon signed-ranks tests. Comparisons between DI-FRC, no DI, and DI-RV were made using Friedman one-way ANOVA by ranks, and paired comparisons were made using Wilcoxon signed-ranks test. \(P\) values of \(<0.05\) were regarded as statistically significant. Summary data are presented as median (interquartile range) unless otherwise stated.

**RESULTS**

Data were obtained from 9 asthmatic and 12 nonasthmatic subjects (Table 1). The asthmatic group did not differ from the nonasthmatic group in age, height, or body mass index but had impaired baseline lung function measured by spirometry. Asthmatic subjects had a higher dose response slope \((P = 0.004)\) than nonasthmatic subjects. Therefore, the single dose of methacholine administered to asthmatic subjects was lower (median [interquartile range] 2.55 [1.43] \(\mu\)mol) than nonasthmatic subjects (102.2 [57] \(\mu\)mol; \(P = 0.004\)).

Avoiding DIs for 20 min had no direct effect on the mechanical properties of the airways, as measured by FOT, in either asthmatic or nonasthmatic subjects. Neither Rrs (Fig. 2) nor Xrs (Fig. 3) measured at 6, 11, and 19 Hz changed significantly between the baseline measurements and those made immediately after the period of DI avoidance in either group. The absence of any change in Rrs in nonasthmatic \((P = 0.57\) at 6 Hz) or asthmatic subjects \((P = 0.20\) at 6 Hz) implies that DI avoidance had no direct effect on airway caliber. Similarly, the absence on any effect of DI avoidance on Xrs in nonasthmatic \((P = 0.57\) at 6 Hz) and asthmatic subjects \((P = 0.07\) at 6 Hz) suggests that DI avoidance had no direct effect on respiratory system elastance.

**Effect of DI avoidance on airway response to methacholine.**

There was significant DI bronchoprotection in the nonasthmatic subjects but not in the asthmatics. The percent fall in FEV\(_1\) was greater during no DI than during DI-FRC in nonasthmatic (mean difference between protocols \(\pm 95\%\) CI \(= 10.3\) \(\pm\) 8.2; \(P = 0.019\)) but not in asthmatic subjects \((1.7\) \(\pm\) 5.6; \(P = 0.51\)).

DIs had no effect on airway narrowing in either nonasthmatic or asthmatic subjects (Fig. 4). There was no difference in the fall in FEV\(_1\)/FVC after bronchoconstriction between DI-FRC and no DI in nonasthmatic \((P = 0.52\) or asthmatic subjects \((P = 0.65\). The decrease in FEV\(_1\)/FVC was of similar magnitude in asthmatic and nonasthmatic subjects during both protocols (DI-FRC \(P = 0.86\) and no DI \(P = 0.75\)).

Methacholine challenge led to airway closure in both asthmatic and nonasthmatic subjects; however, DIs before challenge significantly reduced the development of airway closure in nonasthmatic subjects but had no effect in asthmatic subjects (Fig. 5). In nonasthmatic subjects, the percent fall in FVC was less after DI-FRC compared with no DI \((P = 0.0005)\), whereas, in asthmatic subjects, the percent fall in FVC did not differ between DI-FRC and no DI protocols \((P = 0.43)\).

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**Fig. 1.** Schematic of the modified single-dose protocol used to compare the effects of deep inspirations (DI; A) and DI avoidance (B). FOT, forced oscillation technique; MCh, methacholine.
Measurements of airway mechanical properties, made by the FOT, were consistent with spirometric findings. Inhalation of methacholine increased Rrs and decreased Xrs in both asthmatic and nonasthmatic subjects. In nonasthmatic subjects, DIs reduced the fall in Xrs after methacholine (P = 0.02 at 6 Hz) but had no effect on the increase in Rrs (P = 0.15 at 6 Hz). In asthmatic subjects, there was no difference between DI-FRC and no DI in the fall in Xrs (P = 0.30) or in the increase in Rrs (P = 0.25). There was no evidence of expiratory flow limitation, as measured by FOT, since the index of expiratory flow limitation Xrsinsp-exp did not exceed 2.8 cmH2O/ls in any of the nonasthmatic subjects after methacholine administration during DI-FRC (range: 0.86 – 0.89), no DI (0.53–0.64), or DI-RV (0.32–0.76) protocols.

