Responsiveness of the human airway in vitro during deep inspiration and tidal oscillation

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Noble PB, Jones RL, Thaya Needi E, Cairncross A, Mitchell HW, James AL, McFawn PK. Responsiveness of the human airway in vitro during deep inspiration and tidal oscillation. J Appl Physiol 110: 1510–1518, 2011. First published February 20, 2011; doi:10.1152/japplphysiol.01226.2010.—In healthy individuals, deep inspiration produces bronchodilation and reduced airway responsiveness, which may be a response of the airway wall to mechanical stretch. The aim of this study was to examine the in vitro response of isolated human airways to the dynamic mechanical stretch associated with normal breathing. Human bronchial segments (n = 6) were acquired from patients without airflow obstruction undergoing lung resection for pulmonary neoplasms. The side branches were ligated and the airways were mounted in an organ bath chamber. Airway narrowing to cumulative concentrations of acetylcholine (3 × 10⁻⁶ M to 3 × 10⁻³ M) was measured under static conditions and in the presence of “tidal” oscillations with intermittent “deep inspiration.” Respiratory maneuvers were simulated by varying transmural pressure using a motor-controlled syringe pump (tidal 5 to 10 cmH₂O at 0.25 Hz, deep inspiration 5 to 30 cmH₂O). Airway narrowing was determined from decreases in lumen volume. Tidal oscillation had no effect on airway responses to acetylcholine which was similar to those under static conditions. Deep inspiration in tidally oscillating, acetylcholine-contracted airways produced potent, transient (<1 min) bronchodilation, ranging from full reversal in airway narrowing at low acetylcholine concentrations to ~50% reversal at the highest concentration. This resulted in a temporary reduction in maximal airway response (P < 0.001), without a change in sensitivity to acetylcholine. Our findings are that the mechanical stretch of human airways produced by physiological transmural pressures generated during deep inspiration produces bronchodilation and a transient reduction in airway responsiveness, which can explain the beneficial effects of deep inspiration in bronchial provocation testing in vivo.

bronchoconstriction; mechanical load; airway hyperresponsiveness; asthma

AIRWAY NARROWING IN VIVO is regulated by many physiological factors that may be disrupted in asthma, thereby facilitating excessive airway narrowing in response to bronchoconstrictor stimuli (airway hyperresponsiveness, AHR) (33, 50). There is increased awareness of the fundamental importance of the dynamic mechanical environment of the lung to normal airway function (1). In particular, breathing movements such as deep inspiration (DI) in healthy individuals are potent stimuli for bronchodilation and reduced airway responsiveness (9, 15, 25, 28, 35, 41), whereas this effect is absent or reduced in asthmatic individuals (9, 15, 25). If DI is avoided during a bronchial challenge, airway responsiveness is temporarily increased in healthy individuals and approaches levels comparable within that seen in individuals with asthma (46). Furthermore, several studies suggest that DI can inhibit the response to subsequent bronchoconstrictor stimuli, a phenomenon referred to as bronchoprotection (41). In view of the above it is postulated that there is a link between a failure of the response to DI and the presence of AHR.

While the physiological processes responsible for the beneficial effects of DI are unknown, they are thought at the basic level to involve mechanical stretch of the airway smooth muscle (ASM) during lung inflation (49), although other factors may also be involved including neural and humoral pathways (47), or alteration in parenchymal mechanics (19). In ASM strips, length oscillation produces a reduction in ASM force (17, 45) which if sufficient to modify airway narrowing could explain bronchodilatory responses to DI in vivo. However, the amount of ASM stretch produced in vivo by DI is likely dependent on the prevailing viscoelastic properties of the airway wall that govern the airway distension produced by lung inflation and that may vary between generations, disease states, or different species, animals or humans. We and others have shown that in airway segments from pigs and dogs simulated respiratory movements (e.g., DI) produce strong inhibition of ASM force (in situ) (21, 37) and importantly airway narrowing (32). Notably, reductions in airway narrowing produced by physiological transmural pressure oscillations are typically shorted lived (~1 min) (31, 32) and while they may be relevant within the time course of conventional spirometry and bronchial provocation challenge, they may not induce enduring suppression of airway responsiveness (31). It remains unknown if the response of the human airway wall (and ASM in situ) to physiologically relevant mechanical stretch can explain the short-term beneficial effects of lung inflation observed in vivo and if a failure of this protective mechanism could play a role in the development of AHR.

