Effect of deep inspiration avoidance on ventilation heterogeneity and airway responsiveness in healthy adults

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1Woolcock Institute of Medical Research, Sydney; 2Cooperative Research Centre for Asthma and Airways, Sydney; 3The University of Sydney, Sydney; and 4Department of Respiratory Medicine, Royal North Shore Hospital, St. Leonards, New South Wales, Australia

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Chapman DG, Berend N, King GG, Salome CM. Effect of deep inspiration avoidance on ventilation heterogeneity and airway responsiveness in healthy adults. J Appl Physiol 110: 1400–1405, 2011. First published February 24, 2011; doi:10.1152/japplphysiol.00855.2010.—The mechanisms by which deep inspiration (DI) avoidance increases airway responsiveness in healthy subjects are not known. DI avoidance does not alter respiratory mechanics directly; however, computational modeling has predicted that DI avoidance would increase baseline ventilation heterogeneity. The aim was to determine if DI avoidance increases baseline ventilation heterogeneity and whether this correlated with the increase in airway responsiveness. Twelve healthy subjects had ventilation heterogeneity measured by multiple-breath nitrogen washout (MBNW) before and after 20 min of DI avoidance. This was followed by another 20-min period of DI avoidance before the inhalation of a single methacholine dose. The protocol was repeated on a separate day with the addition of five DIs at the end of each of the two periods of DI avoidance. Baseline ventilation heterogeneity in convection-dependent and diffusion-convection-dependent airways was calculated from MBNW. The response to methacholine was measured by the percent fall in forced expiratory volume in 1 s (forced vital capacity (FVC) (airway narrowing) and percent fall in FVC (airway closure). DI avoidance increased baseline diffusion-convection-dependent airways (P = 0.02) but did not affect convection-dependent airways (P = 0.9). DI avoidance increased both airway closure (P = 0.002) and airway narrowing (P = 0.02) during bronchial challenge. The increase in diffusion-convection-dependent airways due to DI avoidance did not correlate with the increase in either airway narrowing (r = 0.14) or airway closure (r = 0.12). These findings suggest that DI avoidance increases diffusion-convection-dependent ventilation heterogeneity that is not associated with the increase in airway responsiveness. We speculate that DI avoidance reduces surfactant release, which increases peripheral ventilation heterogeneity and also predisposes to peripheral airway closure.

AIRWAY HYPERRESPONSIVENESS (AHR), defined as exaggerated bronchoconstriction in response to low-dose stimulation of airway smooth muscle (ASM) (31), is a characteristic feature of asthma. AHR is associated with an increased risk of exacerbation (20), increased decline in lung function (4), and increased risk for the development of asthma (4). Although AHR has many clinical correlates, the pathophysiological mechanisms leading to AHR are not fully understood. Deep inspirations (DIs) protect against airway responsiveness in normal healthy subjects, so that avoiding DI before bronchial challenge leads to an increased response (18, 25). This bronchoprotective effect of DI is absent in asthma, leading to the speculation that the loss of this protection reflects an important abnormality in the pathophysiology of AHR (25).

The mechanisms by which DIs protect against increased airway responsiveness and the pathophysiology leading to its absence in asthma are not well understood. DI avoidance is unlikely to alter respiratory mechanics directly, since DI avoidance itself does not alter prechallenge specific airway conductance, residual volume, partial expiratory flow (11), respiratory system resistance (Rrs), or respiratory system reactance (Xrs) (5). Instead, it is likely that DI avoidance somehow “primes” the airways for an increased response once the ASM is stimulated. This “priming” effect most likely occurs in peripheral airways since DI avoidance leads to an increase in methacholine-induced airway closure (5, 21). At present, it is unclear how DI avoidance primes the peripheral airways to an increased response, and, as such, a sensitive measure of peripheral airway function may provide important information as to how DI avoidance alters respiratory function.

