Does smooth muscle in an intact airway undergo length adaptation during a sustained change in transmural pressure?

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Ansell TK, McFawn PK, McLaughlin RA, Sampson DD, Eastwood PR, Hillman DR, Mitchell HW, Noble PB. Does smooth muscle in an intact airway undergo length adaptation during a sustained change in transmural pressure? J Appl Physiol 118: 533–543, 2015. First published November 13, 2014; doi:10.1152/japplphysiol.00724.2014.—In isolated airway smooth muscle (ASM) strips, an increase or decrease in ASM length away from its current optimum length causes an immediate reduction in force production followed by a gradual time-dependent recovery in force, a phenomenon termed length adaptation. In situ, length adaptation may be initiated by a change in transmural pressure (Ptm), which is a primary physiological determinant of ASM length. The present study sought to determine the effect of sustained changes in Ptm and therefore, ASM perimeter, on airway function. We measured contractile responses in whole porcine bronchial segments in vitro before and after a sustained inflation from a baseline Ptm of 5 cmH2O to 25 cmH2O, or deflation to −5 cmH2O, for ~50 min in each case. In one group of airways, lumen narrowing and stiffening in response to electrical field stimulation (EFS) were assessed from volume and pressure signals using a servo-controlled syringe pump with pressure feedback. In a second group of airways, lumen narrowing and the perimeter of the ASM in situ were determined by anatomical optical coherence tomography (αOCT); bronchoconstriction is a well-recognized property of isolated airway smooth muscle (ASM) strips that are their capacity to optimize force production following sustained changes in length (8, 44), a phenomenon termed length adaptation (6). Length adaptation manifests as a shift in the active and passive length-tension curves. An increase or decrease in ASM length from its current optimum length for a sustained period of time (by either lengthening or shortening) causes an immediate reduction in force production followed by a gradual time-dependent recovery in force following adaptive rearrangement of the contractile apparatus (34, 49, 56). Length adaptation may also in part explain the attenuation of ASM force production in response to oscillatory (or transient) changes in ASM length (24, 50, 54, 55). Despite evidence of length adaptation in isolated ASM strips, the presence and/or potential role of length adaptation in situ and therefore, the implications for airway function in health and disease, are uncertain. In normal healthy individuals, the length (i.e., perimeter) of the ASM varies with the transmural pressure (Ptm, the pressure difference across the airway wall), which increases and decreases with lung inflation and deflation, respectively. An inflationary airway Ptm is produced by a subatmospheric pleural pressure, and these forces are transmitted to the airway wall by parenchymal attachments. In obstructive lung disease, these lung parenchymal attachment forces are expected to change and, as the magnitude of Ptm is altered, the dependent ASM perimeter will also change. In asthma and nonemphysematous chronic obstructive pulmonary disease, lung hyperinflation favors an increase in ASM perimeter (26, 42). In contrast, reduced elastic lung recoil and mechanical uncoupling of the lung parenchymal attachments in emphysema is likely to shorten ASM perimeter (12, 15). Another possible scenario is that the ASM may be chronically shortened due to persistent levels of basal ASM tone (7). If length adaptation were present in vivo, such disease-related changes in ASM perimeter may be accompanied by changes in contractile capacity, such that force production could be potentiated at these abnormally increased or decreased perimeters. Indeed, length adaptation to a shorter ASM perimeter has been proposed as a potential mechanism underlying airway hyperresponsiveness (i.e., excessive bronchoconstriction in response to an inhaled bronchial challenge) in obstructive lung disease (56).

The aim of the present study was to determine the effect of sustained changes in Ptm and therefore, ASM perimeter, on airway function. Using an intact airway model, we rigorously examined how normal airway function was affected by a change in Ptm through its effect on several physiological properties, including airway narrowing, ASM tension, and airway wall stiffness, under either static or oscillatory conditions. We hypothesized that length adaptation would produce a...
time-dependent increase in contractile response to the physiological determinant of ASM perimeter, $P_{tm}$.

MATERIALS AND METHODS

Animal Handling

All animal experiments conformed to institutional ethics and animal care unit regulations (Animal Ethics Committee, University of Western Australia, Crawley, WA, Australia). Male White Landrace pigs (~35 kg) were initially sedated with tiletamine-zolazepam (4.4 mg/kg im) and xylazine (2.2 mg/kg im) and then exsanguinated under sodium pentobarbitone anaesthesia (30 mg/kg iv). The lungs were immediately removed and transported on ice to the laboratory.

