Airways Dilate to Simulated Inspiratory but not Expiratory Manoeuvres

Adrian R. West¹,², Elangovan Thaya Needi¹, Howard W. Mitchell¹, Peter K. McFawn¹, Peter B. Noble¹,³,⁴

¹Physiology, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia
²School of Biomedical Engineering, Dalhousie University
³School of Women’s and Infants’ Health, The University of Western Australia
⁴Centre for Neonatal Research and Education, The University of Western Australia

Correspondence: Dr Peter B. Noble, M094, School of Women’s and Infants’ Health, The University of Western Australia, 35 Stirling Hwy, Crawley, Australia, 6009
Email: Peter.Noble@uwa.edu.au
Phone: 61-8-6488-7969

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ABSTRACT

In healthy humans deep inspiration (DI) produces bronchodilation of contracted airways which is likely due to transient distension of airway smooth muscle (ASM). We hypothesized that deep expiratory manoeuvres would also produce bronchodilation due to transient airway wall and ASM compression.

We used porcine bronchial segments to assess the effects of DI, maximal expiration and partial expiration (sub-maximal) on airway calibre. Respiratory manoeuvres were simulated by varying transmural pressure using a hydrostatic pressure column: DI, 5 to 30 cmH$_2$O; maximal expiration, 30 to -15 cmH$_2$O; partial expiration, 10 to -15 cmH$_2$O; amidst a background of tidal oscillations, 5 to 10 cmH$_2$O at 0.25 Hz. Changes in luminal cross sectional area in carbachol-contracted airways were measured by videoendoscopy.

DI produced immediate bronchodilation (~40-60%, p=0.0076) lasting up to 1 minute (p=0.0479). In comparison, after maximal expiration, while there was no immediate change in airway calibre, a delayed bronchodilatory response was observed from 4 s after the manoeuvre (p=0.0059) persisting for up to 3 min (p=0.0182). Partial expiration had little to no effect or airway calibre.

Results demonstrate that the airway wall dilates to deep inspiratory manoeuvres but is unresponsive to deep expiratory manoeuvres.
INTRODUCTION

In healthy humans breathing manoeuvres such as deep inspirations (DI) can dilate previously contracted airways [1-6] and represent a potent physiological mechanism for maintaining airway caliber. The importance of this regulatory pathway is demonstrated by observations that DI responses are reduced or absent in obstructive diseases such as asthma [1, 2, 5] and COPD [7, 8]. The bronchodilator actions of DI in vivo can likely be explained by an intrinsic response of the airway wall to stretch as a result of the transmural pressure gradients generated during lung inflation. In vitro, dynamic inflation produces bronchodilation of contracted airway segments [9, 10]. The magnitude and duration of bronchodilation in airway segments is comparable to that observed in vivo suggesting that the actions of DI are initiated at the airway level [3, 10].

The clinical impact of bronchodilation after DI is revealed in tests such as forced expiratory volume in 1 sec (FEV$_1$) or forced vital capacity (FVC). Critically, these manoeuvres are preceded by DI that produces bronchodilation and may itself influence the clinical and experimentally derived lung function data. For instance, when assessing patients with obstructive disease the presence-or-not of DI determines the extent of airway hyperresponsiveness observed in this group [1, 11]. Due to these complicating effects, respiratory manoeuvres that do not involve DI have been used as an alternative, most notably partial forced expiration [2, 12-14]. Although used with the intention of avoiding the DI phase of a FEV$_1$, it is unknown whether expiratory phases of the FEV$_1$, or other expiratory manoeuvres, can themselves influence airway calibre.

While DI and deep expiration initially exert opposing effects on airway calibre (stretch vs compression), evidence from in vitro studies suggest that it is the dynamic nature of the movements which ultimately determines the airway response. In particular length change or
length oscillation of ASM in vitro reduces muscle force [15, 16]. However, adaptive properties (plasticity) of ASM are equally sensitive to both increases and decreases in length [17] and this represents a plausible mechanism underlying the response of the ASM to dynamic stretch associated with breathing manoeuvres [18]. Thus both mechanical stretch and compression may contribute to the effects on airway calibre elicited by breathing manoeuvres. Importantly, if expiration does exert similar effects to inspiration, then the expiratory phases of FEV$_1$, FVC and indeed partial expiration will also favour bronchodilation and influence these measures of airway calibre or responsiveness.