Measurement of the normalized phase III slope during multiple-breath nitrogen washout (MBNW) provides a sensitive measurement of peripheral airway function. Analysis of the MBNW trace allows for ventilation heterogeneity to be partitioned into two distinct airway regions defined by their mode of gas transport as airways where gas transport occurs predominantly by convection and in lung regions where diffusive and convective gas transport interact (24, 29). Computational modeling has predicted that DI avoidance would increase the inequality of airway calibers, preferentially distributing airflow to larger airways (2) and thus increasing ventilation heterogeneity. However, it is unknown whether DI avoidance alters baseline ventilation heterogeneity and whether this is then associated with the increased airway closure during bronchial challenge.

We hypothesized that avoiding DIs in healthy subjects would increase baseline ventilation heterogeneity and that this increase would be associated with an increase in airway closure during bronchial challenge. The aim of the present study was to determine whether DI avoidance increased baseline ventilation heterogeneity and whether this was correlated with the increase in airway responsiveness.

METHODS

Subjects. Subjects were recruited from the staff and students of the University of Sydney and Woolcock Institute of Medical Research and through the research volunteer database at the Woolcock Institute of Medical Research. Subjects had no history or symptoms consistent with asthma. All subjects were lifelong nonsmokers and had no other respiratory or cardiac disease. All subjects were free from upper...
Deep Breaths and Ventilation Heterogeneity

**Fig. 1.** Schematic of the modified single-dose methacholine (MCh) protocol used to compare the effects of deep inspirations (DIs; A) and DI avoidance (B). MBNW, multiple-breath nitrogen washout; FOT, forced oscillation technique; FEV₁, forced expiratory volume in 1 s.

**Table 1. Anthropometric and Lung Function Data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>32.8 (11.1)</td>
</tr>
<tr>
<td>Sex, %women/%men</td>
<td>67/33</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.74 (0.11)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4 (4.0)</td>
</tr>
<tr>
<td>FEV₁, %predicted value</td>
<td>102.9 (12.6)</td>
</tr>
<tr>
<td>FVC, %predicted value</td>
<td>112.8 (13.1)</td>
</tr>
<tr>
<td>Baseline FEV₁/FVC</td>
<td>0.77 (0.03)</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.88 (0.25)</td>
</tr>
<tr>
<td>Total lung capacity, liters</td>
<td>6.80 (1.5)</td>
</tr>
<tr>
<td>Inspiratory reserve volume, liters</td>
<td>3.40 (0.9)</td>
</tr>
<tr>
<td>FRC, liters</td>
<td>3.50 (0.8)</td>
</tr>
<tr>
<td>Residual volume, liters</td>
<td>1.93 (0.4)</td>
</tr>
<tr>
<td>Dose-response slope, %fall/μmol</td>
<td>0.11 (0.06–0.22)</td>
</tr>
</tbody>
</table>

Values are means (SD) except for the dose-response slope, which is shown as the geometric mean (95% confidence interval); n = 12 healthy subjects total. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FRC, functional residual capacity.
Effect of DI avoidance on ventilation heterogeneity.

Avoiding DIs for 20 min increased ventilation heterogeneity arising from the interaction between diffusion and convection but not in convection-dependent airways (\(S_{\text{cond}}\)) between protocols when DIs were taken and avoided (no DI). \(\ast P < 0.05\).

Avoiding DIs for 20 min had no direct effect on respiratory system mechanics as measured by FOT. During the no DI protocol, there was no difference in \(X_{\text{rs}}\) between study protocols \((P = 0.9)\). Compared with when DIs were taken, avoiding DIs reduced FRCMBNW by \(6.7 \pm 2.2\%\) \([2.76 (0.90) \text{ vs. } 2.57 (0.90) \text{ liters, } P = 0.001]\).

Effect of DI avoidance on the airway response to methacholine.