Effect of exhalation to RV on airway response to methacholine. To determine whether the bronchoprotective effect of DIs was determined not only by the magnitude of the inspiration but also by the subsequent expiration, we undertook an additional study visit in nonasthmatic subjects. Since asthmatic subjects had no significant bronchoprotective effect, they were not included in this additional test protocol. Table 2 shows data for eight nonasthmatic subjects after DI-FRC, no DI, and DI-RV protocols. In these nonasthmatic subjects, the protective effect of DIs was abolished when the DI was followed by expiration to RV. Multiple-group analysis showed significant differences between all protocols when the response to methacholine was measured by FEV1 and FVC (P = 0.02 and 0.005, respectively) but not when measured by FEV1/FVC (P = 0.88). When DI were expired to RV, the percent fall in FEV1 was greater than after DI-FRC protocol (P = 0.04) and not different from the no DI protocol (P = 0.46).

These findings were supported by the FOT data, since there was no difference between the change in Rrs during DI-FRC, DI-RV, and no DI protocols (P = 0.88). However, multiple-
of previous studies. We found no protective effect of DIs on Rrs at 6 Hz. Similarly, previous studies have been unable to detect a protective effect of DIs when measured by resistance at 8 Hz (16, 27). However, we found that DIs significantly reduced the methacholine-induced decrease in Xrs at all frequencies. Since methacholine is unlikely to affect lung iner-
tance (22), this protective effect of DIs is likely due to protec-
tion against increases in lung elastance. In nonasthmatic sub-
jects, Lutchen et al. (16) also found that DIs reduced the effect 
of methacholine on lung elastance (the dominant component of 
reactance) measured at 8 Hz. They also measured resistance 
and elastance at very low frequencies down to 0.1 Hz, reflect-
ing changes in very peripheral airways and in lung tissue, and 
found that the response to methacholine in these regions was 
modified by DIs. Their computational modeling suggested that 
avoiding DIs results in uneven constriction involving hetero-
geneous airway closure or near closure. A similar protective 
effect of DIs against increases in respiratory system elastance, 
with no effect on resistance, has been demonstrated in rats (23). 
None of the subjects in the present study had evidence of 
expiratory flow limitation as previously proposed (10). Thus 
the present changes in reactance are most likely a result of 
peripheral airway closure, consistent with modeling predicting 
that peripherally confined airway closure is a feature of the 
response to methacholine (29).

The changes in spirometry in the present study are consistent 
with bronchoprotection as a peripheral airway response and 
strongly suggest that DIs before challenge reduce peripheral 
airway closure. FEV1 can be partitioned into its components of
Table 2. Effect of expiration to RV on the response to methacholine in nonasthmatic subjects (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>DI-FRC</th>
<th>DI-RV</th>
<th>No DI</th>
<th>Friedman P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent fall in FEV₁</td>
<td>15.0 (10.9–24.5)</td>
<td>20.1 (16.3–35.7)*</td>
<td>25.9 (16.6–39.0)*</td>
<td>0.02</td>
</tr>
<tr>
<td>Fall in FEV₁/FVC</td>
<td>0.10 (0.08–0.14)</td>
<td>0.10 (0.08–0.15)</td>
<td>0.09 (0.08–0.18)</td>
<td>0.88</td>
</tr>
<tr>
<td>Percent fall in FVC</td>
<td>2.4 (1.9–6.8)</td>
<td>10.1 (6.4–17.0)*</td>
<td>11.6 (7.1–27.5)*</td>
<td>0.005</td>
</tr>
<tr>
<td>Increase in Rs, cmH₂O·L⁻¹·s⁻¹</td>
<td>2.5 (2.2–2.7)</td>
<td>2.6 (2.2–4.7)</td>
<td>2.3 (1.7–4.0)</td>
<td>0.88</td>
</tr>
<tr>
<td>Fall in Xrs, cmH₂O·L⁻¹·s⁻¹</td>
<td>0.83 (0.7–1.0)</td>
<td>1.5 (0.8–2.8)</td>
<td>1.2 (0.6–3.1)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are median (interquartile range). FRC, functional residual capacity; RV, residual volume; Rs, respiratory system resistance; Xrs, respiratory system reactance. *Significant difference compared with DI-FRC (P < 0.05).

Airway narrowing (FEV₁/FVC) and airway closure (FVC) (12, 28). It is possible that changes in FVC represent near closure rather than complete closure of airways; however, both result in regions of nondetectable flow, representing severely under-ventilated lung units. A recent study combining high-resolution computed tomography and spirometry suggests that changes in FEV₁/FVC are a surrogate for changes in large airway caliber (6); however, changes in FEV₁/FVC likely represent peripheral and central narrowing. The absence of any effect of DIs on the fall in FEV₁/FVC suggests that bronchoprotection is unlikely to be due to an effect on global airway narrowing. However, DIs reduced the fall in FVC after methacholine in nonasthmatic subjects. The use of FVC assumes that any volume change is due to an increase in residual volume reflecting gas trapping (12), and, as such, methacholine-induced changes in FVC reflect airway closure. This strongly suggests that DIs protect against increased airway closure in nonasthmatic but not asthmatic subjects.

