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ORIGINAL RESEARCH

# Individual Canine Airway Response Variability to a Deep Inspiration

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**Abstract:** In healthy individuals, a DI can reverse (bronchodilation) or prevent (bronchoprotection) induced airway constriction. For individuals with asthma or COPD, these effects may be attenuated or absent. Previous work showed that the size and duration of a DI affected the subsequent response of the airways. Also, increased airway tone lead to increased airway size variability. The present study examined how a DI affected the temporal variability in individual airway baseline size and after methacholine challenge in dogs using High-Resolution Computed Tomography. Dogs were anesthetized and ventilated, and on 4 separate days, HRCT scans were acquired before and after a DI at baseline and during a continuous intravenous infusion of methacholine (Mch) at 3 dose rates (17, 67, and 200 µg/min). The Coefficient of Variation was used as an index of temporal variability in airway size.

We found that at baseline and the lowest dose of Mch, variability decreased immediately and 5 minutes after the DI ($P < 0.0001$). In contrast, with higher doses of Mch, the DI caused a variable response. At a rate of 67 µg/min of Mch, the temporal variability increased after 5 minutes, while at a rate of 200 µg/min of Mch, the temporal variability increased immediately after the DI. Increased airway temporal variability has been shown to be associated with asthma. Although the mechanisms underlying this temporal variability are poorly understood, the beneficial effects of a DI to decrease airway temporal variability was eliminated when airway tone was increased. If this effect is absent in asthmatics, this may suggest a possible mechanism for the loss of bronchoprotective and bronchodilatory effects after a DI in asthma.

**Keywords:** airway responsiveness, airway smooth muscle, asthma, deep inspiration, heterogeneity, vagal tone

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## Introduction

A sigh or deep inspiration (DI) causes transient distension and relaxation of the airways. For healthy individuals, a DI can reverse an induced airway constriction (bronchodilation) or prevent a subsequently induced constriction (bronchoprotection).[1,2] However, for individuals with asthma or chronic obstructive pulmonary disease (COPD), these effects may be attenuated or absent.[1,3–11] Underlying reasons for these differences in lung disease remain to be determined.

A deep inspiratory maneuver is also commonly performed to standardize lung volume history prior to measuring lung function. One presumption of this maneuver is that all the airways are distended with the stretch and then return to some stable size based on the balance between the level of tone and inward recoil of the airways with the outward recoil of the parenchyma. What is not known, however, is how uniform is this resultant level of tone. That is, how much heterogeneity exists in the response of individual airways after such a DI.

Although it is well accepted that all airways have some degree of baseline tone,[12] the extent of this tone and thus the airway size changes over some duration of time, but the cause and the interval for these changes are generally not known. Airway narrowing has been shown to be heterogeneous in the airways of asthmatics and normals, being greater in asthmatics.[13] In previous work from our group, we showed that the size of individual airways at baseline in dogs, prior to the administration of any spasmogen, varied widely in the same animals on different days over weeks and months.[14] More recently, we extended these observations to show that with Methacholine (Mch)-induced increases in airway tone, this airway size temporal variability increased even further.[15]

Although we know that the mean airway response to a deep inspiration can vary depending on the size and the duration of the deep inspiration,[16,17] the spatial heterogeneity among individual airways following a DI has not been investigated. Similarly, little is known about the temporal reproducibility of airway responses to a DI on repeated occasions. Therefore, the current study examined how the size of individual canine airways responded to a deep inspiration at baseline and during methacholine (Mch) challenges on 4 different experimental days using High Resolution Computed Tomography (HRCT) to measure airway size.

## Material and Methods

The study protocol was approved by The Johns Hopkins Animal Care and Use Committee, Protocol # DO08H03. All handling of the animals from anesthesia to recovery were done in strict accordance with the guidelines presented in both the Public Health Service Policy on Humane Care and Use of Laboratory Animals (Office of Laboratory Animal Welfare, National Institutes of Health, Bethesda, MD) and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, Washington, DC). Five dogs weighing approximately 20 kg were anesthetized with pharmaceutical grade thiopental sodium (15 mg/kg induction dose followed by 10 mg/kg/hr intravenous maintenance dose). Following endotracheal intubation with an 8.0 mm ID endotracheal tube, the dogs were placed supine and their lungs were ventilated with room air using a volume-cycled ventilator (Harvard Apparatus, Millus, MA) at a tidal volume of 15 ml/kg and a rate of 18 breaths/minute. A stable depth of anesthesia was monitored by lash reflex, heart rate, and respiration, and airway pressure and end tidal $CO_2$ were measured and used to assess the adequacy of ventilation. After induction of anesthesia, during imaging the dogs were paralyzed with 0.5 mg/kg of succinylcholine to ensure no respiratory motion. Following the experimental procedures all animals were kept under direct observation until they were breathing normally. The investigators and/or a trained technician remained as long as necessary to ensure that no animal received less than adequate monitoring until they had fully recovered from the anesthesia and exhibited normal behavior.

