Can breathing-like pressure oscillations reverse or prevent narrowing of small intact airways?

Harvey BC, Parameswaran H, Lutchen KR. Can breathing-like pressure oscillations reverse or prevent narrowing of small intact airways? J Appl Physiol 119: 47–54, 2015. First published May 7, 2015; doi:10.1152/japplphysiol.01100.2014.—Periodic length fluctuations of airway smooth muscle during breathing are thought to modulate airway responsiveness in vivo. Recent animal and human intact airway studies have shown that pressure fluctuations simulating breathing can only marginally reverse airway narrowing and are ineffective at protecting against future narrowing. However, these previous studies were performed on relatively large (>5 mm diameter) airways, which are inherently stiffer than smaller airways for which a preponderance of airway constriction in asthma likely occurs. The goal of this study was to determine the effectiveness of breathing-like transmural pressure oscillations to reverse induced narrowing and/or protect against future narrowing of smaller, more compliant intact airways. We constricted smaller (luminal diameter = 2.92 ± 0.29 mm) intact airway segments twice with ACh (10⁻⁶ M), once while applying tidal-like pressure oscillations (5–15 cmH₂O) before, during, and after inducing constriction (Pre + Post) and again while only imposing the tidal-like pressure oscillation after induced constriction (Post Only). Smaller airways were 128% more compliant than previously studied larger airways. This increased compliance translated into 196% more strain and 76% greater recovery (41 vs. 23%) because of tidal-like pressure oscillations. Larger pressure oscillations (5–25 cmH₂O) caused more recovery (77.5 ± 16.5%). However, pressure oscillations applied before and during constriction resulted in the same steady-state diameter as when pressure oscillations were only applied after constriction. These data show that reduced straining of the airways before a challenge likely does not contribute to the emergence of airway hyperreactivity observed in asthma but may serve to sustain a given level of constriction.

In healthy subjects, deep inspirations (DIs) are capable of reversing airway narrowing (bronchodilation) (14, 38, 47) and have been proposed to be capable of attenuating future narrowing (bronchoprotection) (26, 37, 49, 50). However, these effects are diminished in asthmatics (13, 16, 24, 26). Also, purposely refraining from taking DIs results in amplified sensitivity to methacholine in healthy subjects (53). In animals, bronchocostriction is reduced with increasing tidal volume (12, 48, 52, 54). Additionally, periodic length fluctuations mimicking breathing imposed before (57) or after (5, 17, 21) activation of an isolated strip of airway smooth muscle (ASM) result in a decrease in the amount of force generated by the ASM. These observations, along with the fact that airways from asthmatics are generally stiffer than from healthy subjects (11), spawned the attractive hypothesis that airway hyperreactivity (AHR) in asthma results as a consequence of lack of periodic stretching of airways (53). However, a large structural divide exists between isolated ASM strips and the whole lung in vivo, and it is difficult if not nearly impossible to precisely mimic in situ conditions when designing isolated ASM studies. In short, the connection between these two ends of the biological size spectrum, while enticing, remains ambiguous (35). We previously engineered a system to quantify how ASM-level mechanisms could translate to modulate reactivity at the intact airway level (22, 31). We found that transmural pressure (Ptm) fluctuations showed little capacity to bronchoprotect or bronchodilate intact airways (22, 31). To create >50% reversal of bronchoconstriction, we had to impose large-amplitude Ptm fluctuations (25 cmH₂O) every breath (22). Furthermore, the degree of bronchodilation resulting from Ptm fluctuations depended on the magnitude of strain imposed (22).

Our previous studies were performed on relatively large airways (>5-mm luminal diameter, generations 4–5 in humans) (22, 31). There is strong evidence that a preponderance of airway constriction in asthma occurs in smaller airways (<3-mm diameter, generations 7 or higher) (55). Smaller airways are generally more compliant (30, 51) such that the Ptm oscillations of the same size could result in larger strains and consequently greater bronchodiatory and/or bronchoprotective effects. We therefore advanced our ultrasound imaging and processing techniques to study the effect of breathing-like Ptm oscillations on smaller and more compliant airways that would strain more during breathing or a DI.