The purpose of the present study was to determine if human airways are regulated directly by the mechanical stretch induced by tidal and DI oscillations, and to determine the time course of activity. Human airway segments were acquired from tissue removed after lung surgery in subjects with pulmonary neoplasms, but with normal lung function. The use of isolated airway segments allowed us to determine the independent response of the airway wall under mechanical conditions present during breathing.
Table 1. Airway segment characteristics

<table>
<thead>
<tr>
<th>Lung Lobe</th>
<th>Proximal Diameter, mm</th>
<th>Distal Diameter, mm</th>
<th>Length, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right upper</td>
<td>3.5</td>
<td>2.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Right lower</td>
<td>1.2</td>
<td>1.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Right upper</td>
<td>3</td>
<td>&lt;1</td>
<td>18.2</td>
</tr>
<tr>
<td>Left lower</td>
<td>3.3</td>
<td>&lt;1</td>
<td>19.1</td>
</tr>
<tr>
<td>Left upper</td>
<td>5.1</td>
<td>2.2</td>
<td>15.4</td>
</tr>
<tr>
<td>Left upper</td>
<td>2.3</td>
<td>&lt;1</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Proximal and distal diameters were measured by gently inserting calibrated metal rods (drill bits) before cannulation. Length is the final distended length of the airway segment in the organ bath that is free of the cannula insert.

METHODS

Subject recruitment and spirometry. Subjects undergoing surgery for resection of lung neoplasms were recruited for the study and their permission was obtained to use tissue postoperatively for in vitro studies. Subjects were chosen for the study only if they did not have evidence of fixed airflow obstruction. We defined fixed airflow obstruction as a post bronchodilator forced expiratory volume in 1 s (FEV₁) and FEV₁/forced vital capacity (FVC) below the lower limit of normal (LLN). Reference equations for LLN were from the third National Health and Nutrition Examination Survey (NHANES III) (22). Spirometry was typically performed within 1 wk before surgery using a wedge spirometer (model 570, Med Science) and followed American Thoracic Society and European Respiratory Society guidelines (34). FEV₁ and FVC were measured before and 15 min after three puffs of salbutamol (metered dose inhaler, 300 μg). A positive bronchodilator response was an increase in FEV₁ > 15% and > 200 ml. Subjects also underwent skin prick testing to common aeroallergens (house dust mite, grasses, animal dander, molds) with a wheal diameter of 3 mm or greater indicating a positive response (i.e., atopy). Positive responses to histamine were observed in all subjects. All aspects of the study were approved by the Human Research Ethics Committee, University of Western Australia. All subjects provided written informed consent to participate in the study.

Airway segment preparation. Lung tissue used in the study was macroscopically normal located either proximal to the site of the (peripheral) tumor or in another bronchial segment and was not required for pathological staging (i.e., tissue used was outside the resection margin). A segment of bronchus (cartilaginous at the proximal end) was dissected free of parenchyma, and all side branches were ligated to produce a leak-free airway tube (Table 1), as described previously (36, 37). Airway segments were cannulated and laid horizontally in an organ bath chamber (Fig. 1) containing Krebs solution (mM: 121 NaCl; 5.4 KCl; 1.2 MgSO₄; 25 NaHCO₃; 5.0 sodium morpholinopropane sulfonic acid; 11.5 glucose; and 2.5 CaCl₂) that was warmed to 37°C and gassed (95% O₂,5% CO₂). Segments were stretched in the bath to a length approximating that present at functional residual capacity (FRC) (i.e., 120% of the length of the deflated airway) based on recorded human lung volumes (40) and assuming that the lung expands uniformly in all directions. That is, all airways were studied at a fixed length approximating FRC, while tidal inflation or DI caused radial expansion of the airway (see below). Intraluminal pressure and therefore transmural pressure (Ptm) was initially set at 5 cmH₂O by the height of a reservoir containing Krebs solution connected to one end of the segment.

