

# Measurement of Intra-individual Airway Tone Heterogeneity and its Importance in Asthma

Robert H. Brown<sup>1,2,3,4</sup>, Alkis Togias<sup>5</sup>

<sup>1</sup>Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University

<sup>2</sup>Department of Medicine, Division of Pulmonary and Critical Care Medicine, Johns Hopkins University

<sup>3</sup>Department of Environmental Health Sciences/ Division of Physiology, Johns Hopkins University.

<sup>4</sup>Department of Radiology

<sup>5</sup>Department of Medicine, Divisions of Allergy and Clinical Immunology and Pulmonary and Critical Care Medicine, Johns Hopkins University

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Corresponding Author:

Robert H. Brown, M.D., M.P.H.

Johns Hopkins School of Public Health, Department of Environmental Health Sciences, Room E7614

615 North Wolfe St, Baltimore, Maryland, 21205

Ph: 410-614-5451; FAX: 410-955-0299; rbrown@jhsph.edu

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26 **ABSTRACT:**

27 While airways have some degree of baseline tone, the level and variability of this tone is not known.  
28 It is also unclear whether there is a difference in airway tone or in the variability of airway tone  
29 between asthmatic and healthy individuals. This study examined airway tone and intra-individual  
30 airway tone heterogeneity (variance of airway tone) *in vivo* in 19 individuals with asthma compared  
31 to 9 healthy adults. All participants underwent spirometry, body plethysmography and HRCT at  
32 baseline and after maximum bronchodilation with albuterol. Airway tone was defined as the percent  
33 difference in airway diameter after albuterol at total lung capacity compared to baseline. The amount  
34 of airway tone in each airway varied both within and between subjects. The average airway tone did  
35 not differ significantly between the two groups ( $p=0.09$ ), but the intra-individual airway tone  
36 heterogeneity did ( $p=0.016$ ). Intra-individual airway tone heterogeneity was strongly correlated with  
37 airway tone ( $r= 0.78$ ,  $p<0.0001$ ). Also, it was negatively correlated with the magnitude of the  
38 distension of the airways from FRC to TLC at both baseline ( $r= -0.49$ ,  $p= 0.03$ ) and after maximum  
39 bronchodilation ( $r= -0.51$ ,  $p= 0.02$ ) in the asthma, but not the healthy group. However, we did not  
40 find any relationship between intra-individual airway tone heterogeneity and conventional lung  
41 function outcomes. Intra-individual airway tone heterogeneity appears to be an important  
42 characteristic of airway pathophysiology in asthma.

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45 Key words: airways, bronchodilation, distensibility

## 46 INTRODUCTION

47 Airway smooth muscle tone is one the most important characteristics that determine the state of the  
48 airway lumen, which, in turn, is considered the major factor behind symptoms of airways obstruction  
49 in asthma (5, 34). Bronchodilator-induced changes in airflow have been used as a surrogate in  
50 determining the magnitude of airway smooth muscle tone *in vivo*. However, because airflow is  
51 dependent on several other structural and functional characteristics of the airways and even lung  
52 parenchyma, we have used imaging through high resolution computed tomography (HRCT) to  
53 determine bronchodilator-induced changes in the size of the airway lumen as a more direct surrogate  
54 of airway smooth muscle tone (9, 11). HRCT can determine the luminal diameters, as well the  
55 airway wall thicknesses, in a large number of airways ranging from the trachea down to as small as 2  
56 mm in diameter, and previous work by our group suggested that airway diameter and airway wall  
57 thickness may be important factors in airway tone (12). In addition, HRCT gives us the ability to  
58 measure the same airway locations on multiple occasions and under different physiological  
59 conditions. Using this approach, we have previously shown in both dogs (10) and in humans (12)  
60 that the smooth muscle of all airways has some degree of baseline tone and that the size of individual  
61 canine airways at baseline can vary widely over weeks or months (15) indicating the dynamic state  
62 of baseline tone.

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64 In canine airways, when airway tone is increased with methacholine (Mch), the between-airways  
65 variability in airway size also increases (8) suggesting that tone is not distributed equally to all  
66 airways. This phenomenon is probably reflected in the ventilation heterogeneity that has been  
67 described in asthma using hyperpolarized noble gas MRI (17, 29, 37). In the case of HRCT, with  
68 individual airways being visualizable before and after the administration of a bronchodilator,  
69 between-airway heterogeneity of tone can be measured on the basis of the statistical variance around  
70 the mean change in airway luminal size.

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72 We conducted this study to test the hypothesis that increased baseline tone, and increased

73 heterogeneity in airway tone, both measured through airway imaging, are associated with asthma.

74 We also hypothesized that increased airway wall thickness, a known characteristic of asthma, is

75 associated to airway tone and airway tone heterogeneity. The specific aims of this study were 1) to

76 determine the magnitude of airway tone and the heterogeneity in airway tone in individuals with

77 asthma compared to healthy controls; 2) to examine whether intra-individual airway tone

78 heterogeneity, compared to airway tone per se, best differentiates asthma from the healthy state; and

79 3) to determine whether airway tone heterogeneity relates to lung function parameters or to airway

80 diameter, wall thickness and distensibility.

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## 85    **METHODS**

86

### 87    Subjects

88    The study involved a group of participants with asthma and a group of healthy controls. For the  
89    group with asthma, inclusion criteria were nonsmoker males or females between the ages of 18 and  
90    65, who had typical asthma symptoms and history of exacerbations. In order to test a spectrum of  
91    disease, no asthma severity inclusion criteria were used, other than participant having no  
92    hospitalizations for asthma within the previous 6 months. The presence of bronchial  
93    hyperresponsiveness to methacholine ( $PC_{20} \leq 8\text{mg/ml}$ ) and/or reversibility of airway obstruction by  
94    albuterol (an increase in  $FEV_1$  of  $>200\text{ mL}$  and  $\geq 12\%$  from the baseline or an increase  $\geq 10\%$  in  
95    predicted  $FEV_1$ ) were also used as asthma inclusion criteria. All asthma participants were at least 45  
96    days past the end of their most recent upper respiratory tract infection for all of their visits, and were  
97    asked to withhold inhaled bronchodilators prior to each study visit: 12 hours for short-acting and 48  
98    hours for long-acting beta agonists. The healthy group also included nonsmoker males and females  
99    between the ages of 18 and 65. Current or prior pulmonary symptoms or history of asthma or rhinitis  
100    were exclusionary for this group. The protocol was approved by the Johns Hopkins University IRB  
101    and written informed consents were obtained.

102

### 103    Study Design

104    The study consisted of 3 visits. The first visit was for screening purposes and included a respiratory  
105    questionnaire, epicutaneous skin testing with a panel of common aeroallergens, and baseline  
106    spirometry followed by a conventional methacholine (Mch) inhalation challenge (1). During the  
107    second visit, subjects underwent spirometry and plethysmographic thoracic gas volume  
108    determination followed immediately by two sequential HRCT scans (one at FRC and one at TLC) to  
109    evaluate baseline airway lumen size, wall thickness, and airway distensibility. During the third visit,

after spirometry and lung volumes were again determined, all subjects received albuterol by nebulization until maximal bronchodilation was achieved (see description below). Thereafter, plethysmography was repeated followed by two HRCT scans at FRC and at TLC. For spirometry and body plethysmography outcomes, we used the Hankinson (NHANES III) predicted values (22).

#### *Evaluation of airway tone*

For the purpose of this study, we defined airway tone on the basis of imaging as the percent difference in airway diameter at baseline Total Lung Capacity (TLC) compared to the diameter of the same airway after FEV<sub>1</sub> plateau was reached with albuterol. Albuterol was delivered with at least 2 and up to 4 consecutive nebulizations of 2.5 mg in 3 ml of vehicle until FEV<sub>1</sub> did not increase by more than 5% from its previous value (FEV<sub>1</sub> plateau) (12).