The goal of this study was to quantify the bronchodilatory and bronchoprotective effects of tidal-like (5–15 cmH₂O) Ptm oscillations as well as the bronchodilatory effect of DI-like (5–25 cmH₂O) Ptm oscillations in smaller airways and compare these results to those in larger airways from our previous study (22). Our new airways had luminal diameters of ~3.0 mm with a wall compliance that resulted in a one- to twofold increase in strain attributable to tidal-like Ptm oscillations compared with larger airways. This increased strain resulted in a consequent increase in the ability for these Ptm oscillations to dilate a constricted airway. However, imposing amplified tidal Ptm oscillations before and during the challenge did not reduce the final amount of airway narrowing compared with if the Ptm oscillations were only applied after the airway was constricted. In other words, the Ptm oscillations imbued no substantive a priori bronchoprotection. Taken together, these data suggest that a reduced ability to strain the airways might hamper the capacity to reverse airway narrowing, especially in smaller, more compliant airways, but a reduced straining of airways before a challenge likely does not contribute to AHR observed in asthma.

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**MATERIALS AND METHODS**

**Ultrasound intact airway system.** Experiments were performed on a custom-built intact airway system, as previously described (31). Briefly, intact airways were dissected from fresh bovine lungs (Research 87, Boylston, MA), and side branches were ligated to form a leak-free airway and then mounted inside a tissue bath with Krebs solution (Sigma, St. Louis, MO) (37°C, 5% CO2). The airways were dissected from a more distal region of the tapered main stem bronchus (generations 19–24, ~23 mm long) than previously studied airways (22) and therefore had smaller luminal diameters. Airways were stretched to 110–120% of their resting length to mimic airway lengthening during tidal breathing and held fixed throughout the experiment (42). A computer-controlled syringe pump modulates the height of fluid in a pressure column connected in series with the airway to deliver Ptm oscillations that mimic breathing. An ultrasound system (SonixTablet; Ultrasonix Medical, Richmond, British Columbia, Canada) with high-frequency linear array transducer (L40-8/12, center frequency = 15 MHz) was partially submerged in the tissue bath and mounted <1 cm above the outer edge of the airway and positioned parallel to the long axis of the airway segment. The imaging plane of the transducer cuts through the middle of the airway along its length, thereby allowing for quantification of luminal diameter and wall thickness at each point along the length of the airway. Tissue viability was confirmed with both electric foci at 3 mm and 12 mm), and these images were processed using the segmentation method described above. A linear regression was performed between the known thicknesses (H) of the phantoms and the mean number of pixels between the detected edges (nL) of the corresponding phantoms using $H = c_{\text{tissue}} \Delta t \cdot n_L / n_L - \text{spl}$ ($r^2 = 0.99$), where $c_{\text{tissue}}$ is the speed of sound through the tissue-mimicking phantom. The slope of this line is equal to $c_{\text{tissue}}$ (1.45 mm/μs), and the magnitude of the y-intercept is equal to the spl (0.23 mm). As a result of this correction algorithm, the minimum luminal diameter we can detect is equal to 0.23 mm. An airway at a diameter this small is considered functionally closed because of its high resistance to flow.

The algorithm was validated using two tissue phantoms mimicking the airways walls, which were separated by a known distance. The correction algorithm reduced the luminal diameter measurement error from 492 μm (8.7%) to 51.4 μm (0.9%).

**Experimental protocol.** Airways were constricted twice with a moderate dose of ACh (10⁻⁵ M) while imposing one of the following loading conditions in random order: Pre + Post (Fig. 2A), where sinusoidal Ptm oscillations mimicking amplified tidal breathing (5–15 cmH2O, f = 0.2 Hz) were imposed for 20 min immediately before and during the entire 40 min of the constriction; and Post Only (Fig. 2B), where the airway was constricted statically against a Ptm of 5 cmH2O for 20 min, followed by 20 min of Ptm oscillations from 5–15 cmH2O and then 20 min from 5–25 cmH2O to simulate taking a DI with every breath. The airway was allowed to relax in fresh Krebs for 60 min converted to luminal diameter using a calibration factor and correction constant to account for the ultrasound spatial pulse length (spl) (31) and averaged over the length of a region of interest. Specifically, we calculated the mean luminal diameter ($D$, mm) according to the speed of sound through Krebs solution ($c_{\text{Krebs}} = 1.54$ mm/μs), the total number of pixels within the wall ($n_L$), the time taken to travel between pixels ($\Delta t = 0.232$ μs/pixel), the length of the analyzed region in pixels ($n_L$), and the ultrasound spl:

$$D = \frac{c_{\text{Krebs}} \cdot \Delta t \cdot n_L}{n_L} + \text{spl}$$

To estimate spl, we imaged seven tissue-mimicking phantoms of known thicknesses between 1.0 and 3.7 mm using identical imaging parameters, as used for intact airways (depth = 15 mm, gain = 0.15, foci at 3 mm and 12 mm), and these images were processed using the segmentation method described above. A linear regression was performed between the known thicknesses (H) of the phantoms and the mean number of pixels between the detected edges ($n_L$) of the corresponding phantoms using $H = c_{\text{tissue}} \Delta t \cdot n_L / n_L - \text{spl}$ ($r^2 = 0.99$), where $c_{\text{tissue}}$ is the speed of sound through the tissue-mimicking phantom. The slope of this line is equal to $c_{\text{tissue}}$ (1.45 mm/μs), and the magnitude of the y-intercept is equal to the spl (0.23 mm). As a result of this correction algorithm, the minimum luminal diameter we can detect is equal to 0.23 mm. An airway at a diameter this small is considered functionally closed because of its high resistance to flow.

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Fig. 1. Cross-sectional B-mode ultrasound image of a smaller intact airway. Luminal and outer wall boundaries found using an automated segmentation algorithm are overlaid.

Fig. 2. Intact airways were constricted twice in random order. A: Post Only: airways were constricted statically at a simulated functional residual capacity (transmural pressure, Ptm = 5 cmH2O) for 20 min, followed by 20 min of Ptm oscillations from 5–15 cmH2O for 20 min, and then an additional 20 min from 5–25 cmH2O. B: Pre + Post: airways were exposed to Ptm oscillations (5–15 cmH2O, f = 0.2 Hz) for 20 min immediately before and during the constriction. ACh, acetylcholine.
and larger (gray, \( n = 5 \)) airways before induced constriction (baseline, solid) and after induced narrowing with ACh (constricted, dashed). Data were normalized by the diameter at total lung capacity (TLC) (25 cmH2O) in the baseline state and averaged. Smaller airways are more compliant than larger airways in the large tidal breathing range from 5 to 15 cmH2O (shaded area) both before and after induced narrowing. Larger airway data gathered from Harvey et al. (22).

**RESULTS**

**Airway mechanical properties.** Smaller airways had a baseline luminal diameter of 2.92 ± 0.29 mm at a Ptm of 5 cmH2O (\( n = 5 \)) compared with 5.72 ± 0.52 mm for the larger airways studied previously (22). Our quasistatic Ptm diameter data indicate that smaller airways are more compliant than larger airways above a Ptm corresponding to functional residual capacity (5 cmH2O) both before and after constriction with ACh (Fig. 3).

We calculated \( sC_{\text{dyn}} \) during simulated breathing after induced narrowing to compare the stiffness of smaller and larger airways (22). The \( sC_{\text{dyn}} \) of the smaller airway was 0.026 ± 0.006 cmH2O\(^{-1}\) in the tidal breathing range (Ptm = 5–15 cmH2O) and less compliant during DI-like Ptm excursions from 5–25 cmH2O (0.015 ± 0.002 cmH2O\(^{-1}\), \( P = 0.003 \)). Smaller airways were 128% more compliant from 5–15 cmH2O and 78% more compliant from 5–25 cmH2O than the previously studied larger airways (22).