Airway Segment Preparation

Airway segments were dissected from the main stem bronchus of the left or right lower lobe within ~1 h of being removed. All side branches were ligated with surgical silk and an airway segment was cannulated at both ends as previously described (4, 32). Following cannulation, the airway was mounted horizontally in an organ bath containing gassed (95% O$_2$ and 5% CO$_2$) Krebs solution (in mM: 121 NaCl, 5.4 KCl, 1.2 MgSO$_4$, 25 NaHCO$_3$, 5 sodium morpholinopropyl sulfonic acid, 11.5 glucose, and 2.5 CaCl$_2$, pH 7.3) at 37°C. The horizontal length of the segment was stretched to 105% of its length in the fully deflated lung, shown previously to approximate the length at functional residual capacity (FRC) (39). One end of the airway lumen was connected to a reservoir filled with Krebs solution, the height of which set the initial $P_{tm}$ (5 cmH$_2$O) and which was used to flush the lumen with Krebs solution. Depending on the measurement outcome (see below), the opposite end of the airway was either connected to a liquid-filled syringe pump or used to insert an anatomical optical coherence tomography ($\alpha$OCT) probe to provide an in situ measure of ASM perimeter (41), which was then sealed in place using a latex membrane.

Experimental Protocol

Although the time course of length adaptation may extend for days, it is substantially accelerated by regular stimulation and contraction of the ASM. In studies in which the ASM was stimulated every 5 min, adaptation was evident within a period of ~30 min (10, 11, 24, 44, 55). Therefore, in our study, airways were stimulated to contract every 5 min prior to and during inflationary or deflationary $P_{tm}$ (~50 min).

Airway segments were initially equilibrated to a baseline $P_{tm}$ of 5 cmH$_2$O to simulate FRC in the normal healthy lung. The equilibration period (at 5 cmH$_2$O $P_{tm}$) of 1 h began immediately after the airway was mounted in the organ bath. The Krebs solution in the organ bath contained gassed (95% O$_2$ and 5% CO$_2$) Krebs solution (in mM: 121 NaCl, 5.4 KCl, 1.2 MgSO$_4$, 25 NaHCO$_3$, 5 sodium morpholinopropyl sulfonic acid, 11.5 glucose, and 2.5 CaCl$_2$, pH 7.3) at 37°C. The horizontal length of the segment was stretched to 105% of its length in the fully deflated lung, shown previously to approximate the length at functional residual capacity (FRC) (39). One end of the airway lumen was connected to a reservoir filled with Krebs solution, the height of which set the initial $P_{tm}$ (5 cmH$_2$O) and which was used to flush the lumen with Krebs solution. Depending on the measurement outcome (see below), the opposite end of the airway was either connected to a liquid-filled syringe pump or used to insert an anatomical optical coherence tomography ($\alpha$OCT) probe to provide an in situ measure of ASM perimeter (41), which was then sealed in place using a latex membrane.

Experiment 1: airway narrowing and compliance under oscillatory conditions. A custom-built servocontrolled syringe pump and pressure transducer was used to set $P_{tm}$, apply fixed-$P_{tm}$ oscillations, and measure airway narrowing in response to EFS (% decrease in lumen volume) as previously described (35, 36). Airways were connected to a 1-ml glass syringe driven by a feedback-controlled servomotor and motor controller. $P_{tm}$ was measured via a calibrated pressure transducer with feedback to the servomotor, and changes in airway lumen...
where was cycled from 5 to 10 cmH2O; for 25 cmH2O, pressure was cycled 5 to 0 cmH2O. The resulting pressure and volume oscillations

Airway measurements using penetrates into the tissue and is scattered back from the ASM layer.

refractive index of the medium (1.37 for Krebs solution). The probe was rotated at monitor. Ptm at all times was set by the height of the attached pressure

was withdrawn until airway closure, which likely underestimates vol-

prior to ASM activation was determined from the volume that could be withdrawn until airway closure, which likely understimates volume, as previously discussed (2). Small pressure oscillations were applied (Δ5 cmH2O; i.e., tidal breathing maneuvers) above the target Ptm. Specifically, when the Ptm under study was 5 cmH2O, pressure was cycled from 5 to 10 cmH2O; for 25 cmH2O, pressure was cycled from 25 to 30 cmH2O; and for −5 cmH2O, pressure was cycled from −5 to 0 cmH2O. The resulting pressure and volume oscillations allowed compliance to be calculated as:

\[
\text{specific compliance} = \frac{\Delta\text{tidal volume}}{\Delta\text{tidal Ptm} \times \text{lumen volume}}
\]

where Δtidal volume and Δtidal Ptm are the trough-to-peak changes in volume and pressure during oscillatory conditions, respectively; and lumen volume is volume of the lumen at the trough of the pressure cycle at the time of measurement. A decrease in specific compliance produced by EFS is referred to as airway wall stiffening.