Airway tone was defined by the following formula:

$$\text{Airway tone} = 100 - ((\text{Airway diameter at TLC baseline})/(\text{Airway diameter at TLC relaxed})) \times 100$$

An airway with no tone would be assigned the value of 0% with larger numbers indicating various levels of tone.

#### *Evaluation of airway tone heterogeneity*

Intra-individual airway tone heterogeneity (variability) was defined as the statistical variance of airway tone for all the airways assessed within each subject.

#### *Evaluation of airway distensibility at baseline and after maximum bronchodilation with albuterol*

As described above, subjects were scanned twice in sequence, once at FRC and once at TLC, both at baseline and after maximal bronchodilation with albuterol was achieved. Airway distensibility at baseline and after the FEV<sub>1</sub> plateau was reached was defined by the following formula:

$$\text{airway distensibility} = (((\text{Airway diameter at TLC}) - (\text{Airway diameter at FRC})) / (\text{Airway diameter at FRC})) \times 100$$

Where the airway diameter at TLC is an individual airway diameter measured on HRCT at full lung inflation, and the airway diameter at FRC in the same airway measured on HRCT at end of normal expiration.

#### *Acquisition of CT data*

All scans were performed using the spiral CT (Somatom Plus 4, Siemens) with settings of 120kVp, 170 MAs, 2mm slice thickness, rotation feed of 2 mm/sec, and a reconstruction interval of 1 mm (total 61 scans per set) during a single breath hold for approximately 24 seconds at FRC or TLC and moved caudally. A reference scan was acquired prior to each spiral CT scan set to ensure reproducible image location in the lung. The images were reconstructed as 16bit 512x512 matrix using a field of view of 200 mm. Images were reconstructed with the use of 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. All airways visualized approximately perpendicular to the scan plane (long to short axis ratio less than 1.5:1) were assessed. For repeated airway measurements in a given study participant, adjacent anatomic landmarks, such as airway or vascular branching points, were identified on the HRCT images obtained at FRC and again on the TLC scans.

156

157 *Airway measurements with HRCT*

158 The airway luminal size measurement has previously been described (6) and validated in animals  
159 models (2, 7). A similar range of airway generations (2<sup>nd</sup> through 6<sup>th</sup>) was evaluated in all the  
160 subjects. Briefly, the HRCT images were transferred to a UNIX-based workstation and analyzed  
161 using the airway analysis module of the Volumetric Image and Display Analysis (VIDA) software  
162 package (Dept. of Radiology, Division of Physiologic Imaging, University of Iowa, Iowa City, IA).  
163 To measure the airway areas, the operator drew a rough isocontour estimate of the lumen of the  
164 airway. The software program automatically located a precise isocontour perimeter of the airway  
165 lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that  
166 defined the luminal edge of that airway wall. The length of the rays was set at 6 pixels. The  
167 software program used an algorithm for edge detection based on the “full-width-half-maximum”  
168 principle. The edge of the wall was defined by the program by the points along the lines where the  
169 pixel density changed to half its maximum through the wall. All full and partial pixels (full pixel  
170 size equals 0.1537 mm<sup>2</sup> with our settings) within the adjusted isocontour were counted and  
171 represented the airway area.

172

173 To measure airway wall thickness, at least three lines were randomly drawn through the airway wall.  
174 The VIDA program displayed a histogram of pixel intensity along the line that was drawn through  
175 the airway wall. The inflection points of increasing intensity represented the inner and outer edges of  
176 the airway wall. The line and points were selected manually. The program then automatically  
177 measured the distance in pixels between the two points. The software is capable of measuring  
178 fractions of a pixel, and therefore the measurements of wall thickness were not necessarily quantized  
179 to multiples of the pixel dimension. The number of pixels and any fraction of a pixel that were  
180 counted in the airway wall were then converted to millimeters by multiplying by the size of the pixel

in millimeters based on the scanning parameter. The airway wall measurements were then averaged to give a mean airway wall thickness for each airway. Airway wall thickness was expressed as a percentage of airway diameter measured at the FEV<sub>1</sub> plateau. This was done to eliminate the potentially confounding effect of airway constriction on the apparent airway wall thickness.

#### *Data Analysis*

Data analysis was performed using JMP 11.0.0 software (SAS Institute Inc.). Unpaired t-tests were used to compare the two participant groups in terms of the two primary outcomes, airway tone and airway tone heterogeneity, as well as in terms of spirometric outcomes, lung volumes, airway diameter, and airway wall thickness measurements. To compare changes in pulmonary function after maximum bronchodilation with albuterol and changes in airway dimensions and airway wall thickness after maximum bronchodilation within and between the healthy and asthma groups, a mixed within-between groups ANOVA was used. Simple linear regressions were performed to measure the correlation between airway tone or airway tone heterogeneity and Mch reactivity (PC<sub>20</sub>), spirometric or lung volume outcomes and outcomes of airway morphology. Logistic regressions were performed to examine the independent effects of airway tone and airway tone heterogeneity on the clinical phenotype (asthma and healthy status). Significance was accepted at  $p \leq 0.05$ .

## 200    **RESULTS**

201

202    We screened 44 subjects, 34 with histories consistent with asthma and 10 healthy controls. All 34  
 203    subjects with a history of asthma underwent albuterol reversibility testing, while 16 also underwent  
 204    Mch bronchoprovocation. Methacholine challenge testing was not performed on 7 asthma and 2  
 205    healthy subjects. In 6 asthma subjects, methacholine challenge testing was not performed because  
 206    their baseline FEV<sub>1</sub> was below 70% predicted (a safety requirement). One asthma and 2 healthy  
 207    subjects refused methacholine challenge testing because of previous unpleasant experiences.  
 208    Nineteen of the original 34 asthma participants fulfilled all inclusion criteria and were included in the  
 209    main study (Table 1a). Sixteen subjects were on inhaled beta-agonist bronchodilator, 7 on inhaled  
 210    steroids, 1 on nasal steroids, 3 on oral steroids, 3 on anti-histamines, and 2 on leukotriene receptor  
 211    antagonists. One of the 10 healthy individuals developed a respiratory infection during the study and  
 212    his data were excluded from analysis (Table 1b). Baseline and post-albuterol lung function data are  
 213    shown in Tables 2a & b. Baseline and post-albuterol imaging-based airway measurements are shown  
 214    in Tables 3a & b. Two of the healthy subjects demonstrated 12 and 13% airway reversibility,  
 215    respectively, but this was not an exclusion criterion given that they had no history of asthma or  
 216    rhinitis or any compatible symptoms.

217

### 218    ***Tone***

219    Airway tone, as defined in Methods, was calculated for each volunteer by averaging the tone of all  
 220    measurable airways (mean number of airways measured = 40, range 18 to 64) (Figure 1a & b). A  
 221    trend for higher airway tone was seen in asthma participants, but the difference between the two  
 222    study groups did not reach significance (p=0.088, Figure 2).

223

### 224    ***Intra-individual Tone Heterogeneity***

225 Intra-individual airway tone heterogeneity (variability) was calculated as the variance around the  
 226 mean airway tone for all the airways within each subject (see Methods). We found that the intra-  
 227 individual tone heterogeneity was significantly higher in asthma participants compared to healthy  
 228 controls ( $p=0.016$ ; Figure 3). We also performed logistic regression with “health status” (asthma or  
 229 health) as the outcome variable and with intra-individual airway tone heterogeneity as the  
 230 independent variable. Again, intra-individual airway tone heterogeneity was predictive of asthma  
 231 ( $p=0.0067$ ).