The aim of the present study was to characterize the response of the airway wall separately to both inspiratory and expiratory manoeuvres including DI (inflation to total lung capacity, TLC), maximal expiration (inflation to TLC then deflation to RV) and partial expiration (deflation from end tidal volume to RV). We were particularly interested whether expiratory phases of clinically relevant parameters such as FEV$_1$, FVC or partial expiration could themselves regulate airway calibre by producing bronchodilation. Based on our previous findings [9, 10] we hypothesized that bronchodilatory responses to the different breathing manoeuvres would be expressed at the level of the airway without the need for contributions from airway-parenchymal mechanical interactions or central neural reflexes. Bronchial segments from pigs were contracted to carbachol and bronchodilator responses to each breathing manoeuvre were recorded by video-endoscopy. Respiratory manoeuvres were simulated by varying the transmural pressure of the airway, which were applied by a dynamically oscillating pressure column.
METHODS

Animal handling

All animal experiments conformed to the institutional ethics and animal care unit regulations (University of Western Australia, Animal Ethics Committee, Perth, Australia). White Landrace pigs (~30 Kg) were initially sedated with tiletamine/zolazepam (4.4 mg/Kg im.) and xylazine (2.2 mg/Kg im.) and then exsanguinated under pentobarbitone sodium anaesthesia (30 mg/Kg iv.). Lungs were then removed and transported on ice to the laboratory for dissection of airways.

Airway preparation

A length of the bronchial tree was dissected from the lower lobe of the right lung, beginning from a lobar bronchus and extending distally ~5 cm. All side branches were ligated producing a leak-free preparation. The two ends of the bronchus were cannulated and the tissue preparation was placed horizontally in a custom made Perspex organ bath containing gassed (95% O₂, 5% CO₂) Krebs solution (mM: NaCl 121; KCl 5.4; MgSO₄ 1.2; NaHCO₃ 25; sodium morpholinopropane sulphonic acid 5.0; glucose 11.5; and CaCl₂ 2.5) at 37°C. The airway segment was stretched to a length shown previously to approximate functional residual capacity in the pig lung, i.e., ~105% of the fully deflated length at 0 cmH₂O [19].

Video-endoscopy

Changes in airway lumen caliber (i.e., cross sectional area) to bronchoconstrictor agonist and breathing manoeuvres were recorded by video-endoscopy as previously described [20]. Unlike morphometry, measurements obtained by this approach do not include the area or perimeter contained between interstices. A rigid fibre-optic endoscope (Olympus SES-1711D) coupled to a video camera (SONY DFW-SX900, 7.5 frames/sec) was inserted into the lumen of the airway through the proximal cannula. The endoscope was locked at a position suitable
to visualize generation 10-12. Prior to recordings the airway lumen was stained with a blue ring of dye using a steel applicator to aid visualisation. Colour video images of the lumen were displayed in real time and recorded on a personal computer using video acquisition software (Unibrain Fire-I 1.21).

**Simulation of respiratory manoeuvres**

Respiratory manoeuvres were simulated by controlling airway transmural pressure, which was positive for tidal breathing and DI, and negative for deep expiration. Transmural pressure was set by the height of a hydrostatic pressure column connected in series with the airway lumen. By this approach, airway luminal pressure and therefore transmural pressure was determined by the relative height of the pressure column to the midline of the segment. That is, transmural pressure was positive when the height of the column exceeded the airway midline, and negative when the height of the column was below the airway midline. Dynamic breathing manoeuvres (see below) were simulated by cycling the height of the pressure column using a computer-controlled syringe pump. The syringe plunger was driven by a DC motor (M540, McLennan Servo Supplies) using a BioPWM sequential motor controller (V0.3) and custom designed software (Shane De Catania, 2005), which allowed for sinusoidal or ramp movements at a desired frequency. The syringe pump was calibrated to establish the linear relationship between syringe displacement and the change in fluid height in the pressure column. Pressure changes were confirmed by a transducer (Motorola MPX2010DP) connected to an airway lumen port in the organ bath. Pressure signals were recorded using a Powerlab 2/20 data-acquisition system (ADInstruments).

