RESEARCH ARTICLE

Heterogeneity of airway wall dimensions in humans: a critical determinant of lung function in asthmatics and nonasthmatics

Christopher D. Pascoe,1,2 Chun Y. Seow,3,6 Tillie L. Hackett,4,6 Peter D. Paré,5,6 and Graham M. Donovan7

1Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, Manitoba, Canada; 2Children’s Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada; 3Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; 4Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada; 5Respiratory Division, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada; 6UBC Centre for Heart Lung Innovation, St. Paul’s Hospital, Vancouver, British Columbia, Canada; and 7Department of Mathematics, University of Auckland, Auckland, New Zealand

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Pascoe CD, Seow CY, Hackett TL, Paré PD, Donovan GM. Heterogeneity of airway wall dimensions in humans: A critical determinant of lung function in asthmatics and nonasthmatics. Am J Physiol Lung Cell Mol Physiol 312: L425–L431, 2017. First published January 6, 2017; doi:10.1152/ajplung.00421.2016.—Airway remodeling, a key feature of asthma, alters every layer of the airway wall but most strikingly the airway smooth muscle (ASM) layer. Airway remodeling in asthmatics contributes to fixed airflow obstruction and can amplify airway narrowing caused by ASM activation. Previous modeling studies have shown that the increase in ASM mass has the largest effect on increasing maximal airway narrowing. Simulated heterogeneity in the dimensions and properties of the airway wall can further amplify airway narrowing. Using measurements made on histological sections from donor lungs, we show for the first time that there is profound heterogeneity of ASM area and wall area in both nonasthmatics and asthmatics. Using a mathematical model, we found that this heterogeneity, together with changes in the mean values, contributes to an increased baseline resistance and elastance in asthmatics as well as a leftward shift in the responsiveness of the airways to a simulated agonist in both nonasthmatics and asthmatics. The ability of heterogeneous wall dimensions to shift the dose-response curve is largely due to an increased susceptibility for the small airways to close. This research confirms that heterogeneity of airway wall dimensions can contribute to exaggerated airway narrowing and provides an actual assessment of the magnitude of these effects.

asthma; remodeling; airway hyperresponsiveness; airway resistance

ASTHMA IS A CHRONIC RESPIRATORY DISEASE characterized by profound changes to the structure of the airway wall, termed airway remodeling. Airway remodeling affects every layer of the airway wall and includes epithelial shedding and goblet cell metaplasia (9, 28), thickening of the basal lamina, airway smooth muscle (ASM) hypertrophy and hyperplasia (15, 39), accumulation of connective tissue in the subepithelial and adventitial spaces (5, 17), and angiogenesis of the bronchial vasculature (32). The remodeling represents a repair process in response to chronic inflammation (12) and results in a thickening of the entire airway wall (2). Airway remodeling can contribute to airway narrowing in asthma by encroaching on the airway lumen and, more importantly, by amplifying the airway narrowing caused by smooth muscle contraction (27). Lambert et al. (20) developed a computational model of the tracheobronchial tree to quantify the functional consequences of airway wall remodeling. They predicted that, if the ASM contractile phenotype is preserved, increased airway smooth muscle mass would have the largest impact, largely accounting for the increased maximal airway narrowing in asthma. Lambert et al. (20) used morphometric measurements of the dimensions of the airway wall compartments to construct the model of the asthmatic and nonasthmatic airway tree but assigned only one value for each parameter to each generation of the tree, that is, mean values without any heterogeneity. There is ample evidence that airway properties are heterogeneous, not only in terms of airway length (40), but also in other properties such as wall and ASM thickness (15, 39). A number of investigators have shown that heterogeneity of airway properties and of smooth muscle activation could contribute to exaggerated airway narrowing (7, 34). However, in the absence of data, these theoretical models were based on the assumption that heterogeneity followed a normal or log-normal distribution. In this paper, we directly measured the distributions of airway wall dimensions in both nonasthmatic and asthmatic human lungs and identify greater mean values and variation in the ASM mass and airway wall area in asthmatic compared with nonasthmatic subjects. Wall area and ASM are both log-normally distributed and also significantly correlated. Using these data, we revisit theoretical predictions on the impact of heterogeneity and show that this variation can contribute, not only to an increase in maximal airway narrowing, but also to a shift of the airway dose-response curve to the left, faithfully reproducing characteristics of airway hyperresponsiveness (AHR).
METHODS