232

233 Intra-individual airway tone heterogeneity was strongly correlated with airway tone ( $r= 0.78$ ,  
 234  $p<0.0001$ ). Therefore, we performed logistic regression with “health status” (asthma or health) as the  
 235 outcome variable and airway tone and intra-individual airway tone heterogeneity as the independent  
 236 variables. In addition, we included an interaction term for these two variables in the model. The  
 237 overall model was statistically significant ( $p=0.0003$ ). However, only intra-individual airway tone  
 238 heterogeneity showed an association ( $p=0.03$ ) while airway tone did not ( $p=0.27$ ). The interaction  
 239 term added approximately 10% to the main effect of the intra-individual tone heterogeneity.

240

#### 241 ***Relation of intra-individual airway tone heterogeneity or airway tone with lung function and CT*** 242 ***based airway measurements***

243 We examined the relationships between intra-individual airway tone heterogeneity and pulmonary  
 244 function (Table 4a) or imaging-based airway measurements (Table 4b) at baseline and after  
 245 albuterol. In the group of participants with asthma, intra-individual airway tone heterogeneity  
 246 correlated with baseline airway diameter at TLC ( $r= -0.54$ ,  $p= 0.02$ ), and with airway wall thickness  
 247 at both FRC ( $r= 0.48$ ,  $p= 0.04$ ) and TLC ( $r= 0.59$ ,  $p= 0.01$ ) (Table 4b and Figure 4A). These  
 248 relationships indicated that among our study participants with asthma, those with narrower airways  
 249 and thicker airway walls had greater airway tone heterogeneity. After albuterol, these relationships

were lost. Figure 4B shows that the relationship between airway tone heterogeneity and airway wall thickness was lost because individuals whose airways had the greatest average wall thickness at baseline also had the greatest reductions in wall thickness with maximum bronchodilation, whereas airways with the lowest wall thickness had relatively little change. Interestingly, the change in wall thickness with maximum bronchodilation was strongly correlated with airway tone ( $r=0.97$ ,  $p<0.0001$ ). Intra-individual airway tone heterogeneity did not correlate with any baseline measures of spirometry or body plethysmography in either the asthma or healthy control groups nor did it correlate with the albuterol-induced changes in those parameters (Table 4a).

We also examined the relationships between airway tone and pulmonary function (Table 5a) and airway measurements (Table 5b). There were no significant correlations between airway tone and pulmonary function at baseline, or between airway tone and the albuterol-induced changes in spirometry or plethysmography. However, we found significant correlations between airway tone and airway diameter at baseline FRC ( $r= -0.59$ ,  $p=0.008$ ), airway diameter at baseline TLC ( $r= -0.72$ ,  $p=0.0005$ ), wall thickness at baseline FRC ( $r= 0.70$ ,  $p=0.0012$ ) and wall thickness at baseline TLC ( $r=0.85$ ,  $p<0.0001$ ). As with airway tone heterogeneity, all these relationships were lost after maximum bronchodilation (Table 5b). In participants with asthma, airway tone correlated with Mch reactivity as assessed by the log PC<sub>20</sub> ( $r= -0.61$ ,  $p=0.046$ ), where greater tone was associated with lower PC<sub>20</sub>. Interestingly, there was no correlation between the PC<sub>20</sub> and airway tone heterogeneity ( $r= -0.04$ ,  $p=0.91$ ).

Deep inspirations are thought to be “defective” in protecting or reversing airway constriction in persons with asthma. In the asthma group, we found negative correlations between intra-individual airway tone heterogeneity and the distension of the airways from FRC to TLC with a deep inspiration at both baseline ( $r= -0.49$ ,  $p= 0.03$ ) and after maximum bronchodilation ( $r= -0.51$ ,  $p=$

275 0.02) (Table 4b) with greater intra-individual tone heterogeneity associated with less airways  
276 distention from FRC to TLC. We found no correlation between intra-individual airway tone  
277 heterogeneity and distension of the airways with deep inspiration in the healthy group.

278

279 Finally, we examined the relationships between airway tone and distensibility. As with intra-  
280 individual airway tone heterogeneity, we found negative correlations between airway tone and the  
281 distension of the airways from FRC to TLC with a deep inspiration at both baseline ( $r = -0.60$ ,  $p =$   
282  $0.007$ ) and after maximum bronchodilation with albuterol ( $r = -0.49$ ,  $p = 0.03$ ) in the asthma group.  
283 We also found a correlation between airway tone and distension of the airways with deep inspiration  
284 at baseline in the healthy group ( $r = -0.71$ ,  $p = 0.03$ ).

## 285 **DISCUSSION**

286

287 In this study, we used airway imaging to quantify airway tone and intra-individual airway tone  
288 heterogeneity in participants with asthma of variable severity and in healthy individuals. We found  
289 that, compared to the healthy group, individuals with asthma have larger baseline intra-individual  
290 airway tone heterogeneity. On the other hand, averaging the tone of multiple airways within each  
291 participant did not yield a statistically significant difference between the asthma and the healthy  
292 groups, but a clear trend was present for asthma to have higher tone and the lack of statistical  
293 significance may reflect sample size. We found that airway tone and airway tone heterogeneity  
294 correlated with airway luminal diameter and airway wall thickness at baseline, but not after  
295 maximum bronchodilation. On the other hand, both airway tone and heterogeneity correlated with  
296 airway distensibility from FRC to TLC both at baseline and after maximum bronchodilation with  
297 albuterol. We did not find any relationships between airway tone or airway tone heterogeneity and  
298 measures of pulmonary function assessed by spirometry or body plethysmography.

299

300 Measuring airway smooth muscle tone in vivo is a difficult task. One intuitively assumes that  
301 changes in airflow parameters induced by bronchodilators or by a spasmogenic agent like inhaled  
302 methacholine reflect changes in airway tone. This is true only to some extent because airflow is  
303 dependent on several factors including airway resistance, tissue resistance, elastic recoil, and lung  
304 volume. Furthermore, using spirometry to assess airflow characteristics before and after the  
305 administration of a bronchodilator or before and after the administration of a spasmogen introduces  
306 the effects of deep inspirations on the state of airway smooth muscle, which can influence tone (18).  
307 Measuring airway diameter with the use of HRCT before and after maximal bronchodilation with a  
308 beta-adrenergic agonist offers a different, more direct approach to quantifying tone. We have  
309 previously applied this methodology in a canine model (9) where we showed that, after systemic

administration of atropine at a dose that completely blocks vagal tone, airway diameters reach a plateau at very low transpulmonary pressure and further increases in pressure lead to negligible changes in diameter. With administration of aerosolized histamine, airway diameters were reduced and did not reach maximum relaxed size with increased transpulmonary pressure. In the current study, given the limitations of working with human volunteers, removal of airway tone was achieved with repeated doses of inhaled albuterol until a plateau in FEV<sub>1</sub> was reached. In addition, to ensure maximal physiologic transpulmonary pressure, we used the airway diameter at TLC as the primary outcome to assess changes from baseline. Limitations of this model include the possibility that smaller airways may have not been reached by inhaled albuterol and that smaller airways cannot be adequately visualized by HRCT. We can, however, argue that our measurements were obtained on conducting airways that were most likely reachable by albuterol and maximally relaxed.

Our data indicate that airway tone intra-individual heterogeneity was a strong characteristic of asthma, perhaps stronger than airway tone per se. As we have previously seen in the canine model, where induction of airway tone led to increased airway heterogeneity (8), we found that airway tone intra-individual heterogeneity was strongly correlated to airway tone, but when both parameters were tested in a single predictive model, only intra-individual airway tone heterogeneity was associated with the diagnosis of asthma. We note, however, that, within the asthma group, airway tone did correlate with bronchial responsiveness to methacholine (PC<sub>20</sub>), whereas intra-individual tone heterogeneity did not, indicating that each parameter may play a different role in the behavior of the airways in the context of asthma. It should be noted that not all subjects underwent the methacholine PC<sub>20</sub> provocation test due to various reasons. Whether the inclusion of all the subjects would have changed the results is unknown.