**Bronchodilatory and protective effects in smaller airways.** Figure 4 shows representative traces of a single smaller airway from the Post Only (black) and Pre + Post (gray) protocols. In the Post Only constriction, this airway started at a baseline diameter of 2.93 mm and narrowed to 1.89 mm after 20 min of ACh exposure against a static Ptm of 5 cmH2O (peak constriction). Tidal-like Ptm fluctuations (5–15 cmH2O) resulted in some bronchodilation, and the airway recovered to a diameter of 2.22 mm within 20 min (steady state). DI-like Ptm fluctuations from 5–25 cmH2O (not shown) resulted in dilation to a diameter of 2.78 mm. In the Pre + Post constriction, this airway began at a similar diameter before activation (2.96 mm), and tidal-like Ptm oscillations imposed 11.5% strain per cycle. When the airway was activated, the airway constricted to a diameter of 2.08 mm compared with 1.90 mm when narrowed against a static Ptm. At first, this suggests that applying Ptm oscillations in advance reduced the amount of narrowing (peak constriction). However, these preconstriction oscillations necessarily impose a higher mean Ptm against which the constriction occurs (Ptm = 10 cmH2O in Pre + Post vs. 5 cmH2O in Post Only). In fact, once the oscillations were imposed to the statically constricted airway (at 20 min in the Post Only protocol), its diameter starts to increase and reaches
a steady state level (2.28 mm) very similar to the airway that was oscillated before and during the constriction (steady state). Figure 5 shows compiled data of smaller airway luminal diameters from the Post Only (black) and Pre + Post (gray) loading conditions at baseline, peak constriction, and steady state (i.e., after 5–15 cmH2O Ptm oscillation) time points. The airways start at the same luminal diameters immediately before the addition of ACh, indicating that the Ptm oscillation before activation did not result in any dilation (baseline, $P = 0.601$) despite imposing strains of $10.6 \pm 2.0\%$. Activating the airways against a static Ptm in the Post Only condition resulted in 34.0% narrowing, whereas applying Ptm oscillation during narrowing in the Pre + Post loading condition resulted in only 23.5% initial narrowing (peak constriction, $P = 0.013$). However, once tidal-like Ptm fluctuations were imposed in the Post Only condition, the luminal diameters increased to a similar level as when the Ptm oscillations were applied the entire time (steady state, $P = 0.558$). Thus applying amplified tidal Ptm fluctuations and hence strains in advance of exposure to an agonist did not protect the airways from future constriction compared with that which would have occurred without prior dynamic Ptm oscillations.

In the Post Only protocol, Ptm oscillations of 5–15 cmH2O resulted in $14.6 \pm 3.3\%$ strain per cycle, whereas 5–25 cmH2O imposed $19.4 \pm 3.4\%$ strain ($P = 0.004$). These cyclic strains resulted in 41.2 and 77.6% recovery from the peak constriction diameter for 5–15 cmH2O and 5–25 cmH2O Ptm oscillations, respectively ($P = 0.006$).

**DISCUSSION**

Over the past two decades, the dynamic mechanical environment of the lung has been a major focus in understanding health and disease (39). In particular, results from stretching isolated ASM strips (17, 21, 57) along with in vivo evidence (12, 48, 52–54) led to the hypothesis that a lack of stretch of ASM embedded inside airway walls attributable to breathing could lead to remodeling of the contractile apparatus of the ASM, resulting in AHR associated with asthma (17–19). Furthermore, whereas DIs in healthy subjects are capable of reversing airflow narrowing (bronchodilation) (14, 38, 47) and of attenuating future narrowing (bronchoprotection) (26, 37, 49, 50), these beneficial effects of a DI are diminished or absent in asthmatic subjects (13, 16, 24, 26). This latter phenomenon, however, while distinctive behavior for subjects with existing asthma, does not prove that a lack of dynamics before this led to this individual becoming asthmatic. Indeed, recent evidence from intact airway segments excised from both animal (22, 31) and human lungs (41) has surprisingly shown that Ptm fluctuations mimicking breathing before and during induced constriction do not substantively reduce the final degree of airflow narrowing. In short, there remains no evidence that a lack of dynamic fluctuations of an airway wall can transition intact airways to become hyperreactive.

Therefore, in this study, we wanted to test whether realistic breathing conditions would be effective at 1) reversing narrowing of a constricted isolated airway (bronchodilation), and/or 2) reducing the degree of narrowing compared with if the airway was held at a static Ptm before and during constriction. In particular, we narrowed isolated airways twice with ACh. In the Post Only constriction, the airway was held at a static Ptm (5 cmH2O), stimulated to narrow, and then 20 min later exposed to 5–15 cmH2O of Ptm oscillations. This protocol assessed whether Ptm oscillations imposed after a constriction takes place, but while the ACh is still present, are capable of creating significant and lasting dilation. In the Pre + Post constriction, Ptm oscillations were imposed before, during, and after activation. This assessed whether Ptm oscillations occurring in advance of and then continuing during the ACh exposure protect the airway from future constriction. Our data definitively show a lack of a bronchoprotective impact of Ptm oscillations but indicate a potential for a substantive bronchodilation impact if sufficient strains can be imposed during the oscillations.