Data Collection

Data on airway compartment dimensions were obtained from the airways of lungs obtained by informed, written consent through the University of British Columbia and St. Paul’s Hospital ethics committee. In brief, after surgical removal, the lungs were flushed with Custodiol HTK solution (Odyssy Pharmaceuticals, East Hanover, NJ) and transported on ice by plane to our laboratory. The time between harvesting and arrival at the University of British Columbia was 15–20 h. Tissues from the lungs have been used in previous studies (4, 10). Lungs were inflated with Cryomatrix (Shandon, Pittsburg, PA) that was diluted by 50% with saline until they appeared fully inflated and then rapidly frozen over liquid nitrogen vapors. We have found that this method of fixation approximates an inflation pressure of at least 20 cm H2O. In any case, lung inflation is not a critical factor in determining airway wall compartment volume because the marker of airway size (basement membrane length), is only minimally affected by the degree of lung inflation (18). Incompletely inflated airways fold, but the relationships between basement membrane perimeter (P_bbm) and wall areas are unaffected.

Each lung was systematically, randomly sampled across lung height, resulting in 20 15 mm × 25 mm lung cores. From these cores, two were randomly sampled, formalin fixed, and paraffin embedded in hematoxylin and eosin. Quantification of airway compartment sizes has been previously described (4). In brief, a grid of ~3,500 points was calculated data (denoted by the overbar) are now effectively independent. Median age, yr (range) 20 (4–63) 15 (8–36)

Table 1. Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>Non-asthma</th>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Median age, yr (range)</td>
<td>20 (4–63)</td>
<td>15 (8–36)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>4 (55.6)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Inhaled corticosteroids, n (%)</td>
<td>0 (0)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>2 (22.2)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>End of life steroids, n (%)</td>
<td>3 (33.3)</td>
<td>9 (75.0)</td>
</tr>
<tr>
<td>Fatal asthma, n (%)</td>
<td>N/A</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Average airway size (P_bbm), mm</td>
<td>3.10 ± 0.25</td>
<td>3.50 ± 0.27</td>
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</tbody>
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P_bbm, perimeter of the basement membrane; N/A, not applicable.

of P_bbm and are fitted to a bivariate log-normal distribution, for both the asthmatic and nonasthmatic data. This rescaling allows us to determine how the distributions change with airway size, even though there are not enough data to fit separate distributions for each airway generation. There are then five parameters needed to determine the distribution: μ_ASM, μ_WA, σ_ASM, σ_WA, and μ. These are, respectively, the means (μ) and standard deviations (σ) of the associated normal distributions for each variable, and μ is the correlation between ASM and WA. For nonasthmatic subjects, the parameters are μ_WA = −5.9, σ_WA = 0.6, μ_ASM = −1.6, σ_ASM = 0.4, and the correlation coefficient μ = 0.73. For asthmatic subjects, the parameters are μ_WA = −5.25, σ_WA = 0.56, μ_ASM = −1.35, σ_ASM = 0.3, and the correlation coefficient μ = 0.68. It is important that the two variables (ASM and WA) are correlated and drawn from a bivariate distribution; they are not modeled as independent variables (see Appendix).

