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Effects of lung inflation on airway heterogeneity during histaminergic bronchoconstriction

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¹Harvard Medical School, Boston, Massachusetts; ²Department of Anesthesia, Critical Care, and Pain Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts; ³Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins University, Baltimore, Maryland; ⁴Department of Environmental Health Sciences, The Johns Hopkins University, Baltimore, Maryland; ⁵Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, Maryland; and ⁶Department of Radiology, The Johns Hopkins University, Baltimore, Maryland

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Kaczka DW, Mitzner W, Brown RH. Effects of lung inflation on airway heterogeneity during histaminergic bronchoconstriction. *J Appl Physiol* 115: 626–633, 2013. First published June 27, 2013; doi:10.1152/jappphysiol.00476.2013.—Lung inflation has been shown to dilate airways by altering the mechanical equilibrium between opposing airway and parenchymal forces. However, it is not known how heterogeneously such dilation occurs throughout the airway tree. In six anesthetized dogs, we measured the diameters of five to six central airway segments using high-resolution computed tomography, along with respiratory input impedance (Z_{rs}) during generalized aerosol histamine challenge, and local histamine challenge in which the agonist was instilled directly onto the epithelia of the imaged central airways. Airway diameters and Z_{rs} were measured at 12 and 25 cmH₂O. The Z_{rs} spectra were fitted with a model that incorporated continuous distributions of airway resistances. Airway heterogeneity was quantified using the coefficient of variation for predefined airway distribution functions. Significant reductions in average central airway diameter were observed at 12 cmH₂O for both aerosolized and local challenges, along with significant increases upon inflation to 25 cmH₂O. No significant differences were observed for the coefficient of variation of airway diameters under any condition. Significant increases in effective airway resistance as measured by Z_{rs} were observed only for the aerosolized challenge at 12 cmH₂O, which was completely reversed upon inflation. We conclude that the lung periphery may be the most dominant contributor to increases in airway resistance and tissue elastance during bronchoconstriction induced by aerosolized histamine. However, isolated constriction of only a few central airway segments may also affect tissue stiffness via interdependence with their surrounding parenchyma.

computed tomography; impedance; resistance; elastance; parameter estimation; canine; histamine

DURING BRONCHOCONSTRICTION, lung inflation is known to alter the mechanical equilibrium between opposing airway and parenchymal tissue forces. For example, deep inspirations have been shown to have variable effects on the effective level and distribution of airway caliber during pharmacologically induced bronchoconstriction (18) or spontaneous exacerbations of asthma (32, 41). These variations may reflect pathologic alterations in airway-parenchymal interdependence, or the relative importance of reversible vs. fixed airway obstruction. The extent to which pharmacologically induced bronchoconstriction mimics reactive airway disease depends on several factors,

such as the specific constricting agonist, its potency, dosage, delivery route, and locus of airway constriction (6, 37, 39). Moreover, the magnitude of the constrictor response of the airways to a specific agonist very much depends on the mechanical properties of the surrounding parenchyma. In a previous computed tomography (CT) imaging study (7), we demonstrated that increases in parenchymal stiffness during aerosolized histamine challenge may limit airway constriction under static conditions in situ. Despite such limitation, it is not clear whether increased parenchymal stiffness would lead to alterations in the magnitude or heterogeneity of the resistance pathways throughout the airway tree. We hypothesized that the locus of bronchoconstriction would affect not only estimates of effective airway resistance and its heterogeneity, but also the interdependence between opposing airway and parenchymal tissue forces.

The goals of this study were to determine 1) the effects of lung inflation on the heterogeneity of airway caliber during exogenous bronchoconstriction; and 2) how the locus of constriction affects airway and tissue interactions during lung inflation. In six dogs, we obtained high-resolution CT (HRCT) images and forced oscillatory impedance measurements at baseline and following two different types of histamine challenges. For the first type, we delivered the agonist as an inhaled aerosol, which we hypothesized would affect both central and peripheral airways. For the other, we instilled concentrated histamine locally within the lumens of five to six segmental bronchi using a microcatheter directed under bronchoscopic guidance, resulting in central airway constriction of just a few segments. To examine the effects of lung inflation on these different patterns of airway constriction, the impedance measurements were made at constant mean airway pressures of 12 and 25 cmH₂O for all conditions.

METHODS

Animal preparation and measurements. The protocol was approved by the Johns Hopkins Animal Care and Use Committee to ensure humane treatment of animals. Measurements were made in six male dogs weighing between 20 and 25 kg. Following placement of a peripheral intravenous line, each dog was anesthetized with thiopental (15 mg/kg iv induction dose followed by 10 mg·h⁻¹·kg⁻¹ maintenance infusion). Neuromuscular paralysis was achieved using an initial dose of 0.5 mg/kg succinylcholine, with supplementation as required. Each dog was orally intubated with an endotracheal tube (8.0 to 10.0 mm ID), and mechanically ventilated (Harvard Apparatus Model 600, South Natick, MA) with a rate of ~20 breaths/min and tidal volume of ~15 cc/kg, titrated to achieve an end-tidal CO₂ of 30–40 mmHg.