### Protocol

Each dog served as its own control. The dogs were anesthetized and ventilated as described above. On 4 separate days randomly varying between 1 and 8 weeks apart, baseline HRCT scans were acquired (see below), and the dogs then received a continuous intravenous infusion of methacholine at 3 rates in increasing order (17, 67, and 200 µg/min; Sigma Chemical, St Louis MO), the middle dose, 67 µg/min, was previously demonstrated to decrease the size of the airways to approximately 60% of baseline.[18] In addition, the tubing from the ventilator to the endotracheal tube of the animals had an added large bore Y-connector. One branch of the Y went to the ventilator, and the other branch was connected to a constant pressure source set at 25 $cmH_2O$. This source consisted of an underwater overflow fed by a line from a high flow oxygen supply. At the start of scanning, the ventilator was simultaneously shut off, a solenoid valve to the ventilator was closed, and another solenoid to the pressure source was opened to the dog for a set amount of time (10 seconds). Then solenoids were switched to suddenly expose the trachea to atmospheric pressure and the scans were acquired. Scanning was performed immediately after the DI (approximately 4 seconds) and 5 min after the DI. The dogs were ventilated normally between the scan acquisitions. After completing the DI protocol during the final dose of Mch, intravenous atropine (0.2 mg/kg) was administered, a dose previously shown to completely block vagal tone in the dog.[19] To standardize lung volume history, approximately 10 minutes prior to the first scan series, the airway pressure was increased to 45 $cmH_2O$, held for 5 seconds and then released and the animals were ventilated normally. At each dose and after atropine, HRCT scans were acquired to measure airway areas and lung volumes.

## Imaging and analysis of airways

HRCT scans were obtained with a Sensation-16 scanner (Siemens, Iselin, NJ) using a spiral mode to acquire approximately 300 CT images during an 8 second breath hold (apnea) at 137 kVp, and 165 mA. The images were reconstructed as 1 mm slice thickness and a 512 × 512 matrix using a 175 mm field of view and a high spatial frequency (resolution) algorithm that enhanced edge detection, at a window level of –450 Hounsfield units (HU) and a window width of 1,350 HU. These settings have been shown to provide accurate measurement of luminal size as small as 0.5 mm in diameter.[20,21] For repeated airway measurements in a given dog within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined and used to measure the airway size at the same anatomic cross sections.

The HRCT images were analyzed using the airway analysis module of the Volumetric Image and Display Analysis (VIDA) image analysis software package (Dept. of Radiology, Division of Physiologic Imaging, Univ. of Iowa, Iowa City, IA) as previously described and validated.[19,22] The HRCT images were transferred to a UNIX-based Sun workstation. An initial isocontour was drawn within each airway lumen, and the software program then automatically located the perimeter of the airway lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that defines the luminal edge of the airway wall. Intra- and inter-observer accuracy and variability of the software program using this HRCT technique in phantoms, consisting of rigid tubes to measure known areas, has been previously shown by us[20] and by others[22] to be highly resistant to operator bias. After segmentation of the parenchyma from the chest wall and mediastinum using a semi-automated process, lung volume was calculated in four of the dogs by summing the volume of individual voxel elements contained within the region of interest using the validated[23–25] Pulmonary Workstation 2 software (VIDA diagnostics, Iowa City, IA). The software calculated the tissue/vascular volume and the air volume separately.

## Data Analysis

First, for each airway on each day we calculated the airway area post-DI as a percent the area pre-DI (%DI = the airway area after the DI divided by the airway area before the DI times 100). The %DI was calculated at two time points following the DI: 1) immediately, T0 (technically at 4 sec after returning airway pressure to atmospheric); 2) five min after the DI, T5. To assess airway temporal variability, the coefficient of variation (CV) of the four airway luminal measurements of each airway at baseline and at each dose of methacholine was calculated (the standard deviation of the four airway measurements divided by the mean of the four airway measurements times 100). Data were analyzed by paired t-test and by one-way ANOVA where appropriate and with correction for multiple comparisons, and multiple regression analysis where appropriate (JMP release 7.0.1, SAS Institute, North Carolina). Significance was considered if the $P$-value was $<0.05$.