Previous studies performed on intact airway segments were done on larger airways with luminal diameters >5 mm (22, 31). However, the majority of airway constriction in asthma likely occurs in smaller airways (<3-mm diameter) (55). Because smaller airways are generally more compliant (30, 51), the Ptm oscillations of the same size might impose a larger strain per cycle to the airway wall and the embedded ASM. Furthermore, the degree of recovery is proportional to the magnitude of strain that can be imposed on the airway wall (2, 22). Therefore, we hypothesized that Ptm oscillations might have greater bronchodilatory and bronchoprotective effects in smaller compared with larger airways. To test this hypothesis, we measured the luminal diameter of these smaller airways with and without Ptm oscillations using a state-of-the-art ultrasound system with high-frequency transducer. We compared our results from these smaller airways to the results from Protocol 1 of our previous study, where we applied a similar protocol to larger airways, which were narrowed by a similar percentage (22). Our smaller airways were more than twice as compliant than the larger airways from our previous study (22) in the tidal breathing range (5–15 cmH2O). This increased compliance translated into more strain and greater recovery from induced constriction for a given Ptm oscillation amplitude. Specifically, in our previous study (22), 5–15 cmH2O
Ptm oscillations applied after a similar level of constriction resulted in 5.58% strain and 23.4% recovery in larger airways compared with 14.6% and 41.2% in this study on smaller airways. In other words, tidal-like Ptm oscillations resulted in nearly three times more strain and twice as much recovery in smaller airways compared with previously studied larger airways (22).

Applying larger Ptm oscillations, which mimicked taking a DI with every breath (5–25 cmH2O), after constriction resulted in 19.4% strain and 77.5% reversal of constriction, compared with 8.21% strain and 48.3% recovery in previously studied larger airways (22). These strains are significantly larger than those necessary to cause a similar decrease in force in ASM strip experiments (17) although direct, quantitative comparisons between ASM strip and isolated airway experiments are difficult because the relationship between force and narrowing is complex (46). However, these values are consistent with the relationship between magnitude of strain and degree of reversal observed in human precision-cut lung slices, which were narrowed by a similar amount (33). Therefore, sufficient straining of an airway wall attributable to Ptm oscillations might play an important role in the ability to reverse narrowing, especially in smaller, more compliant airways. Because the magnitude of reversal is dependent on the amount of strain that applied to the airway wall, the increased stiffness of asthmatic airways (11) might be an important ingredient necessary for sustaining bronchoconstriction, whether induced or via natural stimuli in a sustained asthma attack. However, because straining of the airway wall was not capable of full reversal of bronchoconstriction, it is likely one factor among several that contribute to the differential response between healthy and asthmatic subjects.

To assess whether lack of breathing dynamics is a critical trajectory leading to AHR, we compared the constriction achieved when tidal-like Ptm oscillations were applied before and during induced constriction (Pre + Post) to the case when Ptm oscillations were applied only after constriction (Post Only). The Ptm oscillations before ACh were sufficient to strain the walls by 10.6% and at first appeared to reduce the initial degree of constriction by a small amount compared with constriction from the static loading condition. However, this difference was eliminated soon after Ptm oscillations were imposed to the statically constricted airway in Post Only condition. We believe that this transient difference can be explained by the increased mean Ptm against which the airway was narrowed during the Ptm oscillations (i.e., 10 cmH2O for 5–15 cmH2O vs. 5 cmH2O) rather than a bronchoprotective effect of dynamics per se at the level of the ASM. Indeed, in a previous study (31), when we applied Ptm oscillations around a mean of 5 cmH2O rather than from 5–10 cmH2O, there was no evidence of even a transient difference. Our data support the notion that the so-labeled bronchoprotective effect of a DI observed in vivo (26, 37, 49, 50) is not due to a prevention of the ASM from becoming too stiff through decreased cross-bridge cycling or reduced cytoskeleton fluidization (29). These results are consistent with our previously studied larger airways (22). Similarly, in a recent study by Pascoe et al. (45), application of two DI-like stretches before activation with ACh of an isolated ASM strip provided no distinctive protection from eventual ASM shortening compared with stimulating the ASM without prior DI.