Experiment 2: airway narrowing and ASM perimeter under static conditions. Airway lumen cross-sectional area was measured by aOCT as previously described (5). For the present study, the aOCT probe was encased in a transparent catheter (2.2 mm OD), which required a larger airway segment than in experiments 1 and 3 to accommodate the probe and surrounding catheter. During aOCT imaging, broadband near-infrared light is emitted from the optical probe. The same probe is used simultaneously to detect reflections of light from the Krebs-tissue interface of the lumen, which allows the distance to the luminal surface to be determined by low-coherence interferometry (5) image. By rotating the probe within the catheter, a two-dimensional axial (i.e., radial-B scan) of the airway may be reconstructed (Fig. 2). Importantly, the capacity for aOCT to measure Ptm has been previously demonstrated (41) because the light beam penetrates into the tissue and is scattered back from the ASM layer. Airway measurements using aOCT are calibrated to account for the refractive index of the medium (1.37 for Krebs solution). The aOCT probe was rotated at ~0.8 Hz, acquiring quantitative images of A0 and Ptm, which were recorded and displayed in real time on a computer monitor. Ptm at all times was set by the height of the attached pressure column.

Experiment 3: active pressure and ASM tension under static conditions. Isovolumic contractions were measured by closure of a tap located between the airway and the pressure column. Under isovolumic conditions, ASM contraction results in an increase in lumen pressure (i.e., active pressure), which represents ASM tension, thus providing a close comparison of experiments examining adaptive properties in isolated ASM strip studies (18, 24, 44, 54–56). Other than during the measurement of ASM contraction, the tap remained open and Ptm was set by the height of the pressure column, as in experiment 2. Active pressure was subsequently expressed as active tension (Nm⁻¹):

\[
\text{tension} = \text{active pressure} \times \frac{\text{Pmo}}{2 \times \pi}
\]

The Pmo used to calculate tension was the mean Pmo at the same time point measured in experiment 2 using aOCT. Airways used in experiment 2 were larger than those used in experiment 3, so the calculated tension is an overestimation. However, the relative changes in Pmo produced by inflation to 25 cmH2O Ptm and deflation to −5 cmH2O Ptm would be expected to be similar for both experiments.

Analysis and Statistics

Airway narrowing in response to EFS was expressed as % reduction in lumen volume or A0 (i.e., lumen volume immediately prior to EFS; 100% airway narrowing indicates airway closure). Comparisons of airway narrowing during pressure oscillations (experiment 1) were made at the trough of the pressure cycle (i.e., 5, −5, or 25 cmH2O). In experiment 2, A0 and Ptm were determined using custom-designed quantification software (written in C++), which allowed manual measurement of airway dimensions from the aOCT data sets. Inner

![Fig. 2. An example cross-sectional image of a porcine airway recorded by anatomical optical coherence tomography (aOCT) at 5 cmH2O Ptm, before (top) and after (bottom) contraction in response to EFS. The images identify the airway epithelium (AE) and the area contained therein, and the outer border of the airway smooth muscle (ASM) band, which allows measurement of the outer muscle perimeter. The inner wall area corresponds to the area of the dark band surrounding the lumen. Cartilage plates (CP) and the catheter containing the optical probe (PB) are also identifiable.](http://jap.physiology.org/DownloadedFrom/10.1152/japplphysiol.00724.2014)
wall area (WA) was also calculated by subtracting \( A_i \) from the area enclosed by the \( P_{tm} \) (i.e., \( A_{tm} \)). Active pressure in response to EFS (experiment 3) or active tension was expressed as the \( \Delta \) increase in pressure or tension.