Simulated lung function. Following the work of Gillis and Lutchen (7) and Thorpe and Bates (34), we employ a circuit analog model to calculate impedance, using simulated lungs. Here the simulated lungs are constructed using airway tree geometry acquired from CT for the larger airways and generated algorithmically, where CT acquisition is impractical (33); all simulations in this study are performed using a single airway tree geometry (branching pattern and airway lengths) but with heterogeneity of ASM and WA as described below. Thus in the model there is (fixed) heterogeneity in the branching pattern and airway lengths and (variable) heterogeneity in ASM and wall area. The simulated lungs are produced using a model based on that described previously (30). Briefly, for each airway, airway caliber after an ASM stimulus is determined by calculating ASM shortening with the data of Gonem et al. (8). It is important to note that this calibration parameter for encroachment are considered in DISCUSSION.

For each simulation and each airway, ASM and WA are drawn from the bivariate log-normal distribution and scaled to the size of that target airway by reversing the scaling transformation: ASM = ASM × P_bbm^1.875 and WA = WA × P_bbm^1.47.

We also introduce a calibration parameter to assign the direction of wall thickening relative to the average data of Lambert et al. (21). That is, if an airway has thicker or thinner ASM or WA than average for that location, does the extra area impinge inward on the lumen or expand outward? To determine this calibration, airway impedance values were determined from simulated lungs with the calibration parameter ranging from 0% inward (100% outward) up to 100% inward (0% outward) in 5% increments. Using zero ASM tone in the simulations, we compared these model simulations of impedance with the human lung impedance data of Gonem et al. (8) for both asthmatic and nonasthmatic groups; specifically, we use their postbronchodilator data, under the assumption that the bronchodilator eliminates all tone. For each value of the calibration parameter, five simulated lungs were constructed, and their impedances were averaged. Using this method, we found that 85% inward calibration provided the best agreement with the data of Gonem et al (8). It is important to note that this calibration only determines the proportion of the additional airway wall structure that is toward the lumen, relative to the assumed baseline average luminal radius of the Lambert et al. model (21). It is not a direct measure of encroachment in asthmatics relative to nonasthmatics; the implications of this calibration parameter for encroachment are considered in DISCUSSION.
ponent: 1) the maximal isometric ASM force exerted is assumed to be directly proportional to ASM mass (which varies according to the statistical model), and 2) the specific stretch dependence model is removed because here we are not concerned with the response to deep inspiration [following the notation used previously (30), we have the maximal pressure exerted by the smooth muscle $\kappa = \kappa_\times \times$ ASM].

In addition to assessing the effect of airway heterogeneity on baseline airway impedance, simulated dose-response curves were constructed. Four simulated dose-response curves were created: 1) airway dimensions of nonasthmatic subjects without heterogeneity (mean values for dimensions for each generation were used), 2) airway dimensions of nonasthmatic subjects incorporating heterogeneity, 3) airway dimensions of asthmatic subjects without heterogeneity, and 4) airway dimensions of asthmatic subjects incorporating heterogeneity. Progressively increasing smooth muscle activation simulating doubling doses of a contractile agonist and the resulting shortening were determined by calculating the balance between the ASM stress and the loads impeding smooth muscle shortening (airway compliance and lung elastic recoil). To characterize changes in lung function, we measured the airway resistance, elastance, and reactance over the range from 1–32 Hz with data being reported at 5 Hz because this is common in the present literature. The degree of ASM activation was related to methacholine dose using the human ASM data of Ijpma et al. (14).

**Statistical Analysis**

Data on morphometric measurements are presented as means ± SE and taken as the average of multiple airways from each subject. Due to the skewed distributions of the data, statistical comparisons between means were done using an unpaired t-test with Welch’s correction for unequal variances. Results are considered significant at $P < 0.05$. Histograms were plotted using data from individual airways (78 nonasthmatic and 129 asthmatics) and were not normally distributed. An F-test was used to determine whether variances from each distribution were significantly different. Data from the computer model were fit using a dose-response curve. All graphs were plotted in GraphPad Prism 5 (La Jolla, CA).