In our previous study (22), we found a high correlation between the resultant strain imposed on an airway wall and the degree of recovery. In that study (22), we used a slightly different definition of strain. We therefore recalculated the strains based on the definition used in this paper so that direct comparisons could be made. For the larger airways studied previously (22), we found that each additional 1% of strain imposed would result in a 13% increase in recovery. However, a threshold of 2% strain was required to achieve any recovery. If this linear trend held for the smaller airways, the 14.6% strain imposed by the tidal-like Ptm oscillations would have resulted in 97% recovery. Because this strain only caused 41.2% recovery, the relationship between imposed strain and recovery may not be universal for all airways of all sizes but may be distinctive depending on the airway size and airway wall composition.

In this experiment, we were able to test airways significantly smaller and more compliant than our previous system allowed. Nevertheless, there is the potential that even smaller airways deeper into the lung are more compliant and therefore exhibit a larger bronchodilatory or bronchoprotective effect of breathing Ptm oscillations. However, in the relevant Ptm range above 5 cmH2O, the airways we studied here are likely of similar compliance as the terminal bronchioles. Figure 6 compares the normalized Ptm-diameter curves from smaller (black dashed line) and larger (black solid line) (22) bovine airways to predicted curves based on the model developed by Lambert et al. (gray) (30). Note that, whereas airways deeper in the tree become much more compliant than our smaller airways from 0–2 cmH2O, the curves converge for Ptm above 5 cmH2O. This provides evidence that Ptm oscillations applied to even smaller airways would not result in significantly more strain. Nonetheless, even smaller airways might show a differential response to Ptm oscillations if they have a different ratio of ASM to extracellular matrix (ECM) even with comparable compliances with the airways studied here. Future studies performed on smaller airways are necessary to confirm this although some evidence suggests that the strain-recovery rela-

![Fig. 6. Comparison of normalized deflation Ptm-diameter curves from our smaller (black, dashed) and larger (black, solid) (22) bovine airways to the predicted relationship by Lambert et al. (gray) (30) for all generations from 0 (trachea) to 16. In the typical breathing range above 5 cmH2O, generations 6 and above converge onto one curve and closely match the properties of the smaller airways studied here.](http://jap.physiology.org/content/japlphysiol/100/4/51.full)
tionship is similar in even smaller airways embedded within lung slices when constricted by a similar amount (33).

In this study, we chose to impose slightly amplified tidal-like breathing (i.e., $P_{tm} = 5–15 \text{cmH}_{2}\text{O}$). Given the $P_{tm}$-diameter properties of the airways we studied (see Fig. 3), this amplitude of oscillations ensured that we imposed close to the maximum potential strains achievable. Although in vivo experiments assess bronchoprotection by imposing a series of DIs immediately before a challenge (26, 37, 49, 50), we chose not to look at these artificial $P_{tm}$ conditions because people naturally only take a DI every 6 min (6) rather than continuously breathing from 5–30 cmH$_2$O. We decided not to simulate these occasional DIs because their effect is known to be transient, with the effect gone in <6 min and the majority gone within 1–2 min (7, 37, 41).

Among the plethora of studies that have explored the effect of breathing dynamics on reactivity of isolated ASM strips, it turns out that very few have examined the effect of prior oscillations on the effect of future force generation or shortening. The limited number of studies that did attempt to look at this effect found at best a minimal effect of the prior oscillations (45, 57). Taken together with previous (22, 41) intact airway studies, with the current study we can now conclude that, although breathing oscillations can partially reverse the narrowing of constricted airway, there is little evidence that periodic stretching protects airways from abnormally constricting in the first place.

