Articles in PresS. J Appl Physiol (June 25, 2004). 10.1152/japplphysiol.00300.2004

OSCILLATION MECHANICS OF THE HUMAN LUNG PERIPHERY IN ASTHMA

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ABSTRACT

To more precisely measure the mechanical properties of the lung periphery in asthma, we have developed a forced oscillation technique that applies a broad-band flow signal through a wedged bronchoscope. We interpreted the data from 4 healthy and 8 mild asthmatic subjects in terms of an anatomically accurate computer model of the wedged segment. There was substantial overlap in impedance between the two groups, with resistance (R) showing minimal frequency dependence and elastance (E) showing both positive and negative frequency dependence across subjects. Following directly instilled methacholine, R rose in both groups, but compared to healthy subjects, the asthmatic subjects displayed upward, parallel shifts in their dose-response curves. The baseline frequency-response patterns of E were enhanced following methacholine. Frequency-dependencies of R and E were well reproduced in 2 normal subjects by a computational model that employed rigid airways connected to constant-phase tissue units, but were better reproduced in the other 2 normals and 3 asthmatics when the model employed heterogeneous, peripheral airway narrowing and compliant airways. To capture the frequency-dependencies of R and E in the remaining 5 asthmatics, the model was modified by increasing airway wall stiffness. These results indicate that the lung periphery of mild asthmatic subjects is not well distinguished from that of healthy subjects by measurement of mechanical impedance at baseline, but group differences are seen following challenge with methacholine. Modeling of the response suggests that variable contributions of airway narrowing and wall compliance are operative in determining overall mechanical impedance of the lung periphery in humans with asthma, likely reflecting the functional consequences of airway inflammation and remodeling.

KEY WORDS: asthma, forced oscillation technique, impedance, lung periphery, airway remodeling

INTRODUCTION

Traditional pulmonary function tests reflect information about global lung function, but obtaining more precise information from the lung periphery has been a challenge. Previously, we and others have taken advantage of the fiberoptic bronchoscope as a clinical tool that can be used to gain direct access to the lung periphery in living subjects (20-22, 39, 40). Studies to date have used the wedged bronchoscope to deliver a steady-state flow of gas into an isolated lung segment. Measuring the pressure generated by this flow allows calculation of the resistance of the collateral channels connecting the segment to the remainder of the lung. An estimate of segmental compliance can also be obtained from an analysis of the decay in pressure when flow is suddenly stopped (20, 22). However, this approach provides little information about airway resistance within the segment or the viscoelastic properties of the subtended lung region. This information can be obtained, in principle, by applying oscillatory flows into the wedged lung segment to determine the mechanical input impedance (Z_{seg}) of the segment (10).

The purpose of the present study was to develop a bronchoscopic method for determining Z_{seg} in order to further elucidate the nature of the lung periphery in asthma. We specifically sought to test the hypothesis that the responsiveness of the lung periphery to methacholine challenge is greater in subjects with asthma than in normal individuals. This hypothesis arises from the fact that asthmatics are hyperresponsive to methacholine in terms of overall lung function, that asthma has an important inflammatory component which is known to involve the lung periphery, and that airway smooth muscle shortening is predicted to lead to enhanced peripheral constriction in the setting of airway wall inflammation and remodeling (12). It thus seems

reasonable to suspect that the lung periphery would also respond in an exaggerated fashion to methacholine, but this has not yet been directly demonstrated.

METHODS

Human Subjects and Pulmonary Function Testing

Healthy subjects and subjects with asthma were recruited to participate in this study. All subjects were screened by medical history, physical examination and measurement of pulmonary function including spirometry and airways hyperresponsiveness to methacholine. Spirometry (FEV1 and FVC) was performed according to ATS guidelines (1) using a pneumotachograph (Collins GS, Braintree, MA). The PC20 of methacholine was measured according to ATS guidelines (2) using the 5 deep-breath method and doubling doses of methacholine from 0.03 $mg.ml^{-1}$ to 16 $mg.ml^{-1}$, or until the FEV_1 was reduced by at least 20%.

Healthy subjects had no history of smoking or lung disease, no pulmonary complaints, and a forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and ratio of FEV_1/FVC within normal ranges. In addition, all healthy subjects demonstrated no airway hyperresponsiveness to methacholine, defined as a provocative concentration of methacholine causing a 20% fall in FEV_1 (PC20) of >16 $mg.ml^{-1}$. Asthmatics met the NIH definition of asthma (29) were non-smokers, and had a PC20 < 8 $mg.ml^{-1}$. None of the subjects was tested within 4 weeks of any upper respiratory tract infection, and the asthmatic subjects withheld all short-acting bronchodilators for at least 8 hours and long-acting bronchodilators for at least 24 hours prior to testing. The protocol was approved by the Institutional Review Board of the University of Vermont, and all subjects provided written informed consent.

The subjects' characteristics are described in Table 1. A total of 4 healthy subjects and 8 subjects with asthma were studied. There were no significant differences in age, sex or height between the groups. The healthy subjects all had pulmonary function within normal limits (FEV1 = 98 ± 13% predicted) and no evidence of airways hyperresponsiveness. The asthmatics had pulmonary function ranging from normal to moderately reduced (FEV1 = 90 ± 15% predicted), and were hyperresponsive to methacholine (geometric mean PC20 = 1.28 mg/ml, range 0.13 – 6.5 $mg.ml^{-1}$).