DIs may promote homogeneous ventilation throughout the lung and protect nonasthmatic subjects against large-scale airway closure. The present findings indicate that avoiding DIs does not itself alter respiratory system resistance or reactance, but rather “primes” the airways to increased closure once stimulated. This priming effect could occur through an increase in ventilation heterogeneity if, as suggested by Anafi and Wilson (2), avoidance of DIs leads to preferential distribution of airflow to larger airways, resulting in a reduction of caliber of the smaller airways. Computational modeling suggests that increased ventilation heterogeneity would lead to large-scale but localized regions of airway closure in the presence of minimal smooth muscle activation (32). This is consistent with the heterogeneous constriction pattern previously reported (16) and the increased airway closure in the present study following DI avoidance. The loss of DI bronchoprotection in asthmatic subjects could result from uneven airway remodeling, leading to permanent ventilation heterogeneity, which persists even after DIs. Indeed, the severity of AHR in asthmatic subjects has recently been correlated with the degree of ventilation heterogeneity at baseline (11). During expiration to RV, airways close, and during subsequent inspiration, the non-uniform recruitment of airways could lead to heterogeneous ventilation. This could negate the effect of the previous inspiration to total lung capacity and result in widespread airway closure similar to that when DIs were avoided.

Alternatively, surfactant may play a role in the protective effect of DIs against airway closure. It has been hypothesized that the sedimentation of surfactant molecules leading to an increased surface tension (30) would result in the reported decrease in pulmonary compliance with DI avoidance (4, 13). DIs increase the production of pulmonary surfactant in rats (17) by distorting alveolar type I cells, which cause release of surfactant from type II cells (3). The entire store of surfactant is released on reaching the distortion threshold (18). A decrease in surfactant release during DI avoidance, resulting in an increase in surface tension, is likely to lead to increased closure upon airway smooth muscle stimulation. The loss of bronchoprotection in asthma could occur through the known inhibitory effects of inflammation on surfactant (14) or through an abnormality in surfactant release. Furthermore, the loss and/or deactivation of surfactant molecules with expiration to low lung volumes (7–9) could result in the increased airway closure seen with expiration to RV.

Several studies have suggested that bronchoprotection is due to protection against airway smooth muscle (ASM) contraction (15, 16, 24). Changes in length, such as during DIs, alter the organization of contractile filaments within ASM (33), and it has been hypothesized that, during structural reorganization, the airways would be protected against airway narrowing during bronchial challenge. However, DIs did not affect the fall in FEV₁/FVC or Rs, suggesting that global ASM contractility was unaltered. Furthermore, the abolition of the bronchoprotective effect by expiration to RV suggests that a purely dilatory effect of DI on ASM is unlikely to be the underlying mechanism. It must be noted that the present study cannot rule out that DI protection against airway closure occurs through protection against increased contractility of peripheral ASM.

Since the loss of the “bronchoprotective” effect of DIs appears to be a characteristic of AHR (15, 25), an understanding of the mechanisms underpinning the effect is likely to contribute to our understanding of AHR. AHR is characterised by both increased sensitivity to ASM stimulation and an increased maximal response (34). Brusasco et al. (7) reported that avoiding DIs throughout bronchial challenge led to an increase in the maximal response without affecting sensitivity. Although they were unable to disassociate the bronchoprotective effect from the bronchodilatory effect, the study does suggest that the mechanisms affecting the maximal response to challenge may be independent of those affecting ASM sensitivity. In the present study, DI avoidance led to similar magnitudes of airway narrowing in nonasthmatic and asthmatic subjects; however, substantially larger doses of methacholine were required in nonasthmatic subjects, suggesting that the loss of bronchoprotection does not replicate the increased ASM sensitivity characteristic of AHR. This is consistent with previous research suggesting that, although avoiding DIs increases responsiveness in nonasthmatic subjects, it does not induce true AHR (7, 15, 16). Indeed, recent research has highlighted the independent contribution of both airway closure and airway narrowing to AHR (9, 29). We speculate that AHR may be the result of a combination of abnormalities involving airway...
closure and the maximal response plateau due to a loss of DI bronchoprotection and altered ASM sensitivity due to other mechanisms.

The present study suggests that DIs do not alter global airway narrowing in response to methacholine. In contrast, DI bronchoprotection in nonasthmatic subjects is due to an effect on peripheral airway changes, most likely due to a reduction in airway closure. We speculate that DIs protect against airway closure through a reduction in baseline ventilation heterogeneity and/or reduction in surface tension. The inability for DIs to protect against airway closure in asthmatic subjects highlights the need for further investigation into the mechanisms underlying bronchoprotection.

GRANTS

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REFERENCES