Airway narrowing and Ptm oscillation. Airway narrowing to bronchoconstrictor stimulation was derived from the decrease in segment luminal volume under conditions where Ptm was held fixed (static) or oscillated to simulate tidal and DI breathing maneuvers. This was achieved using a custom-built syringe pump oscillator (Fig. 1) comprising a glass syringe (1 ml glass syringe model 1001, Hamilton, NV) driven by a feedback-controlled servomotor (M540, Mclellan Servo Supplies, Surrey, UK) and motor controller that maintained the lumen pressure at the desired static or oscillating value (see below). All driving software was written in C programming language (Shane De Catania, WA, Australia). Changes in airway luminal volume (e.g., airway narrowing, inflation or deflation) were measured by a displacement transducer (HEDS-5540#A06, RS Components, WA, Australia) that measured the rotation of the syringe motor. Volume changes were calibrated to graduations on the syringe. A pressure transducer (MLT0380/D, ADInstruments, NSW, Australia) connected to a luminal port measured intraluminal pressure and therefore Ptm. Transmural pressure was constantly monitored by the motor controller, which added or removed volume to the lumen using the syringe to maintain the set pressure. Airway narrowing (decrease in airway volume) was determined from the volume change required to keep pressure at the set point. Volume and pressure signals were recorded by a PowerLab data-acquisition system (ADInstruments). The measured volume and pressure signals showed no significant phase shift (<40 ms), indicating that time delays in the feedback control and inertial effects were trivial.

Experimental protocol. After dissection, airways were allowed to equilibrate to organ bath conditions for −1 h under a Ptm of 5 cmH₂O. Tissue viability was then confirmed with 10⁻⁴ M acetylcholine (ACh) followed by 30 min of washout and recovery. Cumulative dose-response curves (DRCs) to ACh (3 × 10⁻⁶ M to 3 × 10⁻³ M) were performed (in random order) under static conditions with Ptm held fixed at 5 cmH₂O and dynamic conditions simulating tidal oscillations with intermittent DI. Tidal breathing comprised sinusoidal Ptm oscillations from 5 to 10 cmH₂O at 0.25 Hz, and each DI comprised a 2-s linear ramp up from 5 to 30 cmH₂O, a hold at peak pressure for 2 s, followed by a 2-s linear ramp down to 5 cmH₂O (32). For the dynamic protocol (Fig. 2) DI was applied after airway narrowing at each concentration of ACh had stabilized. Renarrowing after DI was monitored until 1 min before the next dose of ACh was administered. Before each DRC, airways were preconditioned for 12 min: static, Ptm fixed at 5 cmH₂O; dynamic, tidal oscillation with DIs every 6 min (3). Upon completion of the protocols, and once all active ASM tone to ACh was reversed (i.e., ACh was washed out of the bath), theophylline (10⁻² M) was added to the bath to identify intrinsic tone. At the end of the experiment the volume of the relaxed airway at 5 cmH₂O is...

Fig. 1. Schematic of the syringe pump oscillator and organ bath system. Transmural pressure was measured by a pressure transducer (P) that fed back to a servomotor that repositioned a syringe attached to a motor (M). Transmural pressure was constantly monitored by the motor controller, which added or removed volume to the lumen using the syringe to maintain the set pressure. Airway narrowing (decrease in airway volume) was determined from the volume change required to keep pressure at the set point. Volume and pressure signals were recorded by a PowerLab data-acquisition system (ADInstruments). The measured volume and pressure signals showed no significant phase shift (<40 ms), indicating that time delays in the feedback control and inertial effects were trivial.
calculated from the decrease in luminal volume, expressed as %V5.

and deep inspiration (DI; 5 to 30 cmH2O). Before the addition of ACh, airways
simulating tidal breathing (Tidal; 5 to 10 cmH2O trough-to-peak at 0.25 Hz)
line (ACh) was measured in the presence of transmural pressure oscillations
conditions. Airway narrowing (decrease in volume) to cumulative acetylchlo-
response curve, DI was applied after airway narrowing at each concentration
underwent a preconditioning period consisting of tidal oscillation and DI (1
every 6 min), which typically increased airway volume. During the dose-
response curve, DI was applied after airway narrowing at each concentration
had stabilized and renarrowing was monitored for 1 min after which the next
dose was administered. End of the protocol is indicated by drug washout
(WO). The thickness of each trace corresponds to the trough-to-peak changes
in pressure/volume during tidal oscillation (indicated by the bands), which
were interspersed with DI. Measurements of airway narrowing (i.e., volume
change) prior or after DI were made at isopressure points at the trough of each
cycle.

(Vs) was determined from the volume that could be withdrawn until
closure.