Fig. 1. Histograms showing distribution of airway smooth muscle (ASM) mass (A) and wall area (WA) (B) across nonasthmatics and asthmatics. Top: coefficient of variation (%CV) is shown. Differences between distributions were tested using F-test. Distributions were significantly different between nonasthmatics and asthmatics for both ASM mass and WA. Correlation between WA/basement membrane perimeter ($P_{bm}$) and ASM/$P_{bm}$ in airways from nonasthmatics (C) and asthmatics (D). Data are Ln transformed and fit with a linear regression. $R^2$ values and slopes are shown.
RESULTS

Characterization of Airways

The average airway size as measured by $P_{bm}$ was not significantly different between nonasthmatic and asthmatic airways ($3.10 \pm 0.25$ vs. $3.50 \pm 0.27$ mm, $P > 0.05$, respectively). Airways from asthmatic subjects had a greater ASM area/$P_{bm}$ ($0.0170 \pm 0.0024$ mm$^2$/mm) compared with nonasthmatics (ASM area/$P_{bm}$ $0.0085 \pm 0.0010$ mm$^2$/mm, $P < 0.01$) whereas WA/$P_{bm}$ ($0.480 \pm 0.045$ mm$^2$/mm) in asthmatics was not significantly different compared with nonasthmatics (WA/$P_{bm}$ $0.340 \pm 0.035$, $P = 0.09$). In addition to having more ASM, the variation in the mass of ASM around the airways was significantly greater in asthmatics [coefficient of variation (CV), 97.3%] than in nonasthmatic airways (CV, 73.5%, $F$-test $P < 0.05$). The variation in WA asthmatics (CV, 55.9%) was also slightly but significantly greater than in nonasthmatics (CV, 54.6%, $F$-test $P < 0.05$). The distributions of ASM area and WA in asthmatics and nonasthmatics can be seen in Fig. 1, A and B. There was a significant correlation between ASM mass and WA in both nonasthmatics (Fig. 1C) and asthmatics (Fig. 1D).

Impact on Respiratory Impedance

The heterogeneity of the ASM layer and total airway wall influenced baseline airway impedance and had a profound impact on the ability of ASM to narrow the airways and increase airway impedance (Fig. 3); this is true in both asthmatics and nonasthmatics. In concordance with the results of Lambert and Oliver (21, 29), the dose-response curves that were generated using dimensions from asthmatic airways without heterogeneity showed an abrupt and marked increase in impedance in response to increasing ASM activation compared with the dose-response curves generated using nonasthmatic airway dimensions without heterogeneity. When heterogeneity was incorporated into the model, there was, in addition, a substantial leftward shift in the asthmatic curves that mimicked the increase in airway sensitivity to contractile agonists seen in asthmatics. The introduction of heterogeneous airway dimensions altered the amount of methacholine necessary to double the baseline resistance at 5 Hz, alternatively called the PC200. For airways modeled from asthmatic dimensions, the PC200 with the incorporation of heterogeneity was 9.69 nM of methacholine compared with 42.40 nM in the airways with no heterogeneous dimensions. The same was true for PC200 of elastance at 5 Hz (9.42 vs. 51.10 nM methacholine, heterogeneity vs. no heterogeneity) and for PC200 of airway reactance (9.03 vs. 48.10 nM methacholine, heterogeneity vs. no heterogeneity) (Fig. 2). In nonasthmatic airways, the addition of heterogeneous wall dimensions resulted in a PC200 for airway resistance of 94.7 nM methacholine compared with 894.9 nM in airways with no heterogeneity (Fig. 3). A similar result was seen in the nonasthmatic curves for elastance and reactance.