Baseline contractile responses prior to the initiation of the adaptation protocol were averaged (approximately three stimulations) for comparison with contractile responses during and after the adaptation protocol. The immediate effect of rapid changes in \( P_{tm} \) was analyzed using paired \( t \)-tests. The effect of sustained changes in \( P_{tm} \) during the adaptation protocol was analyzed by repeated-measures one-way ANOVA and Dunnett’s post hoc test. Data analysis and statistical tests were performed using Statistica (version 8.0; StatSoft, Tulsa, OK) and GraphPad Prism (version 5.0d; GraphPad Software, La Jolla, CA). Data are presented as means ± SE, where \( n \) = number of animals.

RESULTS

Rapid, hydrostatically driven inflation to 25 cmH2O \( P_{tm} \) or deflation to −5 cmH2O \( P_{tm} \) produced immediate and sustained changes in relaxed airway volume and \( A_i \), and in the level of the contractile response to EFS (airway narrowing, airway wall stiffening, and ASM tension), discussed below.

Experiment 1: Airway Narrowing and Compliance Under Oscillatory Conditions

Inflation immediately increased relaxed airway volume by 39 ± 3% (Fig. 3A), whereas deflation decreased relaxed airway volume by 32 ± 4% (Fig. 3B). During the adaptation protocol, relaxed airway volume continued to increase in inflated airways and decrease in deflated airways. Upon return to a baseline \( P_{tm} \) of 5 cmH2O after 50 min, the volume of airways remained somewhat larger in airways previously inflated (\( P < 0.001 \)) and smaller in airways previously deflated (\( P < 0.001 \)).

At baseline, EFS produced 41 ± 4% and 39 ± 8% airway narrowing in airways subsequently inflated and deflated, respectively (Fig. 3C). During the adaptation protocol, airway narrowing in response to EFS was immediately reduced compared with baseline levels by both inflation and deflation. At 25 cmH2O \( P_{tm} \), EFS produced 32 ± 3% airway narrowing, whereas at −5 cmH2O \( P_{tm} \), airway narrowing response to EFS was 31 ± 6%. Airway narrowing response to EFS continued to gradually decrease during the adaptation protocol. When normalized to the level of airway narrowing at baseline, the reduction in airway narrowing was greater in deflated airways compared with inflated airways (\( P < 0.001 \)). Upon return to baseline, airway narrowing in response to EFS immediately returned to baseline levels in the airways previously inflated, whereas for airways previously deflated, narrowing returned to baseline levels after ~10 min.

Pressure oscillations and the corresponding volume perturbations allowed the calculation of specific compliance of the airway wall in the relaxed and contracted (i.e., during airway narrowing to EFS) states. At baseline, the specific compliance of relaxed airway was 0.0076 ± 0.0010 cmH2O−1 and 0.0095 ± 0.0016 cmH2O−1 in airways prior to inflation (Fig. 4A) and deflation (Fig. 4B), respectively. Compliance of the airway wall was reduced at 25 cmH2O \( P_{tm} \) and increased at −5 cmH2O \( P_{tm} \). Compliance continued to increase during sustained deflation (\( P < 0.001 \)), whereas sustained inflation produced a statistically significant albeit minor decrease in compliance (\( P < 0.001 \)). Compliance returned to baseline levels after ~10 min.
after ∼5 min in previously inflated airways and after ∼35 min in previously deflated airways.

At baseline, EFS produced airway stiffening (i.e., a reduction in specific compliance during contraction). However, with inflation, EFS produced no measureable airway stiffening (i.e., specific compliance was the same for relaxed and contracted airways after inflation). In comparison, airway stiffening in response to EFS was greater after deflation (P < 0.001). Stiffening in response to EFS was not altered during sustained deflation, and upon return to baseline, airway stiffening was the same as it was at baseline levels.

Experiment 2: Airway Narrowing and ASM Perimeter Under Static Conditions

In response to sustained inflation and deflation, changes in relaxed A_i measured using aOCT under static conditions mirrored those observed for lumen volume in experiment 1. Inflation immediately increased A_i by 24 ± 2%, whereas deflation decreased A_i by 43 ± 4% (Fig. 5A). The corresponding change in P_mo (i.e., ASM perimeter) was 11 ± 1% for inflation, and 22 ± 2% decrease during deflation (Fig. 5B). Both A_i and P_mo continued to increase during sustained inflation (A_i, P < 0.001, P_mo P < 0.001) and decrease during sustained deflation (A_i P < 0.001, P_mo P < 0.001). Upon return to baseline, after 50 min, A_i and P_mo were greater in previously inflated airways compared with baseline levels. In previously deflated airways, A_i and P_mo both returned to baseline levels after 30 min. WA_i was unaffected by a short-term or sustained change in P_m.