## Results

A total of 312 airways for 5 dogs were matched and measured with the number of airways measured per dog ranging from 44 to 73. Airway size ranged from

2.3 to 21 mm in diameter. For all dogs the mean relative airway size pre-DI, defined as the percentage of maximum area of the airway after atropine prior to the DI maneuver, was 87% ± 5% (mean ± SD), 74% ± 9%, 65% ± 12%, and 56% ± 10% at baseline and during the Mch infusion of 17, 67, 200 μg/min respectively, ($P < 0.0001$ for all pairwise comparisons). Air volumes in the lungs at FRC pre-DI, as a percentage air volume at FRC after complete relaxation of the airways with atropine, were 93% ± 6%, 91% ± 6%, 90% ± 5%, and 91% ± 5% at baseline and during the Mch infusion of 17, 67, 200 μg/min respectively, ($P = 0.33$ for all pairwise comparisons).

Immediately after the release of the DI (T0), the mean airway size was increased compared to its pre-DI size. The airway size as a percent of pre-DI sizes were 103% ± 4% (mean ± SD), 108% ± 8%, 111% ± 12%, and 114% ± 12% for the baseline and during the Mch infusion of 17, 67, 200 μg/min respectively ($P < 0.0001$ compared to pre-DI). Immediately after the DI (T0), there were no significant changes in the mean air volume in the lungs compared to its pre-DI size ($P > 0.05$). The mean air volume in the lungs as a percentage of maximum air volume were 94% ± 6%, 93% ± 6%, 92% ± 6%, and 92% ± 6% for the baseline and during the Mch infusion of 17, 67, 200 μg/min respectively.

Five minutes after the release of the DI (T5), the mean airway size remained slightly increased compared to its pre-DI size. The mean airway size as a percent of pre-DI sizes were 104% ± 15% (mean ± SD), 107% ± 7%, 104% ± 6%, and 104% ± 7% for the baseline and during the Mch infusion of 17, 67, 200 μg/min respectively $P < 0.0001$ compared to pre-DI). Five minutes after the release of the DI (T5), there were still no significant changes in the mean air volume in the lungs compared to its pre-DI size ($P > 0.05$). The mean air volume in the lungs as a percentage of maximum air volume were 94% ± 6%, 93% ± 6%, 92% ± 6%, and 92% ± 6% for the baseline and during the Mch infusion of 17, 67, 200 μg/min respectively.

To examine the extent of temporal variability after a DI over different days, we calculated the CV for the change in airway size for each airway in each dog across days for each dose of Mch, again calculated as the standard deviation of the airway divided by the mean of the airway measurements times 100. At baseline, the CV pre-DI for the change in airway size for each airway ranged from 0% to 27.7% with a mean of 10.9% ± 0.4% (Fig. 1A). The DI caused a decrease in airway temporal variability with baseline tone. At T0 with the CV for the change in airway size for each airway ranged from 0.3% to 14.6% with a mean of 5.4% ± 0.2% (Fig. 1A). At T5 the CV with baseline tone for the change in airway size for each airway ranged from 0.2% to 20.9% with a mean of 5.7% ± 0.2% (Fig. 1A). After complete relaxation of the airway with atropine, the CV decreased to 4.6% ± 0.2% with a range from 0.3% to 17%.

During exogenous Mch contraction, the CV increased at all concentrations compared to baseline ($P < 0.0001$). There was no difference between the CV at the 2 highest concentrations of Mch. The effect of the DI on airway temporal variability had a different result depending on the level of airway tone. At the lowest dose of Mch, similar to baseline, the DI caused a decrease in airway temporal variability both immediately and 5 minutes later ($P < 0.0001$, Fig. 1B). However, at the two higher concentrations of Mch, the DI caused inconsistent responses in the change in CV. At 67 μg/min of Mch, there was a slight decrease in the CV immediately after the DI, but then an increase in the CV at 5 minutes ($P < 0.0001$, Fig. 1C). At 200 μg/min of Mch, we saw the opposite effect. There was a slight increase in the CV immediately after the DI, but then a small decrease in the CV at 5 minutes ($P < 0.0001$, Fig. 1D). Furthermore, we also noticed differences in CV among the dogs (Table 1A–D).