All individuals with asthma enrolled in this study had a clinical history of asthma, used asthma

335 medications, and showed airway hyperresponsiveness by either bronchoconstriction to methacholine  
336 and/or bronchodilation to albuterol. All healthy subjects had no current or prior pulmonary  
337 symptoms or history of asthma or rhinitis. In retrospect, 3 subjects were identified (asthma subjects  
338 #8 & 17, and healthy #1, Table 1a & b) whose lung function testing could raise questions as to the  
339 correct classification. After excluding these three subjects, there still was no significant difference in  
340 airway tone between the asthma and healthy groups ( $p=0.15$ ), whereas airway tone heterogeneity  
341 remained significantly different ( $p= 0.038$ ).

342

343 Over the years, the potential role of airway heterogeneity in health and disease has attracted  
344 substantial interest (3, 19, 20, 23-25, 27, 28, 31, 33, 38-44). For example, King et al used HRCT to  
345 examine the variability of the airway response to inhaled methacholine in subjects with asthma (28).  
346 Indeed, most studies have examined the heterogeneity of airway responsiveness to a spasmogen and  
347 several have used HRCT or PET imaging to assess airway narrowing. We are not aware of another  
348 study examining the heterogeneity of the bronchodilator response using imaging. Another method of  
349 estimating global heterogeneity in the lung is with lung impedance (19, 20, 27, 32). The degree of  
350 heterogeneity is based on modeling the changes in the frequency dependence of lung resistance and  
351 elastance. With this approach it has been shown that heterogeneity is higher in people with more  
352 severe asthma, compared to moderate asthma and healthy controls (26, 32). However, this method  
353 cannot provide insight into the spatial or anatomic details underlying heterogeneity. These models,  
354 when combined with imaging techniques, can improve estimates regarding which diameters are  
355 involved in producing heterogeneity of ventilation (39). A few studies have been performed in  
356 children using impedance measurements, and their findings support our results. For example, in  
357 clinically stable children with asthma, Goldman et al. (21) demonstrated day-to-day variability in  
358 respiratory impedance not detected by spirometry.

359

Relevant to our findings and a key aspect of asthma is the patchiness of lung ventilation during constriction as well as during quiescent times when symptoms are absent (37). Airway tone heterogeneity may be a major factor in the patchiness that defines asthma. Venegas et al. showed that even for uniform smooth muscle activation of a symmetric bronchial tree, the presence of minimal heterogeneity breaks the symmetry and leads to large clusters of poorly ventilated lung units (30, 38, 42), the hallmark of asthma. Our findings are consistent with this potential mechanism of asthma symptoms. The mechanism behind increased heterogeneity in the behavior of the airways remains to be determined. If one considers all the factors that play into airway tone - central or peripheral drive, activation of preganglionic nerves, synaptic transmission in the parasympathetic ganglia, pre-junctional regulation of acetylcholine release, muscle contraction - and if one also realizes that each airway is likely driven by distinct subsets of these nerves, all of this sets up the possibility that heterogeneity of airway caliber and responses could be easily influenced by continual fluctuations in parasympathetic/cholinergic drive (16).

One of the aims of this study was to examine the associations between airway tone or intra-individual airway tone heterogeneity and baseline spirometry or plethysmography and the changes induced by albuterol on those outcomes. We were not surprised to find that our measure of baseline airway tone did not correlate with baseline spirometric or plethysmographic outcomes or with the albuterol-induced changes on those outcomes. As discussed earlier, spirometric outcomes are influenced by several mechanical and volume factors that seem to be playing an important role at baseline, in addition to airway tone. Furthermore, small airways contribute to airway resistance and to air trapping and increased residual volume, but HRCT imaging cannot assess the geometry of those airways. In some of our previous studies (4, 13, 36), PFT values did correlate with CT measurements, but those studies were usually performed in settings where there were large changes in PFTs, most of the time after induced airway constriction. We have also reported lack of a

relationship between physiologic measurements and imaging outcomes in dogs (14). In those studies, lack of correlation between airway size and lung function measurements after pharmacologically-induced airway relaxation was due to the fact that the changes in diameter with relaxation were relatively small compared to the changes in lung volume which were associated with air flow changes.

Another aim was to assess whether geometrical aspects of the airways were associated with airway tone or with intra-individual airway tone heterogeneity. Airway wall thickness expressed as a fraction of airway diameter correlated with airway tone as well as with intra-individual airway tone heterogeneity at baseline (Figure 4A), but not after albuterol (Figure 4B). This suggests that the statistically significant relationships were secondary to the presence of airway tone at baseline. Notably, albuterol significantly reduced airway wall thickness indicating that tone contributes to wall thickness (Table 3b), probably through the principle of mass conservation with airway narrowing. Furthermore, the loss of the relationship between airway wall thickness and airway tone heterogeneity after albuterol (Figure 4B) was due to the fact that the airways with the thickest walls at baseline showed the largest reductions in wall thickness after maximal bronchodilation. We found a significant difference between the asthma and the healthy groups, in wall thickness, even after removal of tone with albuterol (Tables 3a and b) indicating that remodeling was, indeed, present in our asthma group. However, our data suggest that remodeling is not a major contributor to airway tone or airway tone heterogeneity.

Airway distensibility, the change in airway diameter from FRC to TLC, which is one of the determinants of deep inspiration-induced bronchodilation and is reduced in magnitude in severe asthma (35) was negatively correlated with intra-individual airway tone heterogeneity and airway tone in asthma. This supports the concept that increased tone stiffens the airways and reduces their

ability to distend. However, these relationships remained present even when distensibility was measured after maximum bronchodilation with albuterol. This raises the possibility that airway remodeling also contributes to reduced airway distensibility in some people with asthma.

In summary, we have identified intra-individual airway tone heterogeneity, measured by the variance of the change of airway diameters in response to albuterol, as a parameter strongly associated with the diagnosis of asthma independently of conventional lung function measurements. Our finding is consistent with the previous observations of lung ventilation inhomogeneity in asthma. We believe that this observation and the methodology we have applied can be used to further explore airway pathophysiology in asthma.

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428 The authors declare they have no conflict of interest.

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544 Figure Legend:

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546 Figure 1: Baseline airway tone for all measured airways in each individual with asthma (1a) and  
 547 healthy controls (1b). Each column represents one subject and each circle one airway. The thick  
 548 horizontal line represents mean airway tone for that subject. An airway in which albuterol did not  
 549 change airway diameter is depicted with “zero” tone. \* baseline airway tone is defined as  $100 - ((\text{Airway diameter at TLC baseline})/(\text{Airway diameter at TLC relaxed})) \times 100$ .  
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552

553 Figure 2: Baseline airway tone in the asthma and the healthy groups. Open circles represent the  
 554 mean airway tone for all airways in each study participant in the asthma group and open triangles the  
 555 mean airway tone for all airways in each healthy participant. The horizontal line represents the mean  
 556 airway tone for all the subjects in each group. No significant difference between the two groups was  
 557 found ( $p=0.09$ ). \* baseline airway tone is defined as  $100 - ((\text{Airway diameter at TLC}$   
 558  $\text{baseline})/(\text{Airway diameter at TLC relaxed})) \times 100$ .