AHR in human subjects is typically quantified by calculating the PC$20_0$, the concentration of agonist that results in a 20% decrease in FEV$_1$. Short et al. (31) showed that a 20% decrease in FEV$_1$ is equivalent to an ~43.5% increase in resistance at 5 Hz. Using this threshold, Fig. 4 shows the concentration of methacholine required to reach a 43.5% increase in $R_5$ (PC$R_{43.5}$). When heterogeneity was present, the nonasthmatic lung needed ~1/15 the concentration of methacholine compared with the lung without heterogeneity to reach this threshold value (PC$R_43.5$ 31.2 vs. 475.0 nM M methacholine). The asthmatic modeled lung with heterogeneity needed ~1/9 the concentration of methacholine compared with the lung with heterogeneity to reach this threshold (PC$R_{43.5}$ 3.75 vs. 33.80 nM methacholine, Fig. 4). The difference in PC$R_{43.5}$ between asthmatic airways and nonasthmatic airways actually de-

\[ \text{PC}_{R_{43.5}} = \text{Log MCh Dose} \]

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\[ \text{Log MCh Dose} \]
increased with the incorporation of heterogeneity (14.1 vs. 8.3×). This difference can be appreciated when comparing the EC50 values for methacholine between asthmatic and nonasthmatic lung with heterogeneity, 56.2 nM vs. 4.9 μM (1.94-fold difference), and asthmatic and nonasthmatic airways without heterogeneity, 91.2 nM vs. 16.2 μM (2.25-fold difference).

**DISCUSSION**

Airway remodeling is a key feature of asthmatic airways, and it is known that remodeling can have an important effect on baseline airway distensibility (37) as well as the response of the airway tree to contractile agonists (20). In this study, we have extended the previous analyses by incorporating measures of heterogeneity in airway dimensions and show that heterogeneity further amplifies the effects of remodeling and importantly causes a considerable leftward shift in the airway dose-response curve. However, the model also suggests that it is the increase in airway wall dimensions, rather than increased responsiveness between asthmatics and nonasthmatics. There is also considerable heterogeneity of airway dimensions even in nonasthmatics, and this heterogeneity produces a leftward shift in the dose-response curve, relative to the zero heterogeneity case. This shift is similar in magnitude to the equivalent shift of the asthmatic airway (albeit the nonasthmatic curves still reside in the μM range of the methacholine dose-response curve).

Previous modeling studies (7, 34) have shown the potential impact of heterogeneity on function based on hypothetical distributions of airway dimensions and properties, but here we have shown the functional impact based on measured distributions. Importantly, in terms of functional consequences, both ASM and WA are log-normally distributed, and the two variables have significant correlation (Fig. 1, C and D). On the basis of our data, the increase in baseline resistance seen in asthmatics appears to be dependent on the heterogeneity of airway remodeling and not just on the increase in ASM mass and wall area. A smaller increase in baseline resistance was seen when heterogeneity of wall dimensions was included in the nonasthmatic simulations.

Our data show significant heterogeneity in both nonasthmatic and asthmatic airway wall dimensions and significantly greater heterogeneity in asthmatics. It is unclear how much this variation in wall dimensions is reflected in variation in the lumen diameter. Airways with a thicker wall might be narrower if the thicker wall encroaches on the lumen or the thicker wall could extend outward into the surrounding parenchyma without affecting luminal dimensions. Thicker walls that extended outward might alter mechanical properties but not baseline luminal radius; thicker walls that encroach inward would impinge on the lumen at baseline and increase the impedance of those airways. To assign a degree of baseline luminal impingement by the airway wall, we varied the degree of encroachment and used the computational model to match the impedance values of the lung to those reported by Gonem et al. (8) for in vivo postbronchodilator impedance. The computational model suggests that the balance is skewed toward inward remodeling, with ~85% of the wall and ASM area encroaching in an inward direction and hence contributing to a lower baseline luminal area. A more specific calculation of the additional encroachment driven by remodeling would require generational morphometric data about the remodeling, which is not available in this data set.