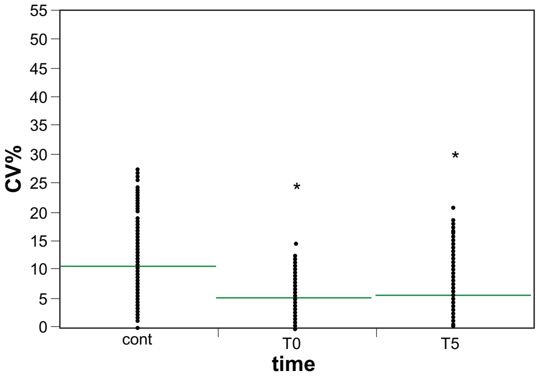

**Figure 1A.** The CV% for all the airways at baseline (pre-DI = control) and immediately (T0) and 5 minutes (T5) after a DI. There was a significant decrease in mean airway CV% immediately and 5 minutes after the DI ($P < 0.0001$).

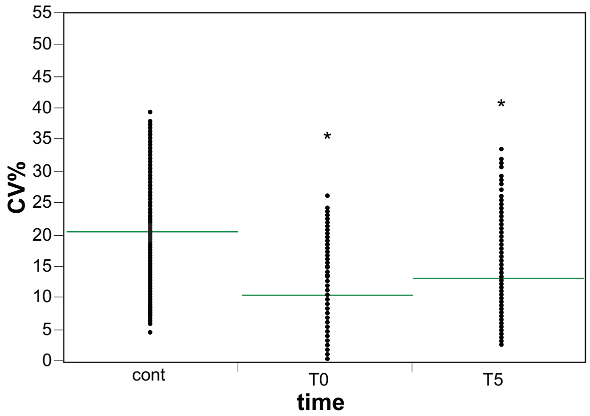

**Figure 1B.** The CV% for all the airways during Mch infusion of 17 µg/min (pre-DI = control) and immediately (T0) and 5 minutes (T5) after a DI. There was a significant decrease in mean airway CV% immediately and 5 minutes after the DI ($P < 0.0001$).

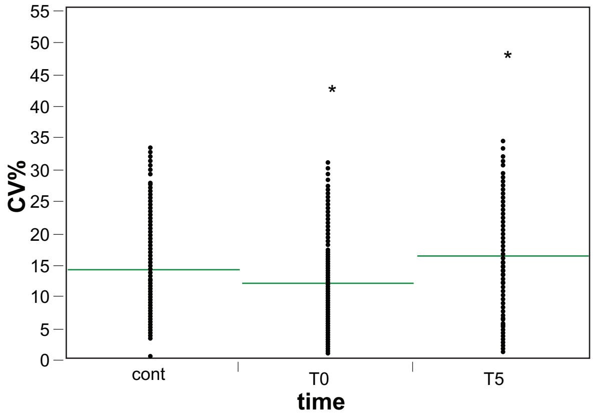

**Figure 1C.** The CV% for all the airways during Mch infusion of 67 µg/min (pre-DI = control) and immediately (T0) and 5 minutes (T5) after a DI. There was a significant decrease in mean airway CV% immediately ($P < 0.0001$) and a significant **increase** in CV% 5 minutes after the DI ($P < 0.0001$).

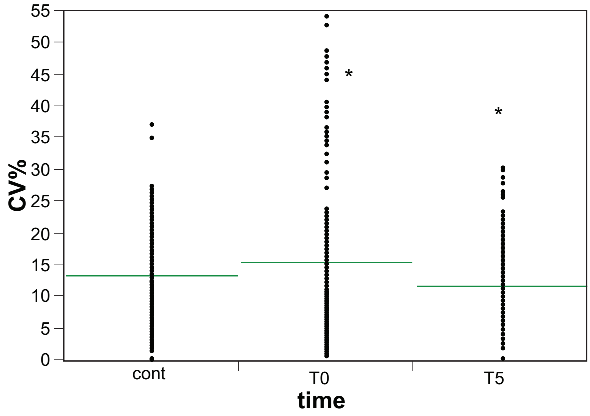

**Figure 1D.** The CV% for all the airways during Mch infusion of 200 µg/min (pre-DI = control) and immediately (T0) and 5 minutes (T5) after a DI. There was a significant increase in mean airway CV% immediately ($P < 0.0001$) and a significant decrease in CV% 5 minutes after the DI ($P < 0.0001$).