**Experimental protocol**

Airway preparations were allowed 1 hour to equilibrate to organ bath conditions before experimentation, during which the lumen and adventitia of the segment were regularly flushed
with fresh Krebs solution. Tissue viability was confirmed by observing airway contractions to acetylcholine (ACh; $10^{-3}$ M) followed by a 30 min washout and recovery period.

The volume history and dynamic environment of the airway was initially set by 3 DIs followed by 20 min of tidal oscillation simulated by sinusoidal transmural pressure cycles between 5 and 10 cmH$_2$O at 0.25 Hz (i.e., the human breathing frequency). Tidal oscillations were continued and airways were contracted to $10^{-6}$ M carbachol administered to the adventitial surface of the airway, an EC$_{50}$ concentration that produced approximately $\sim$35% decrease in lumen area. Preliminary studies showed that airway narrowing was stable 30 min after the addition of carbachol, and remained so for at least a further 10 min (see Results). Our approach was therefore to induce a breathing manoeuvre 30 min after the addition of carbachol and then track airway lumen area for 10 min post manoeuvre. At the end of the recording period, carbachol was replaced with fresh Krebs solution and the airway was allowed to relax for 40 min with regular flushing of both adventitial and luminal surfaces. The entire protocol was then repeated for the next breathing manoeuvre.

**Dynamic breathing manoeuvres**

Three breathing manoeuvres were administered to each airway in random order. A DI comprised a linear ramp up in pressure from 5 to 30 cmH$_2$O, a 2 s pause at ‘end inspiration’, and a ramp down in pressure to 5 cmH$_2$O. Partial expiration comprised a ramp down in pressure from 5 to -15 cmH$_2$O, 2 s pause at ‘end expiration’, and a ramp up in pressure to 5 cmH$_2$O. Maximal expiration effectively combined the two previous manoeuvres, replicating the initial phase of DI including the 2 s pause at 30 cmH$_2$O (end inspiration), followed by a large ramp down in pressure to -15 cmH$_2$O, a 2 s pause (end expiration), and finally a ramp up in pressure to 5 cmH$_2$O. Respective end inspiratory and expiratory pressures of 30 cmH$_2$O and -15 cmH$_2$O meant that pressure amplitudes were comparable between inspiratory and
expiratory manoeuvres (i.e. from a mean pressure of 7.5 cmH₂O during tidal oscillation, inspiration to 30 cmH₂O or expiration to –15 cmH₂O, ΔP was 22.5 cmH₂O). The rate of pressure change was kept constant for all manoeuvres and was 12.5 cmH₂O/sec. Example pressure traces indicating tidal oscillations, all three breathing manoeuvres and associated measurement points are shown in Figure 1.

**Analysis and statistics**

Lumen cross sectional area was quantified by manually tracing an area around the bronchial lumen using ImageJ 1.44. Images were calibrated using a probe of known diameter inserted into the lumen. To assess the potential bronchodilatory effects of each manoeuvre, airway lumen area (A) at different post manoeuvre time points (t) were expressed as the % recovery in airway narrowing to carbachol as follows:

\[
\text{Recovery}(\%) = \frac{A(t) - A_{\text{pre-manoeuvre}}}{A_{\text{pre-carbachol}} - A_{\text{pre-manoeuvre}}}
\]

where \(A_{\text{pre-manoeuvre}}\) is lumen area prior to the initiation of the simulated breathing manoeuvre (contracted to carbachol); \(A_{\text{pre-carbachol}}\) is lumen area of the relaxed airway prior to carbachol. A positive % recovery indicated bronchodilation (i.e., 100% recovery corresponds to complete reversal of constriction), while a negative % recovery indicated additional contraction. To quantify luminal strain (linear) during each respiratory manoeuvre changes in lumen perimeter (P) at end inspiration and expiration were calculated as follows:

\[
\text{Lumen strain (\%)} = \frac{P_{\text{inspir/expir}} - P_{\text{pre-manoeuvre}}}{P_{\text{pre-manoeuvre}}}
\]