We transformed the ASM activation in our model to methacholine dose using published human data for in vitro re-
sponses (14) to facilitate comparisons to in vivo data. Although this mimics the logarithmic relationship between concentration of contractile agonist and activation, the actual concentration scale is much lower in the model than is observed in vivo (PC R4.3,5 ~0.6 μg/ml rather than ~1.0 mg/ml). There are several potential sources for this quantitative discrepancy, but the most likely reason is that the concentration of agonist in the aerosol solution and the actual concentration at the muscle in vivo are very different. In vivo most of the nebulized drug does not reach the lower airway, and the fraction that does needs to penetrate the airway epithelium and avoid local metabolism and uptake in the bronchial microcirculation before acting on the smooth muscle. We did not model heterogeneous deposition of the agonist in the airways or account for heterogeneity in the thickness or composition of the epithelial layer in our model. Nonetheless, the dose-response curves do faithfully reflect the relative changes in sensitivity related to heterogeneity in airway dimensions.

The model assumes that the maximal isometric force the ASM can exert on the airways is proportional to its mass. There is ample evidence from in vitro studies that ASM contractile force is unchanged in the airways of asthmatics compared with nonasthmatics (1, 3, 4, 14) when normalized to the increased ASM mass. There is, however, the possibility that the muscle can undergo a switch to a more synthetic/proliferative state that is marked by a reduction in contractile gene expression (11). In this case, the response of the asthmatic model would be attenuated (relative to the assumption of force being proportional to mass). However, most literature suggests that this is not the case and that ASM from asthmatics has similar or slightly elevated levels of contractile genes and proteins to nonasthmatics (23, 25, 26, 38). There is evidence suggesting that calcium homeostasis is altered in asthma (24, 35), and this may result in elevated ASM contractility; the model does not include any calcium sensitization effects.

The data and analysis presented here suggest several future improvements that would better inform our model and our understanding of the consequences of airway remodeling in asthma. Perhaps the most important is the potential effect of spatial correlations of heterogeneity within the lung. In the absence of spatial correlation data, we have assumed in the model that all airways are uncorrelated; however, theoretical studies have demonstrated that there are several different ways in which spatial correlations could be important. One obvious example is regional or lobar correlations, but it is also possible for more subtle effects relating to variation within and across branching airway pathways to have a major impact (6, 22, 36). It is known that remodeling and wall area depend on airway size, e.g., Hirota et al. (13) identified variation in airway remodeling in different generations in a mouse model of chronic asthma, and in 2012 Kurashima et al. (19) identified the same variation in percent wall area by generation in human asthmatics. Our model accounts for variation in the area distributions with airway size; however, it remains unclear how correlations within and across airway generations might influence flow via pathways in series and parallel. Although the present data set does not allow for specific modeling of these spatial correlations, it is important to acknowledge that they may be important.

The model simulations show a clear leftward shift in the dose-response curve attributable to heterogeneity, but it is also possible to delve deeper into the simulations to understand why this may be the case. By directly examining the distribution of airway caliber, it is clear that this leftward shift is caused primarily by closure (or near closure) of the small airways and that the propensity of the small airways to closure is strongly influenced, not only by mean values of ASM area and WA, but also by the heterogeneity and correlation of ASM area and WA. That is, in a heterogeneous population of small airways, those with thicker walls will be predisposed to close at lower agonist doses, as will those with greater smooth muscle mass. Because these variables are both log-normally distributed and also correlated, there is a “heavy tail” of small airways that have both increased ASM and WA and as such are prone to close at relatively low agonist doses, thus producing the leftward shift in the dose-response curve.

APPENDIX

We have used two different normalizations of measured ASM area and WA: linear normalization by FWA and also normalization by Fbm using a scaling exponent (see Statistical model of ASM and WA variation). The purpose of the scaling exponents is to render the area data effectively independent of Fbm so that the statistical model accounts for changes in the distributions of ASM area and WA with airway size. Figure 4 gives scatter plots of the data to demonstrate the scaling relationships in the data; A and B show that the scaling exponents linearize ASM area and wall area, respectively. Black crosses give nonasthmatic data, and red circles give asthmatic. Linear regression lines are also shown for each group. C shows the ratio of ASM area to wall area, on a log-log scale, with the same data on a linear scale in the inset.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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