Finally, we performed a multiple regression analysis to account for all of the variable at the same time. The CV was the dependent variable, and dog, Mch dose, and the time period of measurement (control, T0, and T5) as independent variables. We are not able to look at airways in different lobes with our current software. We found all three variables were independently and significantly associated with CV ($P < 0.0001$ for all three). We next compared the mean values among each of the independent variables using Tukey's HSD to correct for multiple comparisons. For the Mch dose, controlling for the other independent variables, there was a significant difference in CV among the doses, with the baseline (Mch 0 µg/min) having the lowest CV. The 17 µg/min and the 67 µg/min doses were not significantly different and had the highest CV. The 200 µg/min dose had a CV in the middle that was significant different from the other doses. For the time of measurement, controlling for the other independent variables, there was a significant difference in CV among all three of

**Table 1A–D.** The CV% for each dog at baseline and for each dose of Mch at control (pre DI) and immediately (TO) and 5 minutes (T5) after a DI.

| Dog | Control | T0 | T5 |
|---|---|---|---|
| **A. CV% Baseline** | | | |
| 1 | 12.8 | 6.2 | 4.7 |
| 2 | 11.0 | 7.0 | 9.8 |
| 3 | 7.4 | 4.8 | 6.1 |
| 4 | 3.9 | 4.2 | 3.8 |
| 5 | 19.8 | 4.8 | 3.1 |
| **B. CV% Mch = 17 µg/min** | | | |
| 1 | 13.7 | 9.3 | 16.4 |
| 2 | 24.5 | 9.1 | 18.0 |
| 3 | 22.2 | 7.7 | 14.5 |
| 4 | 15.5 | 8.4 | 7.0 |
| 5 | 23.2 | 15.5 | 8.4 |
| **C. CV% Mch = 67 µg/min** | | | |
| 1 | 16.7 | 19.7 | 26.9 |
| 2 | 11.7 | 9.8 | 10.6 |
| 3 | 21.8 | 17.6 | 22.3 |
| 4 | 12.6 | 12.1 | 14.4 |
| 5 | 9.3 | 5.2 | 14.6 |
| **D. CV% Mch = 200 µg/min** | | | |
| 1 | 13.0 | 35.3 | 9.6 |
| 2 | 17.0 | 12.0 | 12.7 |
| 3 | 16.7 | 15.4 | 19.4 |
| 4 | 12.1 | 11.0 | 10.6 |
| 5 | 8.0 | 10.5 | 5.3 |

the time periods. The highest CV was at the control time period, while the lowest CV was at the T0 time period. At the T5 time period, the CV was intermediate and significantly different from the other two time periods. For the dogs, we found differences in the CV, controlling for the other independent variables, among 4 of the 5 dogs. Dogs 1 and 3 had the highest CV and they were not significantly different. Then in decreasing order of next highest to lowest CV were dogs 2, 5, and finally 4 with the lowest CV, controlling for the other independent variables.

## Discussion

In this study, we measured the size of individual airways on repeated occasions before and after a deep inspiration at baseline and during increasing concentrations of Mch. As far as we are aware there are no comparable data in the literature in humans or animal models that has examined this temporal variability in individual airway responsiveness to a deep inspiration. Although as will be discussed below, there have been studies that have examined the overall airway response to a deep inspiration, these did not provide any insights in the spatial or temporal variability after a deep inspiration. While the temporal variability in baseline airway size,[12] the response to an exogenous challenge,[15] and the single response to a deep inspiration were previously studied,[16,17] the temporal variability in response to a deep inspiration has not been investigated.

We previously demonstrated that airways with any level of Mch stimulation had greater temporal variability than without Mch.[15] Our current results also demonstrate that the temporal variability of the airways decreased after the deep inspiration both at baseline and during low concentrations of Mch stimulation, but this beneficial effect of a deep inspiration was lost at higher concentrations of Mch. Furthermore, we found that the decreased temporal variability at baseline and during a low concentration of Mch stimulation was sustained for at least 5 minutes after the deep inspiration. At higher concentrations of Mch, the temporal variability fluctuated during the 5 minute after the deep inspiration.