where \(P_{\text{inspir/expir}}\) is lumen perimeter at end inspiration or expiration; \(P_{\text{pre-manoeuvre}}\) is lumen perimeter prior to manoeuvre (contracted to carbachol); Positive lumen strain indicated
airway expansion and negative strain compression. Airway shape at baseline, after contraction
to carbachol and during the inspiratory and expiratory manoeuvres was assessed using the
‘circularity index’. The circularity index was calculated from the ratio of the measured lumen
area to that predicted from perimeter assuming circularity (i.e., \(4\pi \text{area}/\text{perimeter}^2\) where a
ratio of 1 indicates a perfect circle).

All data are expressed as mean ± standard error, and all statistics were performed with
GraphPad Prism (v4.03, GraphPad Software, CA, USA) and Statistica (99 Edition, StatSoft
Inc., OK, USA). Repeat measures one-way ANOVA was used to compare relaxed airway
lumen area (i.e. airway size) and the magnitude of airway narrowing prior to the initiation of
the manoeuvres. The presence of bronchodilation or bronchoconstriction following a
manoeuvre was assessed by 2-tailed one sample t-test against a hypothesized mean of zero
(no change in luminal area). Comparisons between bronchodilation following DI and
maximal expiration was assessed by Two-way ANOVA and Newman-Keuls posthoc tests,
with ‘manoeuvre’ and ‘time’ as repeat measures variables. Lumen perimeter strains at end
inspiration (DI v maximal expiration) and end expiration (maximal expiration v partial
expiration) were compared by paired t-test, while repeat measures one-way ANOVA was
used to compare strains measured at end inspiration v end expiration ( NB: for this analysis
\(|\text{absolute strain}|\) was compared, see Results). For consistency, measured lumen perimeter
strains were also assessed by 2-tailed one sample t-test against a hypothesized mean of zero.
P < 0.05 is considered statistically significant.
RESULTS

Airway narrowing to carbachol

In a separate group of airways (n = 4) the airway narrowing time course to a submaximal dose of carbachol was assessed (Fig. 2). In the presence of tidal oscillation, carbachol produced a ~35% reduction in lumen area which reached a plateau at 30 min and remained stable for up to 50 minutes after the addition of carbachol. Based on these observations we chose to assess the response to each manoeuvre at 30 min after the addition of carbachol, and to monitor the resulting changes in lumen area for 10 min. The magnitude of airway narrowing prior to each breathing manoeuvre was the same (Table. 1).

Effect of inspiration and expiration on airway narrowing

We assessed the effects of both inspiratory (DI) and expiratory (maximal and partial expiration) manoeuvres on airway narrowing. A sample image sequence for maximal expiration that comprises airway narrowing to carbachol, an initial DI (airway expansion) and a subsequent expiratory manoeuvre (airway compression) is shown in Figure 3. In the example, airway expansion at end inspiration (increased lumen area) and compression (reduced lumen area) at end expiration are evident.

To examine the potential bronchodilatory effects of each respiratory manoeuvre, changes in airway lumen area after each manoeuvre (DI, maximal and partial expiration) were expressed as the % recovery in airway narrowing (Fig. 4). Consistent with our previous findings [9] DI produced immediate bronchodilation (p=0.0076) that persisted up to 1 min after the manoeuvre (p=0.0479), subsiding by 3 min (p=0.1803). In comparison, immediately after maximal expiration (0 s) the airway was neither contracted nor dilated, but exhibited significant dilation from 4 s (p=0.0059) to 3 min (p=0.0182) but not at 5 min (p=0.0917). The magnitude of bronchodilation to maximal expiration at 4 s was 15.0 ±1.2% recovery which
was less than the 28.7 ±2.2% recovery after DI at the same time point (p=0.0002). At all other subsequent time points there was no significant difference in % recovery between DI and maximal expiration. In stark contrast to both DI and maximal expiration, partial expiration failed to produce any dilation, with some possible initial further constriction (NS, p=0.1367) that was completely absent by 4 s.