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561 Figure 3: Intra-individual airway tone heterogeneity in the asthma and the healthy groups. Open  
 562 circles represent the mean intra-individual airway tone heterogeneity for all airways in each study  
 563 participant in the asthma group and open triangles the mean airway tone for all airways in each  
 564 healthy participant. The horizontal line represents the mean intra-individual airway tone  
 565 heterogeneity for all the subjects in each group. A significant difference in intra-individual airway  
 566 tone heterogeneity between the two groups was found ( $p=0.016$ ). \*Intra-individual airway tone  
 567 heterogeneity is defined as the statistical variance of airway tone for all the airways assessed within  
 568 each subject.

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571 Figure 4: The relationship between airway wall thickness (as a fraction of airway diameter) at TLC  
572 and airway tone heterogeneity. A. baseline and B. after maximum bronchodilation with albuterol.

573 These figures clearly demonstrate that the statistically significant correlation between wall thickness  
574 and heterogeneity at baseline ( $p=0.01$ ) is eliminated after maximum bronchodilation ( $p=0.22$ ).

575 These results strongly support the notion that the relationship at baseline is due to the presence of  
576 airway tone.

577

Figure 1a (asthma)

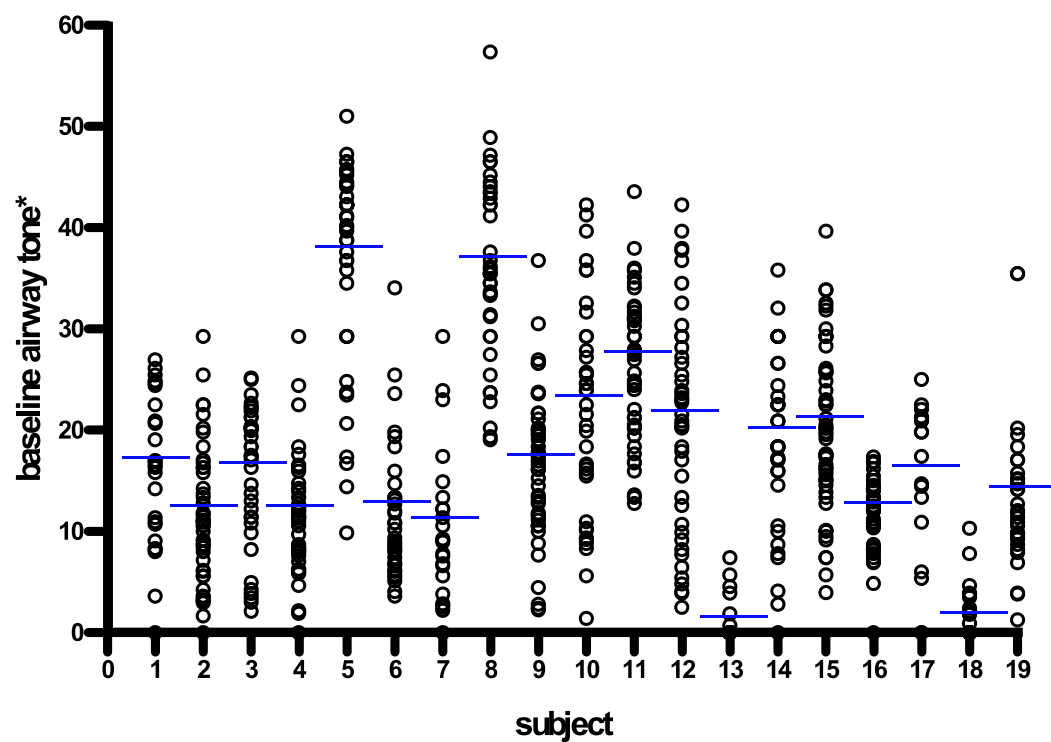
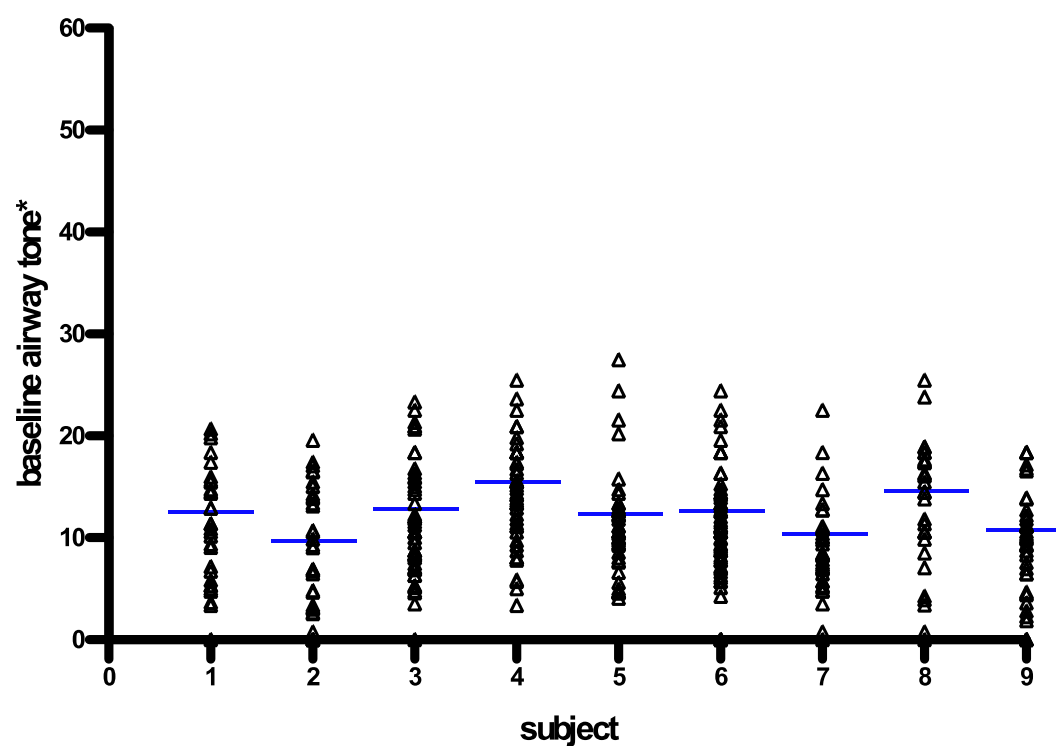


Figure 1b (healthy controls)