Analysis and statistics. Airway narrowing in response to ACh was
calculated from the decrease in luminal volume, expressed as %Vs. Under dynamic conditions, volumes were measured at isopressure
points in the cycle, i.e., trough pressures (Fig. 2). Airway sensitivity
to ACh (i.e., PD2, the negative logarithm of the concentration pro-
ducing half-maximal response) was determined by sigmoidal curve
fits (fixed slope) for which the plateau (Emax) was set as maximal
narrowing to the highest ACh concentration (3 × 10−3 M). Broncho-
dilatory responses at any given time point following DI were ex-
pressed as the amount of reversal of narrowing by DI, relative to the
degree of narrowing from the baseline value induced by the corre-
sponding dose of ACh: %reversal = (post-DI volume − pre-DI
volume)/(baseline volume − pre-DI volume) × 100%. Therefore,
reversal of 100% indicated that post-DI volume had returned to the
baseline value (i.e., full reversal of airway narrowing). Bronchodila-
tion was also assessed from the absolute change in airway volume
produced by DI (µl). Specific airway compliance was determined
from volume strain in relation to the change in pressure during the
inflationary limb of the tidal curve [(ΔV/V)/ΔP, where V is airway
volume at the trough of the pressure cycle and ΔV and ΔP are the
trough to peak volume and pressure changes]. That is, the compliance
of an airway contracted to ACh was normalized to its respective
contracted lumen volume.

Maximum airway narrowing and pD2 between static and/or dynamic
protocols were compared by repeat-measures one-way ANOVA and
Newman-Keuls post hoc analyses. Specific compliance comparisons
with and without DI were made by matched two-way ANOVA and
Newman-Keuls post hoc analyses with time (pre-DI vs. post-DI) and
dose as repeat-measure variables. Comparisons between bronchodila-
tion to D (i.e., %reversal/µl) at different ACh concentrations were made
by matched two-way ANOVA and Newman-Keuls post hoc analyses
with concentration and time as repeat-measures variables. Time con-
stants and DI volume strains were compared by repeat-measures
one-way ANOVA and Newman-Keuls post hoc analyses. Changes in
baseline airway volume (%V5) to Pm oscillation (i.e., precondition-
ing) were compared with changes produced by theophylline by paired
t-test. Data analysis, statistical tests, and curve fitting were achieved
using Graphpad Prism (v4.03, GraphPad Software) and Statistica (99
Edition, StatSoft). Data are presented as means ± SE where n =
number of subjects.

RESULTS

Lung function. Subject characteristics are shown in Table 2,
including pre- and postbronchodilator lung function. There
were four men and two women, ranging in age from 63 to 78
years. No subject had fixed airflow obstruction, based on our
selection criteria for this study (i.e., all subjects had a post-
bronchodilator FEV1 and/or FEV1/FVC > LLN). Two subjects
had a FVC < LLN, which for one subject was attributed to a
previous lobectomy. No subject had doctor-diagnosed asthma,
and no subject exhibited a positive response to bronchodilator.
One subject had a positive skin prick response to grass.

Pm oscillation effects on airway volume. Before each DRC
(see below), airways underwent a preconditioning period (Fig.
2) that set the mechanical history of the airway (static or
dynamic), which for the dynamic protocol comprised tidal Pm
oscillations with regular DIs. Dynamic preconditioning in-
creased airway volume, which occurred rapidly after the initi-
ation of tidal oscillation and reached a plateau before the
administration of ACh. The net increase in volume was 11.2 ±
5.3% V5 (4 of 6 airways dilated). The increase in the volume of
the relaxed segment in response to tidal oscillations (and
periodic DI) suggested the presence of intrinsic ASM tone. The
administration of theophylline at the end of the experiment
increased airway volume by 16.8 ± 3.8% V5 (5 of 6 airways
dilated), an effect similar in magnitude to that produced by Pm
oscillation. Intrinsic ASM tone was stable throughout the
course of the experiment, that is, in the absence of ACh or
oscillation airway volume remained constant.

Pm oscillation effects on airway responsiveness. Maximum
airway narrowing (Fig. 3) was 32.8 ± 3.9% under static
conditions and was not different from that immediately before DI (Dynamic Pre-DI), indicating that tidal $P_{tm}$ oscillations alone had little impact on airway narrowing. In comparison, maximum airway narrowing immediately after DI (Dynamic-DI 0 s) decreased to $14.3 \pm 4.5\%$ ($P < 0.001$). Maximal airway narrowing 1 min after DI (Dynamic-DI 60 s) was not different from that immediately before DI, but was less than under static conditions ($P < 0.05$).