At baseline, airway narrowing in response to EFS measured using aOCT was 64 ± 3% decrease in A_i and 46 ± 5% decrease in A_i in airways subsequently inflated and deflated, respectively (P < 0.001), despite anatomical matching and experimental conditioning. Similarly to experiment 1, airway narrowing and percentage ASM shortening in response to EFS (Fig. 6) were immediately reduced at 25 cmH_2O and at −5 cmH_2O P_m, and continued to gradually decrease, particularly at −5 cmH_2O P_m. There was also a decrease in absolute ASM shortening (i.e., Δmm) following inflation (P < 0.05) or
deflation \((P < 0.001)\). The decrease in airway narrowing \((P < 0.001)\) and muscle shortening \((P < 0.001)\) was more pronounced at \(-5\, \text{cmH}_2\text{O}\) compared with \(25\, \text{cmH}_2\text{O}\) when normalized to baseline levels. Once a \(P_{\text{tm}}\) of \(5\, \text{cmH}_2\text{O}\) was reinstated, airway narrowing and muscle shortening in response to EFS returned immediately to baseline levels in previously inflated airways, whereas previously deflated airways took 15 min for narrowing and shortening to recover to baseline levels.

**Experiment 3: Active Pressure and ASM Tension Under Static Conditions**

Sustained inflationary and deflationary changes in \(P_{\text{tm}}\) had a similar effect on active pressure (Fig. 7A) and ASM tension (Fig. 7B) to that observed for airway narrowing. There was an immediate decrease in active pressure and tension at both \(25\, \text{cmH}_2\text{O}\) and \(-5\, \text{cmH}_2\text{O}\), and a continued gradual decrease in active pressure and tension at \(-5\, \text{cmH}_2\text{O}\) \(P_{\text{tm}}\). Upon return to baseline, active pressure and tension immediately returned to levels observed before the adaptation protocol in previously inflated airways, whereas it took 10 min for previously deflated airways to return to baseline. There was no evidence for an increase in active pressure or tension during the adaptation protocol (either \(25\) or \(-5\, \text{cmH}_2\text{O}\) \(P_{\text{tm}}\)).

**DISCUSSION**

There are several likely scenarios whereby the ASM perimeter may be lengthened (i.e., hyperinflation) or shortened (i.e., parenchymal attachment uncoupling), and the present study set about to examine the potential implications of these changes in ASM perimeter on airway function with a focus on ASM length adaptation. Sustained inflationary and deflationary \(P_{\text{tm}}\) were applied to induce lengthening and shortening, respectively, of the ASM perimeter. Airway caliber, wall compliance, and ASM tension were all immediately modified by the rapid change in \(P_{\text{tm}}\). However, in the presence of regular contractile activation, sustained periods of inflation or deflation failed to induce any changes in airway behavior that would have been
expected with ASM length adaptation, indicating that the ASM response to sustained changes in length observed in isolated ASM strips is not scaled up or down in whole airways subjected to sustained changes in Ptm.

Experimental Design and Rationale

Investigating the applicability of findings in isolated ASM strip preparations to integrated lung function is most necessary because they do not always translate as might be expected. For instance, force inhibition in response to length oscillations is well demonstrated in isolated ASM strips but is scaled down when the ASM is studied in situ (25, 38). Disparity between airway and muscle preparations and that observed in situ are well recognized (27). In the present study, we investigated how ASM length adaptation previously demonstrated in isolated ASM strips (10, 11, 24, 44, 55) alters components of the response in whole bronchial segments, and which may regulate airway responsiveness.