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Whole-lung HRCT scans were obtained for each dog in the supine position during 8-s breath-holds at 12 and 25 cmH₂O using a Sensation-16 scanner (Siemens, Iselin, NJ) in spiral mode to acquire ~300 CT images at 137 kVp and 165 mA. Forced oscillatory measurements of respiratory input impedance (Z_{rs}) were obtained immediately following each scan at the same mean airway pressure using a custom-built servo-controlled pneumatic pressure oscillator (23) whose input driving signal consisted of nine superimposed sinusoids from 0.078 to 8.9 Hz, with amplitude adjusted to yield peak-to-peak pressures of 1–2 cmH₂O (18). Each Z_{rs} measurement lasted ~2 min. The resulting tracheal pressure waveform was measured with a variable reluctance transducer (0–50 cmH₂O; Celesco LCVR, Canoga Park, CA) connected to a small polyethylene catheter inserted into the endotracheal tube and allowed to extend ~2 cm into the trachea (36). Airway flow was obtained with a screen pneumotachograph (4700A; Hans Rudolph, Kansas City, MO) coupled to another pressure transducer (0–2 cmH₂O; Celesco LCVR). Both pressure and flow signals were low-pass filtered at 10 Hz using an 8-pole Butterworth filter (858L8B-2; Frequency Devices, Haverhill, MA) and sampled at 40 Hz by an analog-to-digital converter (DT-2811; Data Translations, Marlborough, MA) for subsequent processing. Phase distortions between A/D channels resulting from multiplexer delays were corrected in real time using a third-order Lagrange polynomial interpolation technique (3).

Protocol. The details of the experimental protocol are outlined in our previous study (7). In brief, each dog served as its own control and was studied under four different conditions on two separate days at least 2 wk apart. On *day 1*, measurements were obtained under baseline conditions and following an aerosolized histamine challenge (200 mg/ml; Sigma Chemical, St Louis MO) using a Hudson 3000 nebulizer (Hudson, Temecula, CA) as described previously (7). For each dog ~1 ml of solution was delivered, with median particle size of 3.1 μ m and geometric SD of 3.2. Three minutes later, HRCT scans were acquired during sustained breath-holds at 12 and 25 cmH₂O, followed immediately by measurements of Z_{rs} at the same corresponding mean airway pressures. The dogs were conventionally ventilated between each HRCT scan and forced oscillation sequence.

On *day 2*, each dog received an iv bolus of atropine (0.2 mg/kg) to achieve complete airway relaxation, and HRCT scans and Z_{rs} measurements were acquired 10 min later. Next, local atomization of histamine was delivered directly to the epithelia of five to six segmental bronchi using a microcatheter inserted under bronchoscopic guidance (7, 9). The catheter had six 0.15-mm side holes drilled circumferentially 1 mm from its distal tip, and rapid bolus injections of concentrated histamine (200 mg/ml, 20 μ l per airway; Sigma Chemical, St Louis MO) sprayed radially on the adjacent airway wall with minimal axial spread. Three minutes later, the HRCT scans and Z_{rs} measurements were obtained.

Image processing. The CT images were reconstructed using a high-spatial-frequency algorithm with 1-mm slice thickness and a 512 $\times$ 512 matrix using a 230-mm field of view at a level of -450 Hounsfield units (HU) and width of 1,350 HU. In each dog, the five to six airway segments constricted during the local histamine challenge on *day 2* were identified for each of the four experimental conditions in approximately the same transverse cross-section using adjacent anatomic landmarks such as airway or vascular branching points (7). The internal diameters (D_n) of these segments were estimated using the airway analysis module of the Pulmonary Workstation 2.0 software package (VIDA Diagnostics, Coralville, IA) by segmenting an initial isocontour within each lumen, and then automatically locating the corresponding airway perimeter using an edge-detection algorithm (43, 45). From the estimated airway luminal area (A), an effective diameter was approximated as $D = 2(A/\pi)^{1/2}$. For each dog and condition, we determined the average diameter of the N identified central airway segments as:

$$\bar{D} = \frac{1}{N} \sum_{n=1}^N D_n \quad (1)$$

along with the coefficient of variation for the airway diameters:

$$CV_D = \frac{1}{\bar{D}} \sqrt{\sum_{n=1}^N (D_n - \bar{D})^2} \quad (2)$$

Total lung volume was determined by segmentation of the parenchyma from the mediastinum and chest wall using a semiautomated process (17) and summing the volume of individual voxel elements contained within the segmented regions (19) using the PASS software package (The University of Iowa, Iowa City, IA).