To assess the effects of DI on airway sensitivity to ACh, a sigmoidal curve was fit to the data for each segment ($r^2 > 0.95$) which allowed calculation of $pD_2$. This was possible in five of six airways, but in one airway DI completely abolished airway narrowing even at the highest ACh concentration so that a sigmoidal fit was not possible. The $pD_2$ for ACh was similar in airways stimulated under static (3.94 ± 0.22) or dynamic conditions before DI (3.72 ± 0.14). Immediately after DI, $pD_2$ was 3.50 ± 0.12, which was not different from that before DI; however, it was less than that under static conditions ($P < 0.05$), suggesting a modest decrease in sensitivity in response to mechanical strain. The $pD_2$ 1 min after DI (3.70 ± 0.13) was not different from that under any other condition.

The above effects of DI on airway narrowing were dose dependent and transient. The magnitude of the bronchodilatory response to DI was assessed from the %reversal of the ACh-induced airway narrowing (Fig. 4A). For this analysis the change in airway volume to DI was expressed as a proportion of the decrease in airway volume produced by ACh before DI. Only the highest three concentrations of ACh ($10^{-4}$ M, $10^{-3}$ M, and $3 \times 10^{-3}$ M) were assessed as these produced narrowing in all airways. The magnitude of %reversal to DI was greatest at the lowest concentration assessed ($10^{-4}$ M) where DI produced full reversal of airway narrowing (Fig. 4A) and in fact the airway was expanded to a volume that was greater than that before the administration of ACh. After renarrowing, the differences in % reversal of airway narrowing between the different concentrations of ACh were no longer present 12 s after DI. When comparing the effects of DI between the highest two concentrations, immediately after DI the %reversal of airway narrowing was greater at $10^{-3}$ M ACh concentration than at $3 \times 10^{-3}$ M ($P < 0.05$) but similar at all other time points.

The magnitude of the bronchodilatory response to DI was also assessed from the absolute increase in airway volume ($\mu l$) produced by DI. The increase in airway volume after DI was greater at the highest two concentrations ($10^{-3}$ M, $3 \times 10^{-3}$ M) compared with $10^{-4}$ M ($P < 0.001$). There was no difference between the increase in airway volume produced by DI at the highest two concentrations.

The kinetics of renarrowing after DI were assessed by fitting a one-phase exponential decay function ($Y = Ae^{-kt} + B$, where $A$ and $B$ are fitted constants and $t$ is time, $r^2 > 0.9$) and computation of the time constant $k$ ($s^{-1}$). Renarrowing after DI was rapid (Fig. 4B). The rate of renarrowing was greater at $10^{-4}$ M ACh concentration compared with $10^{-3}$ M ($P < 0.05$) and $3 \times 10^{-3}$ M ($P < 0.01$) ACh concentrations. There was no difference in the rate of renarrowing at the two highest ACh concentrations.
P\textsubscript{tn} oscillation effects on airway compliance. Airway specific compliance was determined from volume-pressure changes during tidal oscillation. Airway narrowing to ACh was accompanied by a decrease in specific compliance (Fig. 5). Before DI and before the induction of tone, specific compliance was 0.022 ± 0.003 cmH\textsubscript{2}O\textsuperscript{-1}. Compliance at the highest ACh concentration was 0.017 ± 0.004 cmH\textsubscript{2}O\textsuperscript{-1}. The onset of rapid airway renarrowing after DI made it difficult to relate the airway volume-pressure response to the elastic properties of the airway. Specific airway compliance after DI was therefore assessed at a point where the initial rapid phase of renarrowing had subsided (12 s after DI). Specific compliance was significantly increased 12 s after DI but not after 1 min (Fig. 5).

We further assessed whether the above changes in specific airway compliance measured during tidal oscillations influenced the magnitude of airway strain achieved during DI. Airway volume strains at the peak of DI at all concentrations of ACh and before ACh (Baseline) are shown in Fig. 6. Before ACh the magnitude of airway strain during DI was 47.3 ± 6.9%V\textsubscript{s}, which was unaltered by ACh.