Three different experimental approaches were used to assess adaptation to a sustained change in Ptm. In each approach, connections with lung parenchymal attachments were severed in the intact airway model and, as such, results are not influenced by airway-parenchymal attachment interactions that were likely to change with lung inflation and deflation in vivo (13). In experiment 1, we measured airway narrowing during oscillatory pressure perturbation, which allowed us to simultaneously measure specific compliance and airway narrowing, replicating in vivo conditions in which airways are under oscillatory tidal Ptm. A constantly changing ASM length due to breathing is one of the many differences between isolated ASM strips in vitro and the lung in vivo, which could disrupt any length adaptation process. In experiment 2, we measured airway narrowing under static conditions using OCT. Using static conditions allowed us to determine whether tidal oscillations used in experiment 1 inhibited length adaptation. Although OCT required the use of somewhat larger airways than the other approaches (to accommodate the physical dimensions of the probe), it enabled measurement of ASM perimeter in situ, which can be compared with previous studies using isolated ASM (10, 11, 18, 44, 56). In experiment 3, we measured active pressure, an index of ASM tension, because numerous previous studies identifying length adaptation used measurements of muscle tension. By measuring tension, we can rule out any effects from ASM afterloads masking the ASM tension. However, neither approach demonstrated changes consistent with length adaptation. Following a change in Ptm, there was no recovery in airway narrowing (either under static or oscillatory conditions), or ASM shortening or tension. In fact, airway narrowing and ASM shortening and tension tended to further decrease with a sustained period at −5 cmH2O, and to some extent at 25 cmH2O. The lack of any recovery in either airway narrowing, or ASM shortening or tension, argues strongly against ASM length adaptation fulfilling a dominant physiological role at the level of the intact airway.

Changes in ASM perimeter produced by Ptm may have been too small for any adaptive response to be identified when scaled to the whole airway. In experiment 2, under static conditions, inflation to 25 cmH2O produced an ~11% increase in Ptm, and a ~22% decrease after deflation to −5 cmH2O, measured by OCT. This level of ASM perimeter change is at the lower end of the spectrum compared with studies using isolated ASM for which reported length changes that produce length adaptation were in the range of ~30% to 70% (10, 11, 18, 44, 56), which may in some instances exceed physiological limits. The range of Ptm used in the present study encompasses a more physiological range. Inflation to 25 cmH2O is at the plateau of the airway-pressure-volume relationship (i.e., near maximal inflation), whereas −5 cmH2O sits at the inflection point of the airway pressure-volume curve, below which the airway becomes considerably stiffer. Therefore, changes in ASM perimeter with further inflation or deflation beyond that observed in the present study are expected to be small, at least in the porcine airway. We acknowledge that the applicability of findings derived using porcine tissue to the human scenario can...
be debated, and indeed, the human airway is more compliant than the porcine airway (2, 36). However, previous work in our laboratory (2, 36) has demonstrated that in vitro airway responses to changes in $P_{\text{tm}}$ in the porcine airway compare well to the human airway, supporting use of the pig model in the present study.

In experiment 1, the inclusion of pressure oscillations during the measurement of airway narrowing cannot explain a failure to identify length adaptation because results were qualitatively similar under static conditions (experiment 2). Pressure oscillations at $\Delta S \text{ cm}^2\text{H}_2\text{O}$ are a reasonable approximation of tidal oscillations and are not expected to have a major effect on airway narrowing. In airway segments, tidal oscillations are at or below the threshold required to produce bronchodilation (2, 25, 38).

There are several potential methodological explanations for the lack of ASM adaptive response to $P_{\text{tm}}$ in situ. As established in experiment 2, for a constant $P_{\text{tm}}$, there was a statistically significant albeit small change in $P_{\text{mo}}$ with sustained inflation or deflation ($\sim 5\%$ compared with $P_{\text{mo}}$ at the beginning of the adaptation period). An important difference between the present study using whole airways and prior studies using ASM strip preparations is that load (i.e., $P_{\text{tm}}$) was kept constant and the ASM perimeter was free to change in response to the applied load. It is certainly feasible that this moving target may have offset the ASM adaptive response. Flushing of the airway lumen and the resulting changes in $P_{\text{tm}}$ may have similarly disrupted ASM adaptive processes. We nonetheless conclude that compared with the immediate effects of a change in $P_{\text{tm}}$, the proposed physiological driver of ASM perimeter, adaption to a change in ASM perimeter in situ is of much lesser importance to airway function, at least within the context of the short-term but sustained adaptation period studied.