**Luminal strain during inspiration and expiration**

To quantify the extent of airway wall expansion and/or compression during each respiratory manoeuvre, luminal perimeter strain (%) was calculated at end inspiration and end expiration (Figure 5) and these were all statistically significant from zero as assessed by one sample t-tests. Luminal strain was 18.6 ±6.6% and 15.1 ±3.1% at end inspiration for DI and maximal expiration, and -21.2 ±3.4% and -23.9 ±3.0% at end expiration for maximal and partial expiration. There was no statistical difference in the magnitude of strain at end inspiration between DI and maximal expiration (p=0.4599) or at end expiration between maximal and partial expiration (p=0.4376). When direction of strain was ignored (i.e. expansion v compression) there was no difference between the magnitude of strain (|absolute strain|) at end inspiration compared with end expiration (p=0.5753).

Finally, we considered the possibility that deviation from circularity in the lumen particularly during the compressive manoeuvre may impact the strain on the ASM. However we saw no gross changes in lumen dimensions and this was confirmed by the circularity index (>0.94 under all conditions).
DISCUSSION

The present study determined whether airway calibre, which is known to be regulated by deep inspiratory manoeuvres (i.e. DI), is also modulated by deep expiratory manoeuvres. Building on previous findings that airway responses to respiratory movements are initiated by direct stretch on the airway wall [9, 10, 21, 22], we used an isolated airway model in vitro and simulated respiratory manoeuvres by varying transmural pressure. Our findings show that airways exhibit an intrinsic bronchodilatory response to high positive transmural pressures accompanying lung inflation, but not to negative (compressive) transmural pressures achieved during deep expiration.

We evaluated the effects of three different respiratory manoeuvres: DI, involving large positive transmural pressures (30 cmH\textsubscript{2}O) that distended (stretched) the airway wall; partial expiration, that compressed the airway wall to negative transmural pressures (-15 cmH\textsubscript{2}O); and maximal expiration, which essentially combined the expansive and compressive manoeuvres (i.e. 30 cmH\textsubscript{2}O to –15 cmH\textsubscript{2}O). Results show that the airway wall exhibits an intrinsic bronchodilatory response to mechanical stretch accompanying a DI, confirming our earlier findings [9, 10, 22], however there is no such bronchodilatory response to airway compression accompanying deep expiration (partial expiration). In contrast, the airway tends to be more constricted immediately after a partial expiration, although this was not statistically significant (p=0.1367) and any meaningful change is completely absent 4 s later.

Finally, with respect to maximal expiration which involves an initial DI, the dominant response was bronchodilation, albeit delayed, as the magnitude of bronchodilation was partially offset by wall compression achieved during the subsequent deep expiratory manoeuvre.
Our methodological approach closely followed our previous study where positive transmural pressures simulating DI produced bronchodilation in contracted bronchial segments in vitro [9]. The precise transmural pressures that occur during the different breathing manoeuvres in vivo are difficult to predict but we defined DI as inflation to 30 cmH\textsubscript{2}O since this corresponds to the plateau in the pressure-volume curve of the airway [23]. With respect to maximal and partial expiration, we simulated airway wall compression at negative (subatmospheric) transmural pressures present during deep expiratory movements and which may also involve dynamic airway compression. End expiration was arbitrarily defined as −15 cmH\textsubscript{2}O so that inflationary and deflationary manoeuvres produced a similar amplitude change (i.e. ΔP was 22.5 cmH\textsubscript{2}O). We also chose to use a sub maximal but physiologically relevant level of airway narrowing. Given the level of airway contraction (narrowing) impacts considerably on the response to respiratory manoeuvres [22], airways were narrowed to an ~EC50 dose. Carbachol produced a ~35% reduction in lumen area which in vivo would have a meaningful effect on airflow (60% reduction assuming homogenous constriction and laminar flow) while still not maximally contracting ASM.