Signal processing and inverse model analysis. Respiratory impedance Z_{rs} as a function of angular frequency ω was computed from the sampled oscillatory pressure and flow waveforms using a Welch overlap-average periodogram technique with a 25.6-s rectangular window and 80% overlap (22). After neglecting the first 500 points in the data record (~12.5 s) to minimize the influence of transient responses, between 12 and 20 overlapping windows were used to calculate Z_{rs} for each animal. The resistive and reactive spectral components were determined from real and imaginary parts of each Z_{rs} , respectively. Corresponding coherence values (γ^2) were determined at each discrete frequency using appropriate auto- and cross-power spectra (33). We found that $\gamma^2 \geq 0.95$ for all frequencies, suggesting minimal influence of cardiogenic artifacts on our estimates of Z_{rs} . The mechanical properties of the respiratory system were assessed by fitting a viscoelastic distributed airways model to each Z_{rs} spectrum (18, 44), with predicted impedance $\hat{Z}_{rs}$ given by:

$$\hat{Z}_{rs}(\omega) = \left(\int_{R_{\min}}^{R_{\max}} \frac{P(R)}{R + j\omega I + \frac{G - jH}{\omega^\alpha}} dR \right)^{-1} \quad (3)$$

where R is an approximation of airway resistance, which is assumed to vary across a parallel airway tree according to the continuous probability density function, $P(R)$. The integral limits, $R_{\min}$ and $R_{\max}$, denote the lower and upper bounds of $P(R)$, respectively, which was defined a priori according to hyperbolic, uniform, or linear distributions of parallel airway resistances (18, 44). The I parameter predominantly represents central airway gas inertia, whereas G denotes tissue damping, H denotes tissue elastance, $\alpha = (2/\pi)\tan^{-1}(H/G)$, and j = square root (-1). Tissue hysteresivity is approximated as $\eta = G/H$ (13, 16). Thus for a specified $P(R)$, this model will have five independent parameters: $R_{\min}$, $R_{\max}$, I , G , and H . These parameters were estimated using a nonlinear gradient search technique (Matlab v7.0; Mathworks, Natick, MA) that minimized the performance index (15):

$$\Phi = \sum_{k=1}^K [Z_{rs}(\omega_k) - \hat{Z}_{rs}(\omega_k)]^2 \quad (4)$$

where K is the number of discrete frequencies used in pressure excitation waveform. For each Z_{rs} spectrum, we performed three separate simultaneous estimations (for the hyperbolic, uniform, and linear distributions of R) of the five parameters of Equation 3. The most appropriate $P(R)$ for a given Z_{rs} spectrum was established on the basis of minimum Φ (18). For this $P(R)$, an effective airway resistance was determined from its mean value:

$$\bar{R} = \frac{\int_{R_{\min}}^{R_{\max}} RP(R)dR}{\int_{R_{\min}}^{R_{\max}} P(R)dR} \quad (5)$$

whereas the heterogeneity of airway resistances was determined from its coefficient of variation:

$$CV_R = \frac{1}{\bar{R}} \sqrt{\int_{R_{\min}}^{R_{\max}} (R - \bar{R})^2 P(R)dR} \quad (6)$$

Statistical analysis. For each of the four conditions, a one-way ANOVA was used to compare the model parameters G , H , and η from the best form of $P(R)$, and the derived parameters $\bar{R}$ and CV_R (SAS v8.2; SAS Institute, Cary, NC). ANOVA was also used to compare the airway diameters and segmented lung volumes as measured from HRCT. If significance was obtained with ANOVA, post hoc analysis was performed using Tukey's HSD criterion. For each condition, comparisons of all variables were made at 12 and 25 cmH₂O using two-tailed paired t -tests. $P < 0.05$ was considered statistically significant. The percentage difference (Δ) for all variables was determined as the difference between their corresponding values at 12 and 25 cmH₂O multiplied by 100, divided by their average values at the two inflation pressures.

RESULTS

Figure 1 summarizes the mean diameters ($\bar{D}$) of the five to six central airway segments identified from HRCT (Equation 1) and the coefficient of variation for these diameters (CV_D , Equation 2) averaged across all dogs. Data are shown left to right at baseline condition, following aerosolized, then local histamine challenges, and finally, after intravenous atropine. Compared with the baseline and atropinized conditions, significant reductions were observed in the mean diameter of the central airway segments in both the aerosolized and local challenges at each airway pressure. However, significant increases in mean diameter with inflation from 12 to 25 cmH₂O were observed only in the aerosolized and localized challenges. No significant dependencies in the corresponding coefficients of variation were observed with any condition or airway

pressure using ANOVA. The greatest percent difference for $\bar{D}$ between 12 and 25 cmH₂O occurred in the localized condition, with minimal differences occurring in the baseline or atropinized conditions.