An unexpected finding was the gradual decrease in airway narrowing with a sustained $P_{\text{tm}}$ change, particularly at $-5 \text{ cm}^2\text{H}_2\text{O}$, which is in strong contrast to what is predicted to occur with ASM length adaptation. The decrease in airway narrowing was not an issue of viability, which may occur with repeated simulation of ASM and nerve endings, because airway narrowing was observed to recover when $P_{\text{tm}}$ was returned to $5 \text{ cm}^2\text{H}_2\text{O}$ (i.e., baseline). The decrease in airway narrowing may be related to the corresponding change in airway size (volume or $A_t$); specifically, a gradual increase in airway size at $25 \text{ cm}^2\text{H}_2\text{O}$ and a decrease in airway size at $-5 \text{ cm}^2\text{H}_2\text{O}$. The change in airway size theoretically represents a shift to a stiffer region of the pressure-volume curve, above and below $25 \text{ cm}^2\text{H}_2\text{O}$ and $-5 \text{ cm}^2\text{H}_2\text{O}$, respectively (40, 59). Although wall stiffening is expected to limit airway narrowing, there was a similar gradual decrease in ASM tension, which would not be affected by wall compliance. A final possibility is that the change in ASM tension could occur with a corresponding change in ASM preload. As $P_{\text{mo}}$ gradually increases or decreases during the adaption protocol, the ASM is theoretically pushed to a configuration that reduces force production. However, given that the change in $P_{\text{mo}}$ was small, any movement along the ASM length-tension curve would also be small and therefore would not be expected to have a major effect on force production.

Another notable difference between isolated ASM preparations and whole bronchial segments is that in the former, the epithelial membrane is dissected away before study. The airway epithelium is a rich source of bronchoactive mediators (23), and gradual accumulation during the adaptation protocol could alter ASM contraction. There is evidence that the release of prostanoids from the epithelium is modulated by mechanical strain (47), which raises the possibility that mediators were regulated by $P_{\text{tm}}$. In preliminary experiments in which the airway lumen was not flushed during the adaptation protocol, there was a greater decrease in ASM contraction during the adaption protocol (data not shown) than that recorded here, in experiments in which the lumen was flushed. Those preliminary experimental findings do provide some circumstantial evidence for an epithelial-derived response to inflation or deflation. Given this potentially confounding mechanism, it was necessary to regularly flush the airway lumen.

The precise mechanism for the decrease in airway narrowing during the sustained $P_{\text{tm}}$ change remains unclear. This phenomenon may inhibit contraction of passively narrowed bronchi in vivo and could be beneficial by limiting bronchoconstriction in patients with chronic airway narrowing from loss of lung elastic recoil or parenchymal attachments following emphysema. In the context of the present study, the existence of a mechanism that inhibits ASM contraction and therefore opposes ASM length adaptation could explain why length adaptation observed in isolated cells does not appear to affect whole airway mechanics.

Implications for Bronchoprotective and Bronchodilatory Effects of Deep Inspiration

In normal healthy individuals in vivo, deep inspiration (DI) produces a transient reversal of bronchoconstriction (i.e., bronchodilation) (14, 20, 33, 46) and may also transiently reduce the magnitude of subsequent bronchoconstriction, a phenomenon termed bronchoprotection (21, 28, 48, 51). Length adaptation theoretically contributes to both bronchoprotective and bronchodilatory effects of DI (53). However, bronchoprotective effects of DI are not observed using airway segment preparations (19, 37) wherein contractile responses are examined before and after large $P_{\text{tm}}$ oscillations simulating DI. The present lack of evidence for a dominant effect of ASM length adaption in situ is consistent with the failure of simulated DI to induce bronchoprotection in airway segments. The implication of these observations is that bronchoprotective effects of DI in vivo may instead arise from one or more mechanisms other than length adaptation, notably enhanced bronchodilatory effects of DI when bronchoconstriction is assessed by forced expiratory volume in 1 s (FEV$_1$), prevention of airway closure, or both (9, 60). With respect to the bronchodilatory effects elicited by DI in airway segments (36, 58), our present findings favor mechanisms other than length adaptation, such as cross-bridge perturbation (16, 17).

Duration of Length Adaptation

The time needed for ASM length adaptation to occur has varied between studies. Early studies on isolated ASM preparations held ASM length fixed for days to weeks (34, 56), demonstrating large shifts in the ASM passive and active length-tension curves. However, the process of length adaption is accelerated by regular ASM activation and can occur after $\sim 30$ min (10, 11, 24, 44, 55). Therefore, we expect that the $\sim 50$-min duration of altered $P_{\text{tm}}$ with regular ASM activation
every 5 min used in the present study should be sufficient for length adaptation to occur.