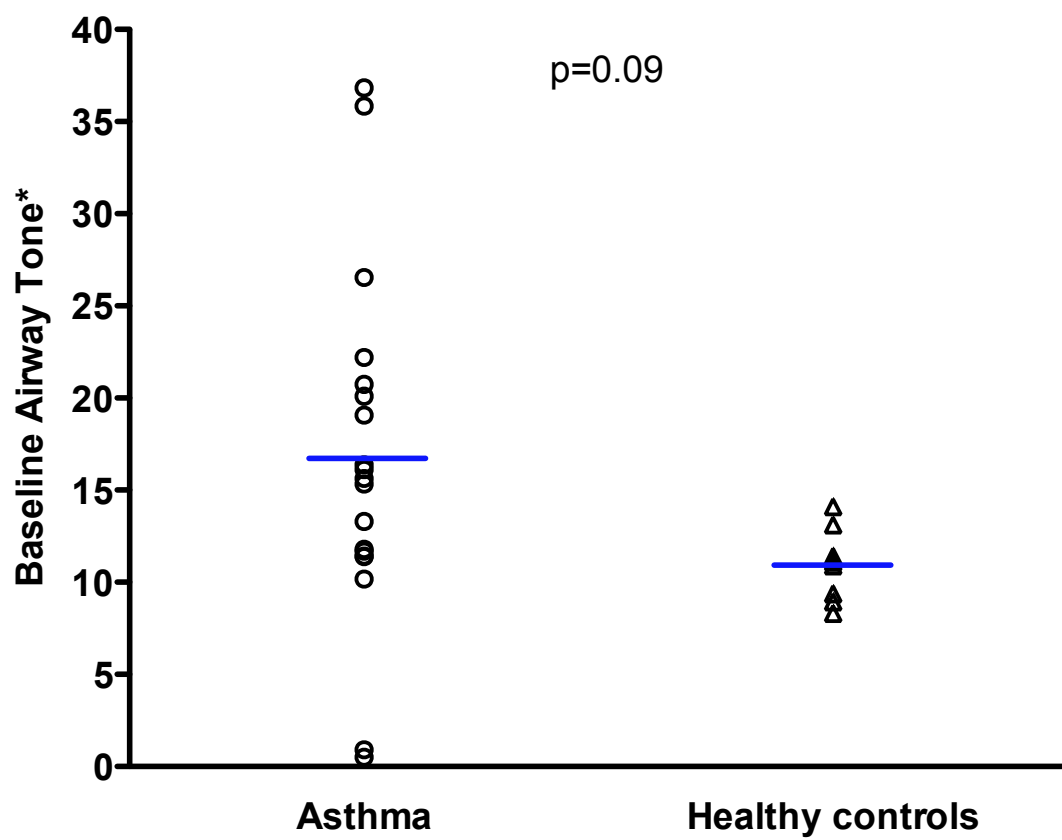


Figure 2

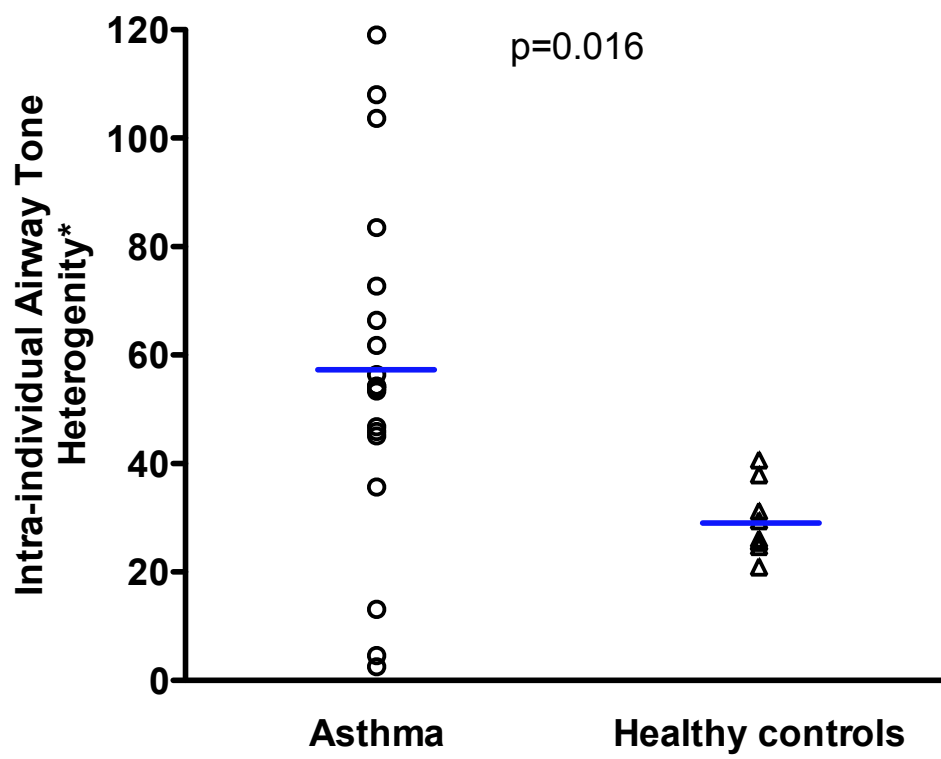


Figure 3

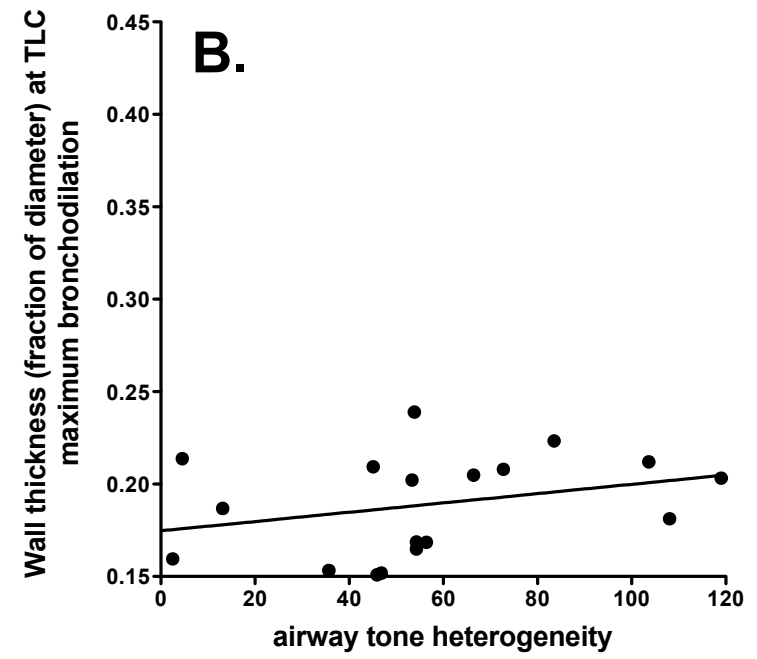
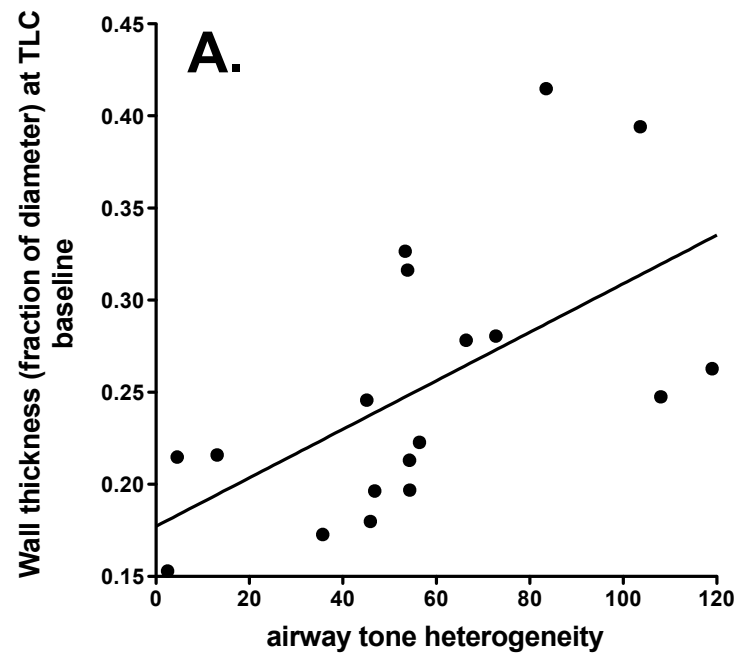