Bronchoscopy

Bronchoscopy was performed in accordance with published guidelines (5) and as previously described (21). Subjects had nothing to eat for at least 8 hours prior to testing, and then were premedicated with an intramuscular injection of 0.6 mg atropine 30 minutes prior to testing to prevent excessive secretions. An intravenous line was started, and supplemental oxygen was provided at 1-6 $liters.min^{-1}$ by nasal cannula to maintain oxygen saturation by pulse oximetry at ≥ 90%. The upper airway was anesthetized with 2-4% lidocaine, and conscious sedation was provided intravenously with 1-4 mg midazolam and 25-100 μg fentanyl, as needed. Heart rate, blood pressure and arterial oxygen saturation were monitored throughout the procedure. We used a conventional bronchoscope (Olympus BF-XT40, Melville, NY) with an outside diameter of 6.3 mm and an instrument channel diameter of 3.2 mm. The bronchoscope was inserted orally or transnasally and passed into the airway tree until it wedged securely into a subsegment of either the right middle or right lower lobe.

Application of Oscillatory Flow

Oscillatory flow was generated by a computer-controlled ventilator (*flexiVent*, SCIREQ, Montreal). The piston of the *flexiVent* was programmed to deliver a broad-band volume perturbation signal composed of the sum of 17 sine waves having mutually prime frequencies between 0.50 and 19.75 Hz. The amplitudes of the sine waves were roughly hyperbolic with frequency and the signal had a duration of 8 s. The phases of the sine waves were chosen by random search to minimize the peak-to-peak amplitude excursion of the signal. The signals were then scaled to have various peak-peak amplitudes between 3 and 20 ml.

The volume displacements generated by the *flexiVent* piston were delivered to the instrument channel of the bronchoscope via plastic tubing with an inner diameter of 1 cm and length of approximately 30 cm, as diagrammed in Fig. 1. The instrument channel of the bronchoscope was cleared of any secretions with a flexible brush prior to measurements. The suction port of the bronchoscope was sealed during application of volume oscillations.

While volume oscillations were being applied to the wedged lung segment, the cylinder volume displacement of the *flexiVent* piston (V_{cyl}) and the pressure inside the *flexiVent* cylinder (P_{cyl}) were measured. All signals were low-pass filtered at 30 Hz and sampled at 128 Hz prior to being stored on a personal computer for later analysis. Before proceeding with the protocol, we dynamically calibrated the system to account for the elastance (compressibility) of the gas and resistive and accelerative losses due to gas flow in the cylinder-bronchoscope system by applying volume oscillations to the system in a closed and open state, respectively (see below).

Protocol

Measurements of V_{cyl} and P_{cyl} in response to the forced oscillations were made while the bronchoscope was maintained in the wedged position and the subjects suspended their breathing

at end-expiration (functional residual capacity, FRC). The absence of a leak was confirmed by the observation of appropriate positive and negative pressure swings when in a properly wedged position, whereas little if any pressure oscillations were recorded in an unwedged situation (since the pressure was quickly dissipated through the leak).

After resuming breathing for 3-5 breaths, repeat measurements were made. Measurements were repeated until 2 stable patterns of V_{cyl} and P_{cyl} were observed. This measurement sequence was used at all time points throughout the protocol.

Baseline measurements were obtained first. Then, after releasing the wedge, subjects took 3 deep breaths to establish volume history, and baseline measurements were again made. Each subject then received methacholine solution instilled directly through the bronchoscope into the wedged segment. The wedge was held for 30 seconds, and then released, allowing the subjects to ventilate the segment freely. The wedge was then re-established after 2 minutes and repeat measurements were made to assess the response of the segment to methacholine. Asthmatics initially received 0.001 mg of methacholine in a total volume of 1 ml of normal saline and, if P_{cyl} did not exceed 20 cm H_20, the instillation and measurement procedure was repeated with 0.01 mg. A further, maximal dose of 0.1 mg was used, again provided $P_{cyl} < 20$ cm H_20. In healthy subjects, the dose sequence administered was 0.01, 0.1, 1.0 mg using the same dose-measurement sequence as for the asthmatic subjects. These doses represented the optimal ranges we determined from earlier experiments in other healthy subjects and subjects with asthma. Measurements were repeated after another series of 3 deep inhalations during which the bronchoscope wedge was again released to allow ventilation of the segment. Lastly, measurements were repeated in all subjects 2 min after 0.25 mg of albuterol in a total volume of 1 ml of normal saline was instilled directly into the wedged segment, and then again after a final

series of 3 deep inhalations. The bronchoscope was then removed and the subjects were placed under direct medical supervision for the next 1-2 hours prior to their discharge from the research suite.

Data Analysis

Impedance of the wedged segment, Z_{seg}, was calculated as follows. First, the ratio between the cross-power spectrum of P_{cyl} with V_{cyl} and the auto-power spectrum of V_{cyl} was calculated in the Fourier domain to yield a transfer function $H(f)$. The calculation was performed by dividing the data records into five 4-second windows, overlapping by 75%. The first window was discarded and the fast Fourier transforms of the remaining windows were calculated. The cross- and auto-power spectra for the remaining blocks were calculated from their transforms and averaged prior to being divided to yield $H(f)$. Only $H(f)$ with coherence values >95% were retained for analysis.

Next, $H(f)$ was corrected for gas compressibility within the ventilator cylinder and for resistive and accelerative losses along the pathway to the distal end of the bronchoscope using the approach described previously (31). Specifically, we first obtained dynamic calibration signals of P_{cyl} and V_{cyl} from the *flexiVent* by applying the volume perturbation through the bronchoscope when its distal end was completely closed. These data were processed in