Several previous studies examining the effect of chronic exposure to increased $P_{\text{tm}}$ on airway function all show that chronic inflation suppresses airway narrowing (52, 61–63). Indeed, continuous positive airway pressure (CPAP) has been suggested as a novel treatment for asthma (43). In vitro, whole rabbit bronchial segments were cultured for 48 h with and without a positive inflationary pressure, which reduced the maximum isometric force produced by the inflated airways (52). In ferrets in vivo, CPAP for 2 to 3 wk reduced airway responsiveness studied at 5 cmH$_2$O (62). Furthermore, the effects of CPAP persisted for at least 24 h (61). In a recent study by Xue and colleagues (63), CPAP for only 2 h was sufficient to alter airway responsiveness in mice.

The evidence to date suggests that the beneficial reduction in airway responsiveness brought about by periodic CPAP may not be mediated by ASM length adaptation. Indeed, in the study in ferrets (62) referred to above, there was reduced force production in isolated ASM strips even when all tissues were studied at their optimum lengths. Instead of length adaptation, CPAP appears to reduce ASM contractility by inhibiting myosin regulatory light chain phosphorylation, suggesting suppressed excitation-contraction coupling rather than length adaptation (62). A separate study by McClean and colleagues (29) showed no change in myosin light chain kinase following a reduction in FRC for 4 wk, suggesting that the effect may be specific to CPAP therapy. In view of our results demonstrating that sustained inflation increases airway caliber (measured via lumen volume and cross-sectional area) and similar findings from in vitro studies on rabbit airways (52), some of the beneficial effects of CPAP may be geometric. In the present study, the increase in airway caliber was observed even $\sim$50 min after the baseline $P_{\text{tm}}$ of 5 cmH$_2$O was reinstated.

**Airway Compliance and $P_{\text{tm}}$**

There was little to no change in specific compliance following sustained inflation, although the increase in airway caliber described above could indicate softening of the airway wall. Following sustained deflation, there was evidence of reduced airway caliber, which is consistent with wall stiffening. In contrast, sustained deflation produced a transient increase in specific airway compliance lasting for $\sim$35 min. Unfortunately, the protocol did not allow for the construction of a full pressure-volume curve, which may have revealed changes in airway compliance at different regions of the curve.

A change in $P_{\text{tm}}$ itself produced pronounces but predictable changes in specific airway compliance. Assessment of compliance between 25 and 30 cmH$_2$O ($\Delta$5 cmH$_2$O) is made in a stiffer region of the pressure-volume curve, whereas compliance between $\sim$5 to 0 cmH$_2$O is at the linear and relatively compliant region of the pressure-volume curve (40, 59). Of more interest was the effect of $P_{\text{tm}}$ on airway wall compliance after ASM contraction in response to EFS. Stiffening of the ASM and airway wall with contractile activation is well established (3, 22, 38). Compared with the reference $P_{\text{tm}}$ of 5 cmH$_2$O at which ASM contraction produced considerable airway stiffening, at 25 cmH$_2$O there was no measureable stiffening with ASM contraction. On the other hand, at $\sim$5 cmH$_2$O $P_{\text{tm}}$, stiffening with ASM contraction increased. These observations likely reflect the load-bearing elements in the airway wall at different $P_{\text{tm}}$. At 25 cmH$_2$O the airway is distended and stiff, with a plateau in its pressure-volume relationship. Given these powerful passive forces, the superimposition of ASM contraction causes little change to overall stiffness. At lower pressures, the passive components of the airway wall are highly compliant, so the compliance changes with ASM have a substantial effect on overall airway compliance. These data are consistent with findings in normal healthy individuals in vivo and suggest that ASM contraction reduces airway compliance at low but not high lung volumes (22).

Airway wall stiffening is of potential relevance in a number of airway diseases, including asthma. Various mechanisms can contribute to airway wall stiffening and include remodeling of the cell structural and/or contractile apparatus (45, 57) and ASM activation (1). In view of the present findings, we propose that lung hyperinflation is another likely contributor to airway wall stiffening, with a potency that at least matches ASM contractile activation at lower lung volumes.

**Conclusions**

The present study showed that whereas evoked airway narrowing is regulated by mechanisms associated with $P_{\text{tm}}$, preload effects (i.e., classic length-tension properties) rather than ASM length adaptation, dominate the contractile response. In addition, an unexpected observation was that airway narrowing decreased rather than increased with sustained $P_{\text{tm}}$ changes, and this was reversible. The physiological limit on ASM contraction and airway narrowing, demonstrated in the present study during sustained inflation or deflation, may be protective and the underlying mechanisms merit further investigation.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


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