**DISCUSSION**

One of the most striking abnormalities distinguishing asthmatic from nonasthmatic individuals is an altered response to
DI that produces potent bronchodilation in healthy individuals (9, 15, 25, 28, 35, 41). The present study is the first demonstration that the human airway wall, separated from the rest of the lung, relaxes in response to DI and therefore produces a transient reduction in airway responsiveness. This finding suggests that in “healthy” airways, the physiological transmural pressures achieved during DI stretch the ASM sufficiently to influence its force-generating capacity, as previously seen in isolated ASM tissues (17, 45). The bronchodilatory/regulatory effects of DI are pronounced and could produce a major shift in the dose-response curve particularly when airway responsiveness is assessed by conventional lung function tests (i.e., FEV₁) that include DI. However, as will be discussed, the effects of DI are short lived and as such dynamic mechanical stretch accompanying DI may have no enduring effect on airway responsiveness.

In an attempt to elucidate the mechanism(s) producing bronchodilation to DI in vivo, numerous laboratories have imposed length perturbations in isolated ASM strip preparations to replicate mechanical strains accompanying breathing (17, 45). The majority of studies in isolated ASM demonstrate a decrease in ASM force following length perturbation, thought to be related to cross-bridge detachment (17, 18) and/or reorganization of the cytoskeleton (43, 45). The effect of dynamic mechanical stretch (radial) on airway narrowing (caliber) is however less clear and is dependent on the viscous and elastic properties of the human airway wall, as well as the cellular response of ASM to the stress. Until now the response of the intact human airway wall to mechanical stretch was unknown. Results indicate that at the height of DI there is a ∼50% increase in airway volume corresponding to a ∼20% increase in luminal circumference, more than sufficient to regulate ASM force (17). Our present findings support the premise that properties of the human airway wall favor or allow sufficient ASM stretch to reduce force and produce bronchodilation to DI.

The magnitude of bronchodilation produced by DI was dependent on the dose of agonist and therefore the level of airway narrowing. The percentage reversal of airway narrowing produced by DI was reduced at higher doses of ACh, although this trend was reversed if bronchodilation was expressed in terms of absolute changes in volume, whereby DI produced a greater change in airway volume at higher doses of ACh. The expectation that bronchodilation to DI may vary with dose is due to the known relationship between the amplitude of ASM stretch and associated reduction in force observed in isolated ASM preparations (17, 45). Intuitively DI may be less effective at higher doses of contractile agonists due to greater ASM and airway wall stiffening (confirmed in the present study), and consequently a reduced magnitude of airway stretch (strain). Yet factors other than ASM strain may also determine the response to DI. In the study by Brown et al. (8) airways of asthmatic individuals were distended by DI to the same degree as healthy individuals; however, after DI bronchoconstriction was observed in the asthmatics and bronchodilation in nonasthmatics.

As discussed above, it was predicted that the magnitude of airway stretch produced by DI would fall with increasing ACh dose due to increased wall stiffness. Indeed in our previous study on porcine airway segments (37) where we simulated DI in a similar manner, the amplitude of airway stretch during DI was less as the dose of agonist, and therefore airway stiffness, increased. However, in the present study the magnitude of airway stretch produced by DI was shown to be independent of dose and was not different from baseline even at the maximal dose of ACh. Differences in methodology could explain the above inconsistencies. In the study on porcine airway segments (37), airways were contracted under isovolumic (isometric) conditions and as such airway narrowing did not occur. It is uncertain how airway behavior under isovolumic conditions relates to the more physiological scenario of airway narrowing. Furthermore, while the magnitude of airway stretch produced by DI will be strongly influenced by wall stiffness before inflation, it is also influenced by ASM relaxation that occurs during the maneuver and is thus not a simple volume-pressure relationship. That is, in the presence of ACh the magnitude of airway stretch produced by DI may not be attenuated due to relaxation and reduced stiffening of ASM that occurs simultaneously during inflation.

The rate of airway renarrowing after DI was also dependent on the dose of agonist and was more rapid at lower levels of activation. In our airway segment preparation the rate of airway renarrowing after DI is determined by viscoelastic mechanical loads arising from the airway wall (23, 24, 26) and the kinetics of ASM constriction including cross-bridge cycling (13). The observed differences in the rate of renarrowing between activation levels are consistent with the expected differences in narrowing against an elastic load. In vivo, and in our experiments, airway narrowing requires compression and deformation of the airway wall (39), the consequence of which is an increased elastic load. Since shortening velocity decreases with greater elastic load (23, 24), this may explain a reduced rate of renarrowing at higher levels of ASM activation. In general, the time course of renarrowing in response to DI observed in the present study compares favorably with human studies that show that the bronchodilatory effects of DI are largely dissipated 1 min after DI (4, 35).