Total lung volumes obtained from segmentation of the imaged parenchyma from the chest wall and mediastinum are summarized in Fig. 2. Similar to the patterns observed in the mean airway diameters in Fig. 1, lung volume was significantly reduced in the aerosolized and localized challenges at each airway pressure compared with baseline and following atropine. Lung volumes also significantly increased with inflation from 12 to 25 cmH₂O under all conditions. The lowest percent difference for lung volumes between 12 and 25 cmH₂O occurred in the aerosolized condition ($P < 0.05$).

Figure 3 summarizes the model parameter values $\bar{R}$, CV_R , I , G , H , and η at baseline, following aerosolized and local histamine challenges, and after atropine. Corresponding percent differences for each parameter between 12 and 25 cmH₂O are also shown. At 12 cmH₂O, the greatest increase in effective airway resistance, $\bar{R}$, occurred during the aerosol histamine challenge. With local central airway constriction, only minor increases in $\bar{R}$ compared with baseline were observed. Inflation to a mean airway pressure of 25 cmH₂O tended to decrease $\bar{R}$ under all conditions, although significant reductions in $\bar{R}$ were achieved only in the aerosolized condition. Whereas the airway heterogeneity index CV_R tended to be higher during the aerosolized histamine condition compared with the other conditions at 12 cmH₂O, this was not statistically significant. The CV_R

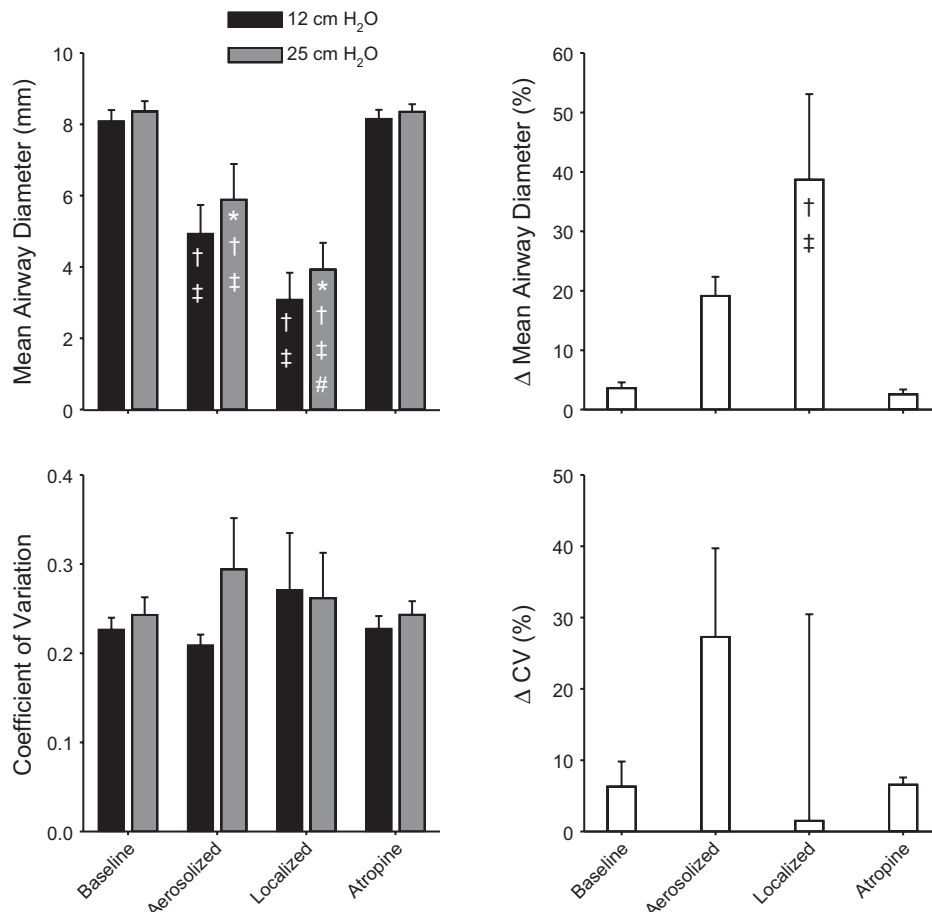


Fig. 1. Summary of the average of five to six central airway diameters (Equation 1) and corresponding coefficients of variation (Equation 2) obtained from HRCT scans during 8-s breath-holds at 12 (black) and 25 (gray) cmH₂O airway pressure. Data are averaged across the six dogs, with error bars denoting SEM. *Significantly higher compared with 12 cmH₂O at same condition; †significantly different compared with baseline condition; ‡significantly different compared with atropine condition; #significantly lower than aerosolized condition. For all comparisons, $P < 0.05$.