During standard lung function testing (i.e. FEV\textsubscript{1} & FVC) a DI that precedes maximal expiration produces bronchodilation and therefore influences subsequent expiratory flow [2, 24]. The potential impact of bronchodilation to DI during the assessment of airway responsiveness/hyperresponsiveness has been documented, although the underlying implications are still not fully appreciated. Since the magnitude of the bronchodilatory response to DI differs in disease including asthma and COPD [1, 2, 5, 7, 8], the presence or not of DI during bronchial challenges will change the severity of hyperresponsiveness observed. Indeed if DIs are removed from bronchial challenges dose-response curves from
healthy and asthmatic individuals converge [1, 11]. Any bronchodilatory response to expiration could further complicate this scenario; however the present data now supports the assumption that expiratory manoeuvres do not influence airway calibre by regulating ASM force.

As discussed, there was also a tendency for airways to be more constricted immediately after the expiratory phase of a manoeuvre which was of borderline significance and lasting only 4 s. Residual airway compression after expiration likely reflects the inertial and viscous properties of the airway wall (i.e. the airway will not return to its pre-compressed lumen area instantly when the compressive load is removed) and thus a transient mechanical effect rather than a biological response to an applied mechanical stimulus. How these mechanical effects would impact lung function testing is unclear since in this clinical scenario flow profiles are assessed during the expiratory manoeuvre rather than after re-inflation as was the case in our protocol. Irrespective of the above considerations, the major conclusion of the present study remains firm: the airway wall does not respond actively to compressive manoeuvres by producing bronchodilation analogous to DI.

There is compelling evidence that bronchodilation to DI observed in vivo [1-6] is mediated by direct stretch to the airway wall which lengthens ASM and produces a reduction in force generation [9, 10, 15, 16, 21, 22]. Bronchodilation to radial stretch simulating DI is the prevailing response in human and porcine bronchial segments [9, 10] although under some conditions, modest contractile responses have been observed in pigs [25] which may bare some relationship to bronchoconstrictor responses to DI observed in asthmatic individuals [26]. Reduced ASM force after mechanical stretch may be due to cross-bridge detachment [15] and/or arise as a result of ‘adaptive’ ASM properties involving reorganisation of
contractile filaments [18]. However, while it is ASM ‘lengthening’ or mechanical ‘stretch’ which is more often linked to the ASM response, it may be length change (lengthening or shortening) that ultimately matters and the underlying cellular mechanisms may also be responsive to muscle shortening/compression. Indeed, adaptive properties of ASM are equally responsive to both lengthening and shortening whereby a chronic increase or decrease in ASM length alters length-tension characteristics [17]. It is also feasible that cross bridge binding may be sensitive to cellular compression although to our knowledge this has not been examined previously. In the present study we hypothesised that expiratory manoeuvres involving ASM compression could also favour a reduction in ASM force and bronchodilation. Our data does not support this possibility.

One possible explanation for the lack of an effect of expiration on airway calibre is that the amplitude of ASM length change during the manoeuvre falls below that required to initiate a reduction in ASM force. Several studies have shown that the magnitude of bronchodilation depends on the amplitude of the applied stretch or pressure change [4, 9, 15, 21, 22]. Although we applied the same change in pressure during both inflation and deflation it is possible that the strain applied to the ASM may have been less in the expiratory manoeuvres for example if the airway wall was more resistant to compression than expansion. While it is very difficult to assess dynamic ASM length change in situ, in the present study we used airway luminal perimeter strain measured during inspiratory (positive strain) and expiratory (negative strain) phases of the manoeuvres. Results suggest that inspiratory and expiratory manoeuvres produced similar airway wall strain, and given bronchodilation was observed in response to inspiration, the level of airway strain was sufficient to modify ASM force.
However it is important to consider that the lumen perimeter strain is not a direct proxy for ASM strain. The airway wall internal to the muscle may become thicker during a compressive manoeuvre, and comparatively thinner during expansion. Luminal perimeter strain may then overestimate ASM strain during expiration. Data from a previous study, which used anatomical optical coherence tomography to measure wall and luminal dimensions [27] was used to account for any disparity between ASM and luminal perimeter due to the thickness of the inner wall. These data were used to predict changes in ASM perimeter from the measured changes in luminal area assuming circularity and constant wall area. The results of the analysis indicated comparable ASM strain during inspiration and expiration (15% and -18% strain respectively). Studies on isolated ASM [15] suggest that length changes exceeding 4% will produce large reductions in ASM force, therefore it seems unlikely that a reduced response to deep expiration (i.e. lack of bronchodilation) can be explained by an applied ASM strain below that sufficient to regulate ASM force. A final possibility for the failure of expiration to produce bronchodilation is that the airway folds on compression without a change in ASM length, although in the present study we saw no evidence for gross changes in lumen shape during deflation (see Fig. 3) and this was confirmed by a lack of change in the circularity index.