Previous studies in healthy individuals demonstrate bronchodilation to DI in vivo (9, 15, 25, 28, 35, 41) an effect that is reduced or absent in individuals with asthma (9, 15, 25). A widely espoused hypothesis is that a failure of the beneficial effects produced by DI is a precursor to AHR. Our findings in human airway segments are that DI produces a strong decrease in maximal response to a contractile agonist. The effect was relatively short lived, and was dissipated 1 min after DI. In the context of conventional lung function test and provocation challenge, where airway responsiveness is assessed by lung function maneuvers that include DI (i.e., FEV₁), an impairment in the airway’s response to DI in asthma could certainly contribute to the appearance of AHR. The inclusion of DI during bronchial challenge produces a strong suppression of airway responsiveness in healthy but not in asthmatic individuals and consequently differences between healthy and asthmatic individuals are reduced if DI is removed from challenge (9, 46). However, the transient nature of the bronchodilation produced by DI may mean that there is no enduring effect on airway responsiveness. In a recent study by LaPrad et al. (31) airway narrowing was measured in bovine airway segments under static and dynamic conditions that included DI simulated every 6 min, the rate at which humans spontaneously sigh (3). While DIIs did produce transient bronchodilation, airway renarrowing was rapid and there was no lasting reduction in...
airway responsiveness. Given that that the present study shows that renarrowing of human airways is also rapid, these findings suggest that outside the context of conventional lung function tests and challenge, DI may have no lasting impact on airway responsiveness.

In addition to maximal bronchoconstrictor response, the second broad determinant of airway responsiveness is sensitivity, which is also increased in asthma, an effect that may be unrelated to any impairment in the response to dynamic mechanical stretch. To our knowledge, no study has demonstrated an effect of DI on airway sensitivity in vivo (9, 10, 12). In a previous study in vitro (37), we observed a reduction in airway sensitivity after DI in porcine airway segments, and while there was also some evidence for a reduction in sensitivity under dynamic conditions in the present study, in both instances the changes were modest. Removal of the beneficial effects of dynamic strain is of little consequence to airway sensitivity, and as such impairment of the response to DI cannot explain the shift in sensitivity seen in asthma (50).

The possibility that regular mechanical stretch during tidal oscillations reduces the capacity of airways to narrow has been highlighted in several studies (5, 17, 44). In the present study tidal oscillations had no effect on airway responsiveness (maximal response or sensitivity), consistent with our earlier findings on airway segments from pig lungs (37). In that study we concluded that airway wall load prevents the critical level of ASM stretch required to reduce force and thus narrowing, which may also be true for these sized human airways. LaPrad et al. (31) similarly observed no effect of physiological tidal oscillations on airway narrowing in bovine airway segments. On the other hand, while we observed no effect of tidal oscillation on airway responsiveness, resting airway volume (i.e., absence of ACh) was increased by some ~10% when tidal oscillation was combined with periodic DI (i.e., during the preconditioning period). Reversal of intrinsic ASM tone by breathing movements is the most likely explanation for the increase in airway volume, since theophylline caused relaxation of unstimulated bronchi. The apparent bronchodilatory effect of tidal oscillations (with periodic DI) in the absence of ACh is somewhat contrary to the lack of an effect of tidal oscillations on ACh-induced airway tone. Dynamic mechanical stretch may have inhibited spontaneous tone not by acting on ASM but instead by inhibiting the release of contractile mediators such as leukotrienes (20). Further, we cannot rule out the possibility that the increase in airway volume during this period arose due to hysteresis of passive wall components and was therefore not due to reversal of ASM tone.

The major innovation of the present study is the use of human airway tissue, which we acquired from patients with lung neoplasms but without airflow obstruction. There are several limitations of this approach that require comment. Our subjects were elderly (63–78 yr old), which may have affected the response of the airway wall to DI. Indeed it has previously been shown that the bronchodilatory response to DI diminishes with age (42). Most of our subjects had a history of smoking (3 exsmokers, 1 current smoker, and 2 nonsmokers), which may have produced airway inflammation, although inflammation is minimal in exsmokers without airflow obstruction or symptoms of chronic bronchitis (48). Airway tissue was sampled away from the site of the tumor in an attempt to limit any direct effects such as inflammatory cell infiltration, edema, obstruction, or atelectasis. There were however constraints on the amount of tissue available to us and as a result the length of some airway segments was short. Despite the above limitations, which potentially hindered our capacity to examine responses to DI, we observed potent bronchodilatory responses to DI in human airways consistent with that observed in vivo.