Our results refute the notion that an otherwise healthy airway can transition to one that is hyperreactive in a fashion similar to asthma simply by prohibiting its dynamic wall movements associated with breathing. Consequently, we conjecture that there is something fundamentally different about asthmatic airways other than simply a transition of their ASM attributable to altered airway dynamics. Several alternative hypotheses have been proposed (35). One hypothesis states that chronic inflammatory conditions might lead to hyperresponsive airways through a phenomenon known as force adaptation (8–10). In particular, Bossé et al. (8–10) hypothesized that chronic low levels of airway inflammation can lead to sustained increased basal ASM tone, resulting in the force-adaptation process and ASM that is capable of generating increased force in response to an additional contractile stimulus. This phenomenon still needs to be tested at the intact airway level. Of course, chronic exposure to increased tension can result in remodeling of several other constituents within the walls of airways besides the ASM. Hence, it is likely that, for an airway to transition from healthy to hyperreactive, it requires a confluence of phenomena (including but not exclusively dynamics) that alter the ASM and the constituents of the ECM within which it contracts when exposed to an agonist. Because this experiment was designed to study the short-term effects of $P_{tm}$ oscillations, the consequences of airway wall remodeling could not be observed.

The change in caliber of an airway to agonist and indeed the force and stiffness of the ASM itself should be codependent on the composition and stiffness of the ECM in the airway wall (1, 23, 28, 44). Hence, mechanisms of exaggerated airway narrowing and force generation proposed at the level of ASM isolated from its native ECM may not necessarily translate to the level of the airway, let alone the intact human. Also, most experiments examining the effect of stretching on ASM force generation use tracheal ASM strips that may have distinct properties and cell phenotypes from bronchial ASM (36).

Importantly, testing hypotheses generated from isolated ASM strips or even isolated intact airways in vitro is problematic because of the plethora of confounding factors at the level of the whole organ. We have recently described and cited evidence of several explanations for how lack of periodic volume changes in healthy subjects would amplify reactivity in ways independent of ASM dynamics (35), including heterogeneity (20, 25, 34, 56), emergent atelectasis (7), and increased mean pressures with larger tidal volumes (52). Furthermore, a relatively minor change in airway caliber can potentially synergize with other factors to be significant at the whole lung level, especially because airway resistance is inversely proportional to diameter to the fourth power (45).

Following a challenge in vivo, there is clearance of spasmodgens, and therefore concentration decreases over time. In this study, airways were exposed to a constant concentration of ACh throughout our protocols. We chose to do this because very little quantitative information is known on important parameters such as clearance rate, and addition of an extra variable would make interpretation of our results more difficult. Moreover, it is not clear how long ASM agonists persist in the airways during an actual asthma attack. Interestingly, in the absence of DIs, airway resistance remains elevated for at least 30 min following a challenge, but the bronchodilatory effect of DI increases over this time, presumably because the concentration of agonist has decreased (15). Similarly, in isolated airways, the effect of $P_{tm}$ oscillations is greater when a bronchodilator is added to a constricted airway (4). Future studies are needed to explore the effect of a decreasing concentration of the effectiveness of a DI to reverse narrowing of an isolated airway.

All of the experiments presented here and our previous studies were performed on bovine airways (22, 31). Human airways have slightly different compositions and mechanical properties. In particular, the specific compliance during $P_{tm}$ oscillation from 5–10 cmH$_2$O is 0.02–0.03 cmH$_2$O$^{-1}$ in human airways (40, 41), whereas similar-sized bovine airways have a specific compliance of 0.01 cmH$_2$O$^{-1}$ (22). Nevertheless, Noble et al. (40, 41) have done similar experiments on larger-sized human airways with results similar to our bovine larger airways. It remains to be shown whether the same will happen when comparing analogous small human airways to our smaller bovine ones.

In conclusion, we showed that, compared with previously studied larger airways, the smaller airways were more compliant, and therefore $P_{tm}$ oscillations resulted in more strain and an increased recovery from constriction compared with larger, stiffer airways. By implication, then, loss of a capacity to impose strain on a constricted airway could contribute to the inability for asthmatics to partially reverse an attack. Conversely, our data do not support the notion that asthmatic airways emerge simply because of phenomena that would diminish airway wall fluctuations during breathing and a DI.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: B.C.H., H.P., and K.R.L. conception and design of research; B.C.H. performed experiments; B.C.H. analyzed data; B.C.H., H.P., and K.R.L. interpreted results of experiments; B.C.H. prepared figures; B.C.H. drafted manuscript; B.C.H., H.P., and K.R.L. edited and revised manuscript; B.C.H., H.P., and K.R.L. approved final version of manuscript.

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