Figure 4

Table 1a: Participants with Asthma: Baseline Characteristics

| Asthmatic Subject | Sex | Age   | Race | FEV <sub>1</sub> % pred | FVC % pred | FEV <sub>1</sub> /FVC baseline | PC <sub>20</sub> (mg/ml) | Reversibility (% Δ from baseline) | RV % pred | TLC % pred | RV/TLC    |
|-------------------|-----|-------|------|-------------------------|------------|--------------------------------|--------------------------|-----------------------------------|-----------|------------|-----------|
| 1                 | M   | 25    | AA   | 75                      | 97         | 66                             | 0.22                     | 30                                | 179       | 131        | 0.40      |
| 2                 | M   | 23    | AA   | 104                     | 121        | 74                             | 23.39                    | 12                                | 104       | 108        | 0.25      |
| 3                 | M   | 35    | AA   | 68                      | 111        | 51                             | #                        | 54                                | #         | #          | #         |
| 4                 | M   | 23    | C    | 90                      | 104        | 71                             | 1.5                      | 18                                | 220       | 164        | 0.32      |
| 5                 | M   | 33    | C    | 72                      | 87         | 67                             | 1.61                     | 24                                | 98        | 128        | 0.28      |
| 6                 | F   | 28    | AA   | 88                      | 110        | 69                             | 0.02                     | 12                                | 63        | 144        | 0.18      |
| 7                 | F   | 25    | C    | 85                      | 98         | 75                             | 2.63                     | 8                                 | 135       | 124        | 0.44      |
| 8                 | M   | 38    | C    | 85                      | 97         | 70                             | #                        | 16                                | 283       | 193        | 0.43      |
| 9                 | F   | 44    | AA   | 75                      | 89         | 69                             | 2.14                     | 16                                | #         | #          | #         |
| 10                | F   | 22    | C    | 109                     | 121        | 79                             | >25                      | 16                                | #         | #          | #         |
| 11                | M   | 28    | HSP  | 83                      | 94         | 75                             | 5.76                     | 3                                 | 98        | 95         | 0.24      |
| 12                | M   | 43    | AA   | 75                      | 80         | 76                             | 4.109                    | 6                                 | 100       | 73         | 0.38      |
| 13                | F   | 48    | AA   | 69                      | 73         | 77                             | #                        | 21                                | 124       | 85         | 0.52      |
| 14                | F   | 34    | AA   | 87                      | 87         | 84                             | 0.58                     | 8                                 | 303       | 140        | 0.64      |
| 15                | M   | 60    | C    | 60                      | 82         | 56                             | #                        | 12                                | 173       | 110        | 0.51      |
| 16                | M   | 34    | AA   | 43                      | 73         | 49                             | #                        | 22                                | #         | #          | #         |
| 17                | F   | 50    | AA   | 69                      | 75         | 74                             | #                        | 15                                | 111       | 53         | 0.60      |
| 18                | M   | 45    | AA   | 43                      | 94         | 37                             | #                        | 22                                | 234       | 150        | 0.50      |
| 19                | F   | 19    | C    | 74                      | 83         | 79                             | 0.45                     | 7                                 | 93        | 87         | 0.23      |
| Mean±SD           |     | 35±11 |      | 77±17                   | 93±15      | 68±12                          |                          | 17±11                             | 154±74    | 119±37     | .039±0.14 |

Table 1b: Healthy Controls: Baseline Characteristics

| Healthy Subject | sex | Age   | Race | FEV <sub>1</sub> % pred | FVC % pred | FEV <sub>1</sub> /FVC baseline | PC <sub>20</sub> (mg/ml) | Reversibility (% Δ from baseline) | RV % pred | TLC % pred | RV/TLC   |
|-----------------|-----|-------|------|-------------------------|------------|--------------------------------|--------------------------|-----------------------------------|-----------|------------|----------|
| 1               | M   | 21    | C    | 81                      | 100        | 68                             | >25                      | 12                                | 97        | 116        | 0.17     |
| 2               | M   | 52    | C    | 86                      | 97         | 69                             | >25                      | 4                                 | 100       | 101        | 0.30     |
| 3               | F   | 20    | C    | 89                      | 84         | 93                             | >25                      | 4                                 | 119       | 89         | 0.32     |
| 4               | F   | 23    | C    | 82                      | 86         | 82                             | >25                      | 13                                | #         | #          | #        |
| 5               | M   | 47    | C    | 81                      | 89         | 71                             | >25                      | 1                                 | 147       | 97         | 0.44     |
| 6               | M   | 41    | C    | 111                     | 116        | 75                             | #                        | 5                                 | 88        | 111        | 0.22     |
| 7               | M   | 20    | HSP  | 88                      | 93         | 81                             | >25                      | 5                                 | 94        | 107        | 0.18     |
| 8               | M   | 27    | C    | 102                     | 102        | 81                             | #                        | 0                                 | 85        | 107        | 0.19     |
| 9               | F   | 35    | C    | 101                     | 110        | 78                             | 12.9                     | 8                                 | 88        | 88         | 0.30     |
| Mean±SD         |     | 32±12 |      | 91±11                   | 98±11      | 78±8                           |                          | 4±4                               | 107±25    | 101±10     | 0.28±0.1 |

# not performed

**Table 2a: Baseline pulmonary function (Mean±SD)**

| Measurement                  | Healthy Controls (n=9) | Asthma (n=19) | Between groups t-test p-value |
|------------------------------|------------------------|---------------|-------------------------------|
| FEV <sub>1</sub> % predicted | 91±10.6%               | 77±17%        | <b>0.03</b>                   |
| FVC % predicted              | 98±10.7%               | 93±15%        | 0.47                          |
| FEV <sub>1</sub> /FVC        | 0.78±0.08              | 0.68±0.12     | <b>0.05</b>                   |
| RV % predicted               | 102±21%                | 155±74%       | 0.07                          |
| FRC % predicted              | 124±27%                | 129±38%       | 0.77                          |
| TLC % predicted              | 102±10%                | 108±32%       | 0.60                          |
| RV/TLC                       | 0.27±0.09%             | 0.40±0.12%    | <b>0.03</b>                   |

**Table 2b: Change in pulmonary function after maximal relaxation with albuterol (Mean±SD)**

| Measurement              | Healthy Controls (n=9)    | Asthma (n=19)                    | Between groups t-test p-value |
|--------------------------|---------------------------|----------------------------------|-------------------------------|
| % Δ FEV <sub>1</sub>     | 5±5%<br>(p=0.17)          | 17±11%<br>( <b>p&lt;0.0001</b> ) | <b>0.02</b>                   |
| % Δ FVC                  | -0.5±2.2%<br>(p=0.97)     | 6±6%<br>( <b>p&lt;0.0002</b> )   | 0.09                          |
| % ΔFEV <sub>1</sub> /FVC | 6±4%<br>( <b>p=0.03</b> ) | 10±8%<br>( <b>p&lt;0.0001</b> )  | 0.07                          |
| % Δ RV                   | 4±18%<br>(p=0.99)         | -15±24%<br>( <b>p=0.01</b> )     | 0.21                          |
| % Δ FRC                  | -8±8%<br>(p=0.18)         | -9±10%<br>( <b>p=0.01</b> )      | 0.69                          |
| % Δ TLC                  | 2±3%<br>(p=0.73)          | -5±6%<br>( <b>p=0.009</b> )      | 0.36                          |
| % Δ RV/TLC               | 2±15%<br>(p=0.99)         | -11±20%<br>(p=0.07)              | 0.06                          |

P values within groups represent paired comparisons of baseline versus post-albuterol maximum relaxation measurements using a mixed within-between groups ANOVA

**Table 3a: Baseline airway dimensions (Mean±SD)**

| Measurement                               | Healthy Controls (n=9) | Asthma (n=19) | Between groups t-test (p-value) |
|-------------------------------------------|------------------------|---------------|---------------------------------|
| Baseline diameter FRC                     | 5.19±0.64              | 5.06±1.0      | 0.74                            |
| Baseline diameter TLC                     | 5.91±0.83              | 5.68±1.3      | 0.63                            |
| Wall thickness (fraction of diameter) FRC | 0.24±0.04              | 0.31±0.06     | <b>0.006</b>                    |
| Wall thickness (fraction of diameter) TLC | 0.19±0.03              | 0.25±0.07     | <b>0.03</b>                     |
| % Δ diameter from FRC to TLC baseline     | 16±8%                  | 14±11%        | 0.62                            |