Avoiding DIs for 20 min had no direct effect on respiratory system mechanics as measured by FOT. During the DI avoidance period, \(S_{\text{acin}}\) was \(0.016 \pm 0.01 \text{ liters}^{-1}\) \([\text{means } \pm 95\% \text{ confidence interval (CI)}]\) greater without DIs than when DIs were taken \((P = 0.02)\), whereas there was no difference in \(S_{\text{cond}}\) between protocols \((P = 0.9)\). Compared with when DIs were taken, avoiding DIs reduced FRCMBNW by \(6.7 \pm 2.2\%\) \([2.76 (0.90) \text{ vs. } 2.57 (0.90) \text{ liters, } P = 0.001]\).

Effect of DI avoidance on ventilation heterogeneity. Avoiding DIs for 20 min increased ventilation heterogeneity arising from the interaction between diffusion and convection but not in convection-dependent airways (\(S_{\text{cond}}\)) between protocols when DIs were taken and avoided (no DI). \(\ast P < 0.05\).

![Figure 2](image-url) Comparison of ventilation heterogeneity occurring in regions where diffusive and convective gas transport interact (\(S_{\text{acin}}\)) and in convection-dependent airways (\(S_{\text{cond}}\)) between protocols when DIs were taken and avoided (no DI). \(\ast P < 0.05\).

![Figure 3](image-url) Comparison of methacholine-induced airway closure (A) and airway narrowing (B) measured by spirometry between protocols when DIs were taken or avoided. FVC, forced vital capacity. \(\ast P < 0.05; **P < 0.01\).

Table 2. Correlation coefficients (\(r_s\) values) between the effect of DI avoidance on baseline MBNW parameters and the effect of DI avoidance on the response to challenge

<table>
<thead>
<tr>
<th>MBNW Parameter</th>
<th>(S_{\text{acin}})</th>
<th>(S_{\text{cond}})</th>
<th>Percent Decrease in FRCMBNW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent fall in FEV(_1)</td>
<td>0.27</td>
<td>-0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Percent fall in FEV(_1)/FVC</td>
<td>0.14</td>
<td>-0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>Percent fall in FVC</td>
<td>0.12</td>
<td>-0.24</td>
<td>0.26</td>
</tr>
</tbody>
</table>

The effect of DI avoidance was calculated as the value with the no deep inspiration (DI) protocol minus the value with the DI protocol. Correlations were analyzed using Spearman coefficients (\(r_s\) values). MBNW, multiple-breath nitrogen washout; \(S_{\text{acin}}\), ventilation heterogeneity in airways where gas transport occurs predominantly by diffusion; \(S_{\text{cond}}\), ventilation heterogeneity in airways where gas transport occurs predominantly by convection.
spirometry (Fig. 4), nor did it correlate with the other spirometric measures of the response to methacholine. The percent decrease in FRC_{MBNW} after DI avoidance did not correlate with the increase in S_{acin} (r_s = -0.35, P = 0.27). There was also no correlation between the percent decrease in FRC_{MBNW} after DI avoidance and the increased response to methacholine measured by the percent fall in FEV_1, percent fall in FEV_1/FVC, or percent fall in FVC (Table 2).

DISCUSSION

In the present study, we report that DI avoidance increased baseline ventilation heterogeneity arising in regions of interaction between diffusive and convective gas transport (S_{acin}) but not in convection-dependent airways (S_{cond}). Furthermore, DI avoidance increased the response to bronchial challenge by increasing airway closure and airway narrowing. However, the increased baseline ventilation heterogeneity did not correlate with the increase in airway closure or airway narrowing. These findings imply that the effect of DI avoidance is due to mechanisms occurring in very peripheral airways. In addition, these findings suggest that the increased airway responsiveness after DI avoidance is not a direct result of the increase in baseline ventilation heterogeneity but rather that both changes may reflect a common mechanism.