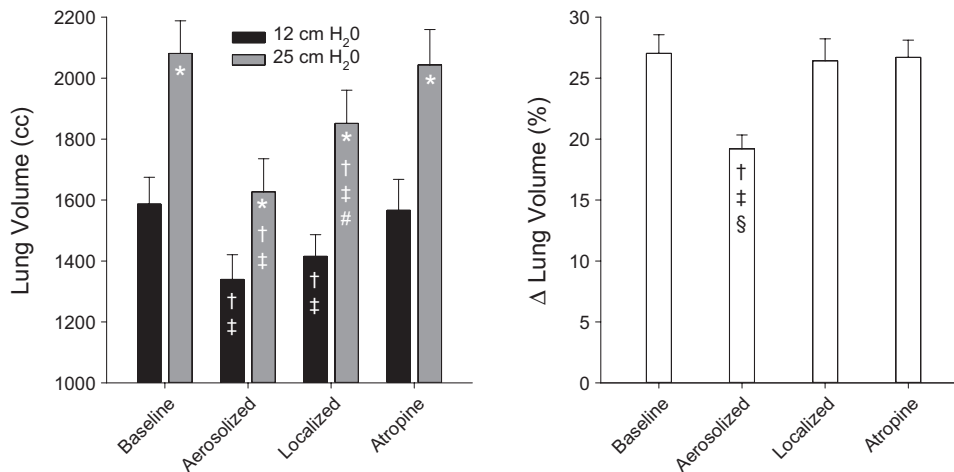


Fig. 2. Lung volumes obtained from segmented CT images for the four different experimental conditions during 8-s breath-holds at 12 (black) and 25 (gray) cmH₂O airway pressure, along with corresponding percent differences between 12 and 25 cmH₂O. Data are averaged across six dogs, with error bars denoting SEM. *Significantly higher compared with 12 cmH₂O at same condition; †significantly different compared with baseline condition; ‡significantly different compared with atropine condition; #significantly different compared with aerosolized condition; §significantly different compared with localized condition. For all comparisons, $P < 0.05$.

was slightly reduced in all conditions with inflation from 12 to 25 cmH₂O, but this reduction also did not achieve significance. The airway inertance parameter, I , in the aerosolized condition was significantly higher compared with the localized and atropinized conditions at both 12 and 25 cmH₂O. In the aerosolized condition, the tissue parameters G , H , and η were significantly higher than their corresponding baseline and atropinized values at both 12 and 25 cmH₂O. However, in the localized condition, only η was significantly higher than its baseline and atropinized values. Significant increases in H and decreases in η were observed with inflation from 12 to 25 cmH₂O in all four conditions, whereas G significantly increased only in the aerosolized and localized conditions. Significant percent differences between 12 and 25 cm were observed only in $\bar{R}$ and I during the aerosolized condition.

Figure 4 shows the association between tissue compliance (i.e., $1/H$) as measured using forced oscillations, and the segmented lung volume as measured with HRCT. Data are shown for all individual dogs under all conditions. These data were clustered into two groups corresponding to airway pressures of 12 and 25 cmH₂O. We observed statistically significant correlations between compliance and lung volume in each group.

DISCUSSION

In this study we have demonstrated that the locus of airway constriction has influence on the magnitude and distribution of airway resistances during histaminergic bronchoconstriction. We used HRCT to assess central airway diameters during localized and generalized aerosol histamine challenges. We also determined the parallel distribution of airway resistances using forced oscillatory measurement of respiratory impedance and inverse modeling. Although increased lung tissue stiffness may result in an attenuated response to bronchoconstriction by augmenting airway transmural pressure (7), such alterations of airway tethering forces do not necessarily result in decreased airway or tissue resistances. Indeed, our HRCT data indicate that for our selected doses of histamine, direct local challenges to the central airways resulted in slightly greater reductions in mean diameter compared with the generalized aerosol challenge (Fig. 1). However, on the basis of impedance data, only the generalized aerosol histamine challenge resulted in a significant increase in effective airway resistance, $\bar{R}$ (Fig. 3).

Assuming that the aerosol challenge resulted in both central and peripheral airway constriction (5), these results indicate that a substantial portion of the increased $\bar{R}$ was due to constriction of peripheral airways. Moreover, the increase in $\bar{R}$ with generalized aerosol histamine challenge to the whole lung could be reversed with inflation of the lungs to 25 cmH₂O (Fig. 3), consistent with parenchymal tethering forces pulling open these constricted peripheral airways. In addition, the greatest percent difference in $\bar{R}$ between 12 and 25 cmH₂O occurred with the aerosol challenge.

Histamine is known to cause bronchoconstriction by direct action on airway smooth muscle, or via reflex vagal discharge following stimulation of irritant receptors (40). Generally, the response to any aerosolized bronchoconstricting agents is heterogeneous throughout the airway tree (11, 18, 32), which may reflect the variability in agonist delivery to the lung periphery, or the pharmacologic responsiveness of airway smooth muscle. In a previous study using HRCT, Brown et al. (6) demonstrated minimal differences in the heterogeneity of constriction in airway segments ranging from about 1 to 10 mm in diameter, whether induced by aerosolized or intravenous histamine. They concluded that the increased heterogeneity of constriction following histamine exposure is predominated by local pharmacologic responses in airway smooth muscle. Whereas our imaging data are also consistent with these findings, our impedance measurements would indicate slightly increased airway heterogeneity with aerosolized histamine exposure, possibly occurring in airways smaller than 1 mm in diameter and below the resolution of HRCT. This is consistent with the aerosolized histamine reaching the most peripheral regions of the lung, resulting in more widespread constriction throughout the airway tree. However, the deposition of the aerosol may have been less uniform in these most peripheral regions, due to variation in the path lengths from trachea to alveoli. This may have contributed to the apparent increase in airway heterogeneity as detected from Z_{rs} .