Consistent with our previous observations, we saw significant differences in the temporal variability among dogs and between days. We also saw significant individual airway temporal variability within each dog. Since we were infusing the Mch at a steady-state constant concentration, the temporal variability must be related to intrinsic differences at the individual airway level.

## Experimental methods

To minimize any potential variability associated with the methods, all dogs were anesthetized, intubated, and ventilated in the same manner at the same time of day on each of the four occasions. Furthermore, to avoid a varying time course associated with an acute aerosol challenge, we administered the spasmogen as a continuous intravenous administration that should have reached all the airways with the same concentration. In addition, we waited 20 minutes after beginning each dose of the continuous infusion, about 4 half-lives longer than the response time for the airways to Mch, to assure we were at steady-state before acquiring the scans. Locating the same airway on different days was straightforward, and has been documented in several previous studies.[16,17,26–30]

The intervals between the repeated studies ranged from 1 to 8 weeks and were based on HRCT scanner availability. While we strived to maintain comparable intervals between the studies, due to scheduling constraints associated with the clinical CT scanner, it was not always possible. While constant intervals between each study session may have some advantages, there were no indications that the random time interval between measurements had any consistent effect on our results. This is consistent with our previous work on baseline sizes,[14] where the time interval between the baseline studies also varied widely, with no correlation between the length of time and the extent of baseline tone. What we still do not know is the limits of this time frame of temporal variability outside of the 1–8 weeks used here. That is, how much of this temporal variability might occur over shorter intervals of hours or days remains to be determined.

To standardize the measurement of airway area, all measurements were made by the same person (KF). We also only measured airways with a baseline diameter greater than 2 mm in diameter, which has been shown to be a size above which there is sufficient signal to noise and limited measurement variability even after contraction.[13] In addition, all the measurements were made at FRC, which was not significantly changed either immediately or 5 minutes after the DI.

In previous work,[16] we studied the mean effect of a DI on the subsequent response of the airways. That work showed that a short duration or small size DI caused an immediate distension of the airways but led to subsequent narrowing of the airways relative to the pre-DI size. In contrast, a long duration or large DI caused a larger immediate distension of the airways and a subsequent maintained dilation of the airways up to 5 minutes after the DI. In the current work, we chose both a small size and a short duration DI, with the expectation we would observe a subsequent contraction of the airways 5 minutes after the DI. This subsequent contraction at the 5 min point, however, was not found in the present study. We believe that methodological differences in the two studies may account for this difference. In the previous work, we acquired the CT scans immediately after the release of the DI, every 30 seconds for the next 2 minutes, and then every minute for the next 3 minutes. In the current study, we only acquired the CT scans immediately after the DI and after 5 minutes. To acquire the CT scans, it is necessary to stop the ventilation for the duration of the scanning. The previous study was performed on an older model scanner that had only 4 detectors and thus required a significantly longer apneic period, about 15 seconds versus about 7 seconds for the current study. The fact that we also stopped the ventilation more times (8 times or ≈ 120 s) in the previous studies compared to the current study (2 times or ≈ 14 s) may have accounted for the subsequent bronchoconstriction we saw previously, since, it is well documented that a lack of tidal stretching of the airways can lead to spontaneous constriction.[31,32] This effect of tidal volume stretching (or lack thereof) also may be relevant to the mechanisms of the DI induced bronchoconstriction and bronchodilation after a DI in individuals with asthma.[33]

Another difference compared to our previous findings is that in the current study, the distension of the airways immediately after the DI was smaller than what was previously observed.[16,17] This is also likely due to methodological differences. Our previous studies examined either changes in duration[17] or changes in size[16] of the DI, but not both. At the shorter durations, we used a larger size DI, and for the experiments with the smaller DIs, we used a longer duration. In the current study, we used both a short duration and a small size, and this combination could have contributed to the small residual airway dilation at 5 min after the DI.