It is well known that avoiding DIs before challenge increases airway responsiveness in healthy subjects; however, the mechanisms leading to this increased responsiveness are unknown. Avoiding DIs itself does not alter prechallenge specific airway conductance, residual volume, partial expiratory flow, R_{ns}, or X_{rs} (5, 11). In contrast to these measures, we presently report that avoiding DIs increases ventilation heterogeneity, suggesting that DI avoidance promotes airway instability in peripheral regions of the lung. The increase in ventilation heterogeneity occurred in the lung region where diffusion and convection gas transport interact, as measured by an increase in S_{acin}. While the exact anatomic site corresponding to the increase in S_{acin} is unknown, modeling of multiple-breath washout tests using inert gases has suggested that in normal, healthy subjects, S_{acin} reflects ventilation heterogeneity arising in intra-acinar airways (10). While DI avoidance increased both baseline S_{acin} and methacholine-induced airway closure in the present study, there was no correlation between the increase in S_{acin} and the increase in airway closure. This suggests that the increase in baseline ventilation heterogeneity itself is not the priming effect of DI avoidance but rather that changes in baseline ventilation heterogeneity and airway closure during challenge may reflect a common mechanism. Although the correlation coefficient was extremely small, we cannot exclude the contribution of a relatively small sample size to the lack of significant association reported in the present study.

DI avoidance also decreased baseline FRC; however, it is unlikely that this change contributed to either the increase in ventilation heterogeneity or increase in airway responsiveness. The increase in S_{acin} after DI avoidance did not correlate with the decrease in FRC. This is consistent with reports (7, 8) showing that reducing end-expiratory lung volume to a comparable level to that induced by DI avoidance in the present study reduces ventilation heterogeneity; however, it must be noted that this measure of ventilation heterogeneity comprised contributions from both S_{acin} and S_{cond} so that the independent effect of reducing FRC on S_{acin} is unclear. Nonetheless, any reduction in FRC is likely to reduce S_{acin}, since reducing lung volume would place intra-acinar airways on the steeper portion of their individual pressure-volume curves, thus minimizing inequalities in mechanical properties. On the other hand, it is possible that the reduction in FRC with DI avoidance may have led to the closure of some intra-acinar airways. These poorly ventilated airways would then reopen during inspiration, ultimately leading to an increase in S_{acin}; however, the presence of airway closure at FRC is unlikely in the present study since the small reduction in FRC (~200 ml) would still place the population of young, healthy subjects well above closing volume. Reducing end-expiratory lung volume increases responsiveness, as measured by pulmonary resistance, in normal, healthy subjects (12), albeit with reductions in FRC considerably greater than seen with DI avoidance in the present study. However, we found no effect of DI avoidance on the increase in K_{rs} as well as no association between the decrease in FRC and the increase in responsiveness measured by spirometry, suggesting that the increase in airway responsiveness after DI avoidance is not due to the reduction in end-expiratory lung volume.

The mechanism by which DI avoidance increases airway responsiveness in healthy subjects is not well understood; however, the findings of the present study highlight two possible mechanisms. ASM is highly adaptable to acute alterations in resting length, so that the maximal force-generating capacity of ASM is always maintained (15, 30). During DI avoidance, ASM adaptation may increase airway responsiveness through either plastic adaptation of the contractile elements (30) or alterations in cross-bridge dynamics (15). Increased ventilation heterogeneity after DI avoidance may lead to greater adaptation of ASM in slowly ventilating airways, due to the reduced change in caliber during tidal breathing. This would be consistent with the increased heterogeneous constriction pattern after DI avoidance (21). In contrast, DI avoidance increased ventilation heterogeneity only in the diffusion-convective-dependent, intra-acinar airways. Since ASM is only minimally present within the acinus (19), it appears unlikely that the increased airway responsiveness after DI avoidance is due to increased ASM contractility; however, we cannot exclude the notion that the increase in baseline diffusion-convective-de-
dependent ventilation heterogeneity and the increase in airway responsiveness are due to independent mechanisms. On the other hand, DI avoidance may lead to an inadequate release of surfactant. Distortion of alveolar cells by DI causes the release of pulmonary surfactant in rats (22, 23). If the release of surfactant in healthy subjects is similarly dependent upon distortion, then DI avoidance could result in an inadequate supply of surfactant. Surface tension would increase in affected airways, causing them to narrow, and ultimately lead to an increase in ventilation heterogeneity in peripheral airways (i.e., increase in \( S_{\text{acin}} \)). An increase in surface tension is likely to predispose airways to closure, consistent with the increase in airway closure after DI avoidance found in the present study and elsewhere (5, 21). Abnormalities in surfactant composition during DI avoidance lead to alveolar collapse and atelectasis (17, 26), consistent with the small reduction in FRC after DI avoidance in the present study. However, it is unknown whether DI increases surfactant release in humans, and, therefore, the role of surfactant in DI bronchoprotection remains speculative.