Our direct localized histamine challenge involved only five to six airway segments per dog, and resulted in minimal changes in $\bar{R}$ or CV_R (Fig. 3). Nonetheless, both the tissue damping (G) and elastance (H) parameters trended higher for this direct localized challenge compared with baseline, but a value of $P \leq 0.05$ was not achieved. The significant reductions in effective lung volume at 12 and 25 cmH₂O were also consistent with increased lung tissue stiffness for this chal-

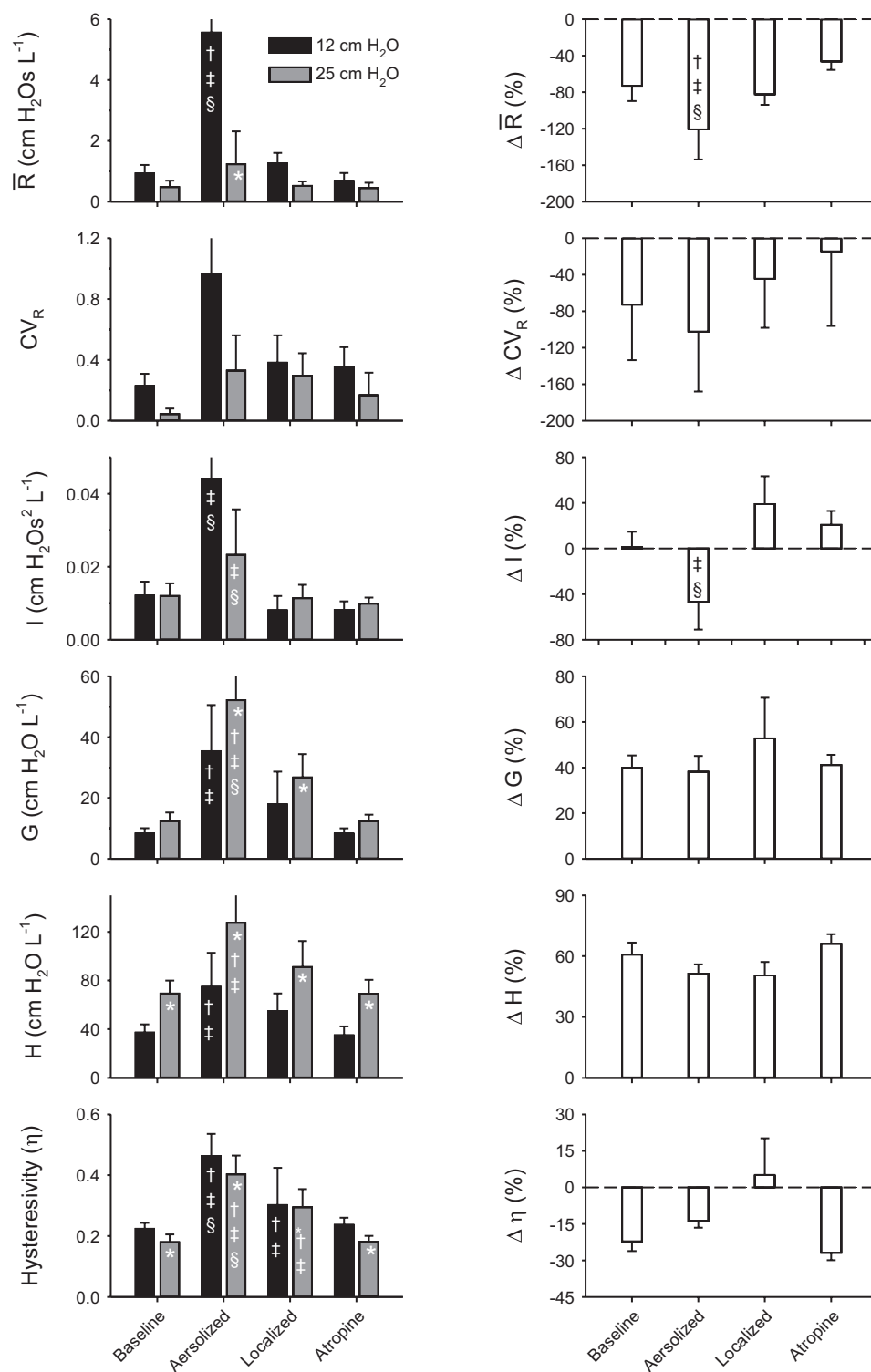


Fig. 3. *Left*: model parameters of effective airway resistance ($\bar{R}$) and its coefficient of variation (CV_R), central airway inertia (I), tissue damping (G), tissue elastance (H), and tissue hysteresivity (η) for the four different experimental conditions obtained using forced oscillations at mean airway pressures of 12 (black) and 25 (gray) cmH₂O. *Right*: corresponding percent differences (Δ) between 12 and 25 cmH₂O. Data are averaged across the six dogs, with error bars denoting SEM. *Significantly different compared with 12 cmH₂O at same condition using two-tailed paired t -test; †significantly different compared with baseline condition; ‡significantly different compared with atropine condition; §significantly different compared with localized condition. For all comparisons, $P < 0.05$.

lunge. Remarkably, this isolated constriction of only five to six central airway segments was sufficient to reduce the effective lung volume at 12 cmH₂O to a similar magnitude to that obtained during aerosolized constriction (Fig. 2). This implies that these segments possess a considerable degree of interdependence with their surrounding parenchyma (35). In addition, tissue hysteresivity (η) achieved significantly higher values for the local challenge compared with the baseline and atropinized

conditions at both 12 and 25 cmH₂O. The slight increase in mean central diameter with mean airway pressure as measured with HRCT in these segments is also consistent with previous studies by Brown and co-workers (7, 8) who also demonstrated similar effects of lung inflation on airway diameters.

The greatest reductions in lung volume at 12 and 25 cmH₂O, or the corresponding percent difference in volume between these pressures, occurred in the generalized aerosol histamine

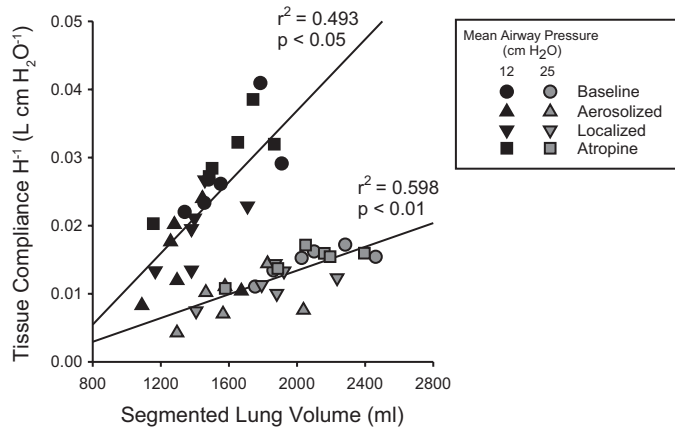


Fig. 4. Correlations between tissue compliance (i.e., $1/H$) as measured with forced oscillations, and the segmented lung volume as measured with HRCT. Data are shown for all individual dogs under all conditions for mean airway pressure of 12 (black symbols) and 25 cmH₂O (gray symbols). Lines denote corresponding linear regressions at 12 and 25 cmH₂O.