**Table 3b: Airway dimensions and airway wall thickness after maximum relaxation (Mean±SD)**

| Measurement                                                           | Healthy Controls (n=9)             | Asthma (n=19)                     | Between groups t-test (p-value) |
|-----------------------------------------------------------------------|------------------------------------|-----------------------------------|---------------------------------|
| Relaxed diameter FRC                                                  | 6.4±0.8                            | 6.2±1.0                           | 0.53                            |
| Relaxed diameter TLC                                                  | 6.6±0.9                            | 6.6±1.1                           | 0.91                            |
| % Δ diameter at FRC (baseline to relaxed)                             | 27±7%<br>( <b>p&lt;0.0001</b> )    | 27±16%<br>( <b>p&lt;0.0001</b> )  | 0.62*                           |
| % Δ diameter at TLC (baseline to relaxed)<br>(defined as airway tone) | 13±3%<br>( <b>p&lt;0.0001</b> )    | 22±17%<br>( <b>p&lt;0.0001</b> )  | 0.83*                           |
| Wall thickness (fraction of diameter) FRC after relaxation            | 0.18±0.03                          | 0.22±0.03                         | <b>0.002</b>                    |
| Wall thickness (fraction of diameter) TLC after relaxation            | 0.16±0.02                          | 0.19±0.03                         | <b>0.03</b>                     |
| % Δ Wall thickness (fraction of diameter) FRC (baseline to relaxed)   | 24.4±4.8<br>( <b>p&lt;0.0001</b> ) | 24.9±14<br>( <b>p&lt;0.0001</b> ) | 0.11                            |
| % Δ Wall thickness (fraction of diameter) TLC (baseline to relaxed)   | 14.4±3.6<br>( <b>p&lt;0.0001</b> ) | 21.8±13<br>( <b>p&lt;0.0001</b> ) | 0.92                            |
| % Δ diameter from FRC to TLC relaxed                                  | 3±4%                               | 10±9%                             | <b>0.046</b>                    |

P values within groups represent paired comparisons of baseline versus post-albuterol maximum relaxation measurements

\* analysis by mixed within-between groups ANOVA

Table 4a: Correlations between intra-individual airway tone heterogeneity and pulmonary function

|                                          | asthma            | health            |
|------------------------------------------|-------------------|-------------------|
| Baseline FEV <sub>1</sub> (% pred)       | r= 0.18, p= 0.45  | r= 0.11, p= 0.77  |
| Baseline FVC (% pred)                    | r= 0.17, p= 0.48  | r= 0.12, p= 0.76  |
| Baseline FEV <sub>1</sub> /FVC           | r= -0.15, p= 0.54 | r= -0.15, p= 0.69 |
| Baseline RV (% pred)                     | r= -0.16, p= 0.58 | r= -0.23, p= 0.58 |
| Baseline TLC (% pred)                    | r= -0.13, p= 0.65 | r= 0.13, p= 0.77  |
| Baseline RV/TLC                          | r= -0.17, p= 0.53 | r= -0.14, p= 0.73 |
| Post-albuterol FEV <sub>1</sub> (% pred) | r= 0.29, p= 0.23  | r= 0.00, p= 0.99  |
| Post-albuterol FVC (% pred)              | r= 0.24, p= 0.33  | r= 0.08, p= 0.84  |
| Post-albuterol FEV <sub>1</sub> /FVC     | r= 0.24, p= 0.32  | r= -0.26, p= 0.49 |
| Post-albuterol RV (% pred)               | r= -0.11, p= 0.69 | r= -0.44, p= 0.28 |
| Post-albuterol TLC (% pred)              | r= -0.15, p= 0.60 | r= 0.07, p= 0.87  |
| Post-albuterol RV/TLC                    | r= -0.05, p= 0.87 | r= -0.15, p= 0.72 |

Table 4b: Correlations between intra-individual airway tone heterogeneity and airway measurements

|                                                   | asthma                     | health            |
|---------------------------------------------------|----------------------------|-------------------|
| Diameter baseline FRC                             | r= -0.42, p= 0.07          | r= 0.48, p= 0.20  |
| Diameter baseline TLC                             | <b>r= -0.54, p= 0.02</b>   | r= 0.30, p= 0.44  |
| Diameter post-albuterol FRC                       | r= -0.04, p=0.87           | r= 0.21, p= 0.60  |
| Diameter post-albuterol TLC                       | r= -0.24, p= 0.33          | r= 0.35, p= 0.35  |
| WT (fraction dia) baseline FRC                    | <b>r= 0.48, p= 0.04</b>    | r= -0.31, p= 0.42 |
| WT (fraction dia) baseline TLC                    | <b>r= 0.59, p= 0.01</b>    | r= -0.34, p= 0.36 |
| WT (fraction dia) post- albuterol FRC             | r= -0.13, p= 0.61          | r= -0.25, p= 0.52 |
| WT (fraction dia) post-albuterol TLC              | r= 0.30, p= 0.22           | r= -0.43, p= 0.25 |
| %Δ diameter FRC-TLC baseline                      | <b>r= -0.49, p= 0.03</b>   | r= -0.14, p= 0.72 |
| %Δ diameter FRC-TLC post-albuterol                | <b>r= -0.51, p= 0.02</b>   | r= 0.50, p= 0.17  |
| %Δ diameter TLC baseline to post-albuterol (tone) | <b>r= 0.78, p&lt;0.001</b> | r= 0.09, p= 0.82  |

Table 5a: Correlations between airway tone and pulmonary function

|                                          | asthma  | health  |
|------------------------------------------|---------|---------|
| Baseline FEV <sub>1</sub> (% pred)       | p= 0.56 | p= 0.99 |
| Baseline FVC (% pred)                    | p= 0.43 | p= 0.57 |
| Baseline FEV <sub>1</sub> /FVC           | p= 0.75 | p= 0.32 |
| Baseline RV (% pred)                     | p= 0.32 | p= 0.93 |
| Baseline TLC (% pred)                    | p= 0.24 | p= 0.63 |
| Baseline RV/TLC                          | p= 0.59 | p= 0.72 |
| Post-albuterol FEV <sub>1</sub> (% pred) | p= 0.22 | p= 0.78 |
| Post-albuterol FVC (% pred)              | p= 0.23 | p= 0.59 |
| Post-albuterol FEV <sub>1</sub> /FVC     | p= 0.43 | p= 0.15 |
| Post-albuterol RV (% pred)               | p= 0.51 | p= 0.10 |
| Post-albuterol TLC (% pred)              | p= 0.16 | p= 0.92 |
| Post-albuterol RV/TLC                    | p= 0.71 | p= 0.19 |

Table 5b: Correlations between airway tone and airway measurements

|                              | asthma                       | health                   |
|------------------------------|------------------------------|--------------------------|
| Diameter baseline FRC        | <b>r= -0.59, p= 0.008</b>    | p= 0.39                  |
| Diameter baseline TLC        | <b>r= -0.72, p= 0.0005</b>   | p= 0.13                  |
| Diameter post-albuterol FRC  | p=0.53                       | p= 0.16                  |
| Diameter post-albuterol TLC  | p= 0.15                      | p= 0.27                  |
| WT (frac dia) baseline FRC   | <b>r= 0.70, p= 0.0012</b>    | p= 0.82                  |
| WT (frac dia) baseline TLC   | <b>r= 0.85, p&lt; 0.0001</b> | p= 0.78                  |
| WT (frac dia) post-alb FRC   | p= 0.72                      | p= 0.81                  |
| WT (frac dia) post-alb TLC   | p= 0.11                      | p= 0.75                  |
| %Δ diameter FRC-TLC baseline | <b>r= -0.60, p= 0.007</b>    | <b>r= -0.71, p= 0.03</b> |
| %Δ diameter FRC-TLC post-alb | <b>r= -0.49, p= 0.03</b>     | r= 0.21, p= 0.59         |