We (5) have previously reported that DI avoidance increases airway closure but has little effect on airway narrowing. Similarly, in the present study, we found a major effect of DI avoidance on airway closure; however, we also found a small but significant effect on airway narrowing. It is not clear why the findings of our two studies differ since the inclusion criteria and study protocol for both studies were similar. Nevertheless, the findings from both studies are consistent with the notion that DI avoidance affects the peripheral airways. In the present study, we found that DI avoidance increased \( S_{\text{acin}} \), a peripheral measure of ventilation heterogeneity, as well as the tendency toward an increased response measured by \( X_{\text{rs}} \) with no effect on \( R_{\text{rs}} \). Taken together, this suggests that the effect of DI avoidance is due to peripheral mechanisms, most likely involving increased airway closure during methacholine challenge, as previously reported (5, 21).

Ventilation heterogeneity was measured while \( V_T \) was maintained at 1 liter, and this fixed volume may have had an effect on the present study. First, \( V_T \) is known to influence the measurement of ventilation heterogeneity, so that ventilation heterogeneity increases in convection-dependent airways and decreases in diffusion-convective-dependent regions with increasing \( V_T \) (9, 14). However, MBNW \( V_T \) was consistent between study protocols, so that the effect of \( V_T \) on ventilation heterogeneity is unlikely to have altered the finding that DI avoidance increases \( S_{\text{acin}} \). Nonetheless, it is possible that the 1-liter breaths led to an underestimation of the magnitude of the effect of DI avoidance on \( S_{\text{acin}} \). Second, the fixed \( V_T \) during MBNWs led to inherent variability in the \( V_T \) to-inspiratory reserve volume (IRV) ratio during measurements of ventilation heterogeneity (range: 20.5–39.5%). It is possible that in those subjects with a lower IRV, the MBNWs themselves may have reduced the effect of DI avoidance; however, it is unknown whether inspirations to below total lung capacity are able to confer bronchoprotection. Although there was no association between the increase in \( S_{\text{acin}} \) after DI avoidance and the \( V_T \)-to-IRV ratio (\( r_s = 0.07, P = 0.82 \)), we cannot exclude the possibility that the fixed MBNW \( V_T \) introduced nonbiological variation into the measurement of the effect of DI avoidance on \( S_{\text{acin}} \) and, therefore, may explain the lack of correlation between the effect of DI avoidance on baseline ventilation heterogeneity and on airway closure during challenge.

The novel finding from the present study is that in healthy subjects, simply avoiding DIs by itself increases baseline ventilation heterogeneity occurring in lung regions of interaction between diffusive and convective gas transport. This suggests that the effect of DI avoidance is due to mechanisms that have their functional consequence on peripheral airways, most likely those proximal to the acinus entrance. However, the increase in ventilation heterogeneity after DI avoidance did not correlate with the increase in airway closure during challenge, suggesting that the two were not causally associated. Instead, it is likely that the effects of DI avoidance on baseline ventilation heterogeneity and on airway closure during challenge are due to a common mechanism. We speculate that DI avoidance leads to a reduction in surfactant release, thereby increasing baseline ventilation heterogeneity in diffusion-convective-dependent airways as well as predisposing to increased airway closure during bronchial challenge.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**