In conclusion we have found strong support for the view that deep expiratory manoeuvres do not cause bronchodilation supporting the notion that partial expiratory manoeuvres can be used to assess bronchoconstriction without contaminating the measurement by themselves changing ASM tone. These findings suggest that while the ASM is sensitive to dynamic mechanical stretch, it is unresponsive to transient compressive events.
REFERENCES


ACKNOWLEDGEMENTS

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**Table 1.** Airway lumen area before and after narrowing to carbachol

<table>
<thead>
<tr>
<th>Manoeuver</th>
<th>n</th>
<th>Relaxed, mm$^2$</th>
<th>Contracted, mm$^2$</th>
<th>Airway Narrowing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digital (DI)</td>
<td>5</td>
<td>14.2 ±2.7</td>
<td>9.2 ±2.6</td>
<td>38.6 ±8.1</td>
</tr>
<tr>
<td>Partial expiration</td>
<td>5</td>
<td>14.8 ±2.6</td>
<td>8.7 ±1.9</td>
<td>35.3 ±6.4</td>
</tr>
<tr>
<td>Maximal expiration</td>
<td>5</td>
<td>13.4 ±2.9</td>
<td>9.5 ±1.9</td>
<td>35.1 ±4.9</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Airway lumen area before (Relaxed) and after (Contracted) the addition of carbachol. Airway narrowing was calculated from the % reduction in lumen area. Prior to each manoeuvre lumen area (p=0.6166) and airway narrowing (p=0.8005) were similar between groups.
FIGURE LEGENDS

**Figure 1.**
Schematic showing airway transmural pressures and points of measurement before, during and after DI, maximal and partial expiration manoeuvres. ‘a’ pre manoeuvre; ‘b’ end inspiration to 30 cmH\(_2\)O (DI and maximal expiration); ‘c’ end expiration to –15 cmH\(_2\)O (maximal and partial expiration); ‘d’ first measurement after manoeuvre (0 s); e) measurement 4 s after manoeuvre. Other measurements were also acquired at 30 s, 1 min, 3 min, 5 min and 10 min after manoeuvres, and prior to the administration of carbachol (i.e. relaxed airway).

**Figure 2.**
Airway narrowing time course study (n=4). Lumen area (% of Relaxed) to carbachol was measured during tidal oscillations alone. Lumen area plateaued 30 min after contraction and remained stable thereafter.
Figure 3.
Sample image sequence for a maximal expiration manoeuvre. Lumen images were taken prior to (A) and after (B) narrowing to carbachol, at end inspiration (C) and end expiration (D) and at several time points after the manoeuvre (E, 4 s). Typically there was no change in airway lumen immediately after maximal expiration, but dilation was apparent at 4 s.

Figure 4.
% recovery (n=5) in airway narrowing after DI, maximal and partial expiration. DI produced immediate bronchodilation (p=0.0076) that persisted for at least 1 min. The airway was neither dilated nor contracted immediately after maximal expiration, however a delayed bronchodilatory response was observed from 4 s (p=0.0059) to 3 min after manoeuvre (p=0.0182). In contrast, partial expiration did not produce a statistically significant change in airway calibre (and importantly no bronchodilation), although there was a trend towards
enhanced narrowing immediately after the manoeuvre (NS, p=0.1367), but which was absent 4 s later.

**Figure 5.**
Lumen perimeter strain (n=5) measured at end inspiration, during deep inspiration (DI) and maximal expiration, and at end expiration, during maximal and partial expiration. There was no statistical difference in the magnitude of strain at end inspiration between DI and maximal expiration (p=0.4599) or at end expiration between maximal expiration and partial expiration (p=0.4376). There was also no difference between the magnitude of strain (|absolute strain|) at end inspiration compared with end expiration (p=0.5753).