challenge (Fig. 2). These decreases were similar to those reported by Loring and co-workers (29, 30), and are consistent with increases in tissue elastance (H) that accompany bronchoconstriction (7, 32). For each specified inflation pressure, we also observed significant correlations between segmented lung volume and Z_{rs} -based measures of tissue compliance (i.e., the reciprocal of elastance, $1/H$). Such correlations are reflective of the general relationship between the size of the lung and its mechanical properties (Fig. 4). As expected, lung inflation from 12 to 25 cmH₂O mean airway pressure significantly increased H for all conditions and decreased the slope of the correlation between lung volume and compliance. Such behavior is consistent with parenchymal strain stiffening during lung inflation (19, 26).

In a previous study, Mitzner et al. (35) presumed that increases in tissue elastance during bronchoconstriction were reflective of interdependent tethering between airways and parenchyma. They further speculated that increases in lung tissue resistance during bronchoconstriction may reflect the physical properties of the central conducting airways. Similarly, we observed significant increases in tissue damping, G , during the generalized aerosol histamine challenge, although such increases were minimal for the direct localized challenge. Histamine is known to directly influence lung tissue viscosity and stiffness, perhaps through modulation of cross-bridge cycling of various contractile elements in the parenchyma (10). The increases we observed in tissue hysteresivity, η , in both the local and generalized aerosol challenges may reflect relative increases in energy dissipation in the lung tissues with respect to energy storage during bronchoconstriction. Nonetheless, mechanical heterogeneity of the airways and tissues may also influence η (4, 18, 20, 21). This may explain why the greatest increases in η occurred during the generalized aerosol challenge, for which airway resistance heterogeneity tended to be highest. Decreases in η with lung inflation have also been noted to occur over similar pressure ranges in dogs and other species (14, 26, 38, 42), and may reflect decreases in airway or lung tissue heterogeneity (4, 18, 20). To the extent that our distributed airways model can account for parallel heterogeneity in the lungs (18, 44), our data suggest that histaminergic

exposure alters the coupling between energy dissipation and storage in the parenchyma or chest wall (2, 13, 26, 31).