Results from the present work show that when there was a substantially increased airway tone induced by the highest concentrations of Mch, there was a loss of the DI's ability to decrease the airway temporal variability. However, we did not see a dose response relationship between the increase in airways tone and the temporal variability after the DI. This was surprising, since the mean level of airway smooth muscle constriction continued to increase with increasing doses of Mch, so we would have expected the temporal variability to also change accordingly from the lowest to the highest dose of Mch. This observation suggests that there might be a threshold beyond which additional muscle tone no longer leads to a beneficial effect of a DI. It is interesting that this lack of a dose response relationship with regard to airway tone is consistent with the response to a DI in subjects with asthma of varying severity compared to healthy individuals.[2,8] Scichilone et al demonstrated no difference in either the bronchodilatory or bronchoprotective effects of a DI in the individuals with mild asthma compared to those with moderate to severe disease.[8] The lack of a dose-response effect is also consistent with our recent work in dogs examining airway temporal variability with increased airway tone.[34] In a related study, Que, et al[35] found increased temporal variability in human respiratory resistance after Mch challenge. They assumed this resulted from intrinsic rapid fluctuation in each of the hundreds of conducting airways lying in series and parallel that was increased with Mch. How this temporal variability would be altered by a DI was not studied by Que, et al, but if their untested assumption about the smooth muscle tone is true, the expectation would be that if the airways are dilated (as with a DI), there would be less temporal variability. Why there is an all or none response with regard to Mch dose, however, is not clear and is something that warrants further study.

While we did not measure transpulmonary pressure, we did measure lung volumes from the HRCT scans at baseline and immediately and 5 minutes after the DI. Colebatch et al[36] showed that when boluses of smooth muscle agonists were administered in the pulmonary artery of cats and dogs, there were transient decreases in lung volume and dynamic compliance, and increases in transpulmonary pressure. However, we did not see any decrease in lung volume at any of the steady state doses of Mch that we administered, either immediately or 5 minutes after the DI maneuvers. The difference in these studies may reflect the effect that a bolus injection into the pulmonary artery causes extremely high acute concentrations that are quickly diluted, so even our highest steady state doses may not be comparable with the boluses given by Colebatch et al.[36]

In asthma, the loss of the bronchoprotective effect of a DI is one the earliest pathologic changes observed.[8] Even individuals with mild asthma show a loss of bronchoprotection.[6] If we can extrapolate from our data, we would predict that while a large change in tone is not required to increase the temporal variability after a DI, in order to lose the bronchoprotective effects of a DI, even modest changes in tone may be sufficient. However, this extrapolation cannot be carried too far, since, the remodeling and changes in airway smooth muscle mass that have been shown to exist in asthmatic individuals[37–39] could not possibly explain the differences we observed in the same airways of healthy dogs after a DI.

At the present time, we can only offer several speculations for the difference in temporal variability in the airway response to DI observed with Mch challenges. Variations in local vagal tone and its response to a large stretch could result in release of local mediators that would contribute to this temporal variability, but unfortunately there is no information on either the spatial distribution of vagal tone to the airways or its temporal variation even in the absence of DIs. Similarly, ignorance exists with regard to temporal and spatial variations in the interstitial milieu bathing the airways, so the effect of any local mediator or nitric oxide release is unknown. One source of temporal variability relevant to the large stresses associated with a DI is perhaps related to the slowly adapting (SAR) and rapidly adapting pulmonary stretch receptors in the lung. Many SARs are known to be active at FRC,[40] and since increased SAR activity with the lung inflation phase of a DI would surely cause bronchodilation, any decrease in SAR activity during the relaxation phase will lead to a variable degree of bronchoconstriction. Perhaps the most likely explanation is that the airway smooth muscle itself is responsible for both the widely varying response to exogenous stimulation and DI, as suggested by work of Que, et al.[35] In a study by Frey et al[41] using a fractal model, it was shown that increased temporal variability in peak expiratory flows was associated with the most severe asthmatics and with an increased risk of unstable airway function. King et al using HRCT scans also demonstrated increase airway narrowing heterogeneity in asthma compared to healthy individuals.[13] The potential relevance of the temporal variability in responsiveness to Mch and to DIs could be resolved with studies that follow individual airway responses over time in asthmatic or normal subjects.

In conclusion, results from this study document a differential temporal variability in airway size following a DI depending on the level of airway tone. Increased airway tone led to increased airway temporal variability. At high levels of airway tone, the beneficial effects of a DI were abolished. While the mechanisms underlying this temporal variability are poorly understood, if we consider that 1) increased heterogeneity may exacerbate clinical disease, and 2) that the bronchoprotective and bronchodilatory effect of a DI on the airways is lost in individuals with asthma, then we speculate that we would likely find a smaller decrease in airway temporal variability after a DI in asthmatic subjects compared to healthy individuals.

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## Disclosures

This manuscript has been read and approved by all authors. This paper is unique and not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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