In the present study, bronchoconstriction and response to DI were assessed in the absence of parenchymal attachments. This enabled us to assess the behavior of the isolated airway wall. Responses to DI in vivo may be influenced by airway-parenchymal mechanical interactions, in particular relative hysteresis of the lung parenchyma and the airway wall (19). All other factors being equal, bronchodilatory responses to DI might be somewhat attenuated in vivo due to a loss of elastic recoil pressure and therefore airway transmural pressure associated with parenchymal hysteresis. However, the effects of parenchymal hysteresis may be offset by an increase in airway transmural pressure produced by distortion of lung parenchyma as the external surface of the airway narrows (29, 30). Consequently airways in vivo may encounter fractionally different transmural pressures than those simulated in the present study. An increase in transmural pressure produced by distortion of the lung parenchyma may also reduce the magnitude of airway narrowing before DI although a recent study suggests that this effect will be small (38). Other than the lung parenchyma, the response to DI could be modified in vivo by additional extrinsic factors including stretch-activated neural or humoral pathways (46).

Direct imaging of canine airways in vivo demonstrates the capacity for airway closure (6, 7) and while this has not been confirmed in humans, data derived from forced oscillation measurements are suggestive of airway closure or at least functional closure in the absence of DI (11). We found no evidence of airway closure following contractile stimulation, even after maximal activation to ACh under a static transmural pressure. It is possible that in our liquid-filled airway segments the removal of surface tension forces (that may play a role in the narrowed airway) would favor a more patent airway lumen and therefore limit narrowing, or perhaps airway closure simply does not occur in healthy human airways > 1 mm in diameter.

An additional effect of DI was the reversal of airway stiffening produced by ASM contraction, which could itself impact on normal physiological regulation of airway caliber. As proposed by Fredberg (16), reduced airway stiffness should facilitate greater ASM stretch and greater bronchodilation to subsequent dynamic respiratory movements including DI. The capacity for DI to reduce airway stiffness shown here is consistent with the predictions from isolated ASM studies (17, 18). An increase in airway stiffness (or reduced compliance) following contractile activation is believed to be due to recruitment of bound cross bridges, but also involves polymerization of contractile filaments (2). Interestingly, the magnitude of airway stiffening in human airways (~25% decrease in compliance) is small compared with that shown previously in animal airway segments (37). While there are obvious physiological and structural differences between species that may account for this discrepancy, the changes in specific compliance reported here provide a better indication of what is likely to occur in vivo. In the present study airway compliance was measured as the airway narrowed, accompanied by distortion
of ASM and other mural structures (39) and presumably impacting on wall stiffness. This approach differs from previous studies (37) where compliance was measured in airways during isometric or isovolumetric contractions, which are less likely to reflect physiological behavior.

The primary aim of the present study was to examine the bronchodilatory response of the human airway wall to DI. In healthy humans in vivo, it has been established that in addition to producing bronchodilation, DI taken before bronchial challenge attenuates bronchoconstriction evoked subsequent to DI, a phenomenon dubbed bronchoprotection (41). The cumulative nature of our dose-response curve effectively meant that under dynamic conditions, subsequent doses of ACh were influenced by prior DI, which may have produced some bronchoprotection. While it is difficult to discern possible bronchoprotective effects in our human airway segments from the effects of periodic bronchodilation to DI and tidal oscillation, there appears to be no enduring protective effect of prior DI. In previous studies on mid-sized porcine airway segments (27, 36) we also found no evidence that DI modified subsequent constriction, suggesting that bronchoprotective effects of DI are due to factors other than direct modulation of airway narrowing in conducting airways (11, 14).

We conclude that the human airway wall responds to mechanical stretch (radial) produced by physiological transmural pressures generated during DI and suggest that the resulting bronchodilation and transient reduction in airway responsiveness explains many of the beneficial effects of DI observed in vivo. In comparison, the change in transmural pressure accompanying tidal breathing seems unimportant to airway responsiveness. If the primary mode of action of DI is via direct mechanical stretch to the airway wall, then the pathological changes such as airway remodeling and stiffening that occur in asthma are likely mechanisms causing dissociation of the airway response from the effects of DI. With respect to AHR in asthma, the transient nature of the changes produced by dynamic mechanical stretch suggests that long-term enhancement of airway responsiveness is due to mechanisms unrelated to a failure of the bronchodilatory effects of DI.

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DISCLOSURES

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

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