The airway heterogeneity index, CV_R , at 12 cmH₂O suggests that airway heterogeneity was maximized during the aerosolized condition. At 25 cmH₂O, we may speculate that slight reductions in such heterogeneity were observed for all conditions, as indicated by the tendency for CV_R to decrease with lung inflation, although this was not statistically significant. This suggests that parenchymal tethering forces make the width of the resistance distribution more narrow, or equivalently, make the lungs more mechanically homogenous. Fredberg et al. (11) also used forced oscillations from 2 to 60 Hz to detect increases in airway heterogeneity following aerosolized histamine in dogs. They concluded that the frequency-dependent changes in impedance above 2 Hz were consistent with widespread peripheral airway constriction and the shunting of oscillatory flow into the central airway walls (21, 32, 34). Our data were not entirely consistent with this behavior because the frequency dependence we observed in our impedance spectra could largely be accounted for by tissue viscoelasticity and parallel airway heterogeneity. This discrepancy may be due to the frequency range of our impedance spectra being much lower than that of Fredberg et al. (0.078–8.9 Hz vs. 2–60 Hz), and that we used a much higher concentration of aerosolized histamine (30 mg/ml vs. 200 mg/ml).

Compared with the baseline condition, tissue hysteresivity significantly increased for both the aerosolized and localized challenges. Such increases are consistent with increased parallel time constant heterogeneity for which the model of Equation 3 does not entirely account (18, 25, 44). As noted above, we cannot rule out that either the generalized aerosol challenge or the direct local challenge directly altered the parenchymal coupling between energy dissipation and storage (13, 26). For example, most constricting agonists affect the contractile state of both airway smooth muscle (12, 40) and the parenchyma (10, 27, 47). Nonetheless, increases in apparent η and G may also result from enhanced interregional flow during bronchoconstriction (4, 31).

Finally, atropine is known to result in airway relaxation via blockade of cholinergic receptors in airway smooth muscle, and neither our imaging nor impedance data were sensitive enough to detect significant differences in airway caliber or tissue properties between the baseline and atropinized conditions. This is consistent with there being minimal existing baseline cholinergic tone in airway smooth muscle or the lung parenchyma (7).

It is important to realize that our imaging and impedance measurements were obtained over near-static conditions, and thus may not reflect actual obstructive patterns that may emerge during tidal excursions (24, 28). Recent modeling studies suggest that during breathing, intraparenchymal airways self-segregate into two distinct populations due to instabilities in the pressure-volume relationship of their subtending acini: one with largely opened airways, and the other with nearly closed ones (1, 46). Whereas such behavior may certainly be observed during physiologic tidal volumes and distending pressures, it is not clear whether it would be expected during small-amplitude pressure oscillations at constant mean airway pressures. Thus our model of Equation 3, which assumes a continuous distribution of parallel airway resistances,

may not accurately reflect such a binary pattern of bronchoconstriction.

To investigate this issue further, we refitted all of our impedance data to an alternative model consisting of only two parallel branches. Each branch consisted of a unique airway resistance parameter, but were subtended by identical inertance parameters and constant-phase tissue compartments (22). We hypothesized that such a topology might be more appropriate to describe a heterogeneous pattern of highly constricted vs. fully opened airway segments (46). However, we found that the application of this two-pathway model to our Z_{rs} data resulted in no statistical improvement in the quality of fit for the vast majority of the dogs (25), and frequently yielded nonphysical (i.e., negative) parameter values. Thus at least during small-amplitude forced oscillations at constant mean airway pressure, our modeling analysis would suggest that airway resistance values can be largely approximated as a continuum, rather than being polarized into two distinct groups.

Conclusions. In summary, we have demonstrated that lung inflation leads to decreases in both average level of airway constriction and a slight narrowing of resistance distribution. Changes in effective airway resistance and its heterogeneity depend on the locus of constriction, and these changes may be the result of central vs. peripheral differences in airway-parenchymal interdependence. Changes in the effective airway resistance, airway heterogeneity, or tissue elastance are also sensitive to the locus of airway constriction. Although these data indicate that the lung periphery may be the dominant contributor to increases in airway resistance and tissue elastance during bronchoconstriction induced by aerosolized histamine, isolated constriction of a few central airway segments may also affect tissue stiffness via interdependence with their surrounding parenchyma.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: D.W.K., W.M., and R.H.B. conception and design of research; D.W.K. and R.H.B. performed experiments; D.W.K. analyzed data; D.W.K., W.M., and R.H.B. interpreted results of experiments; D.W.K. prepared figures; D.W.K. drafted manuscript; D.W.K., W.M., and R.H.B. edited and revised manuscript; D.W.K., W.M., and R.H.B. approved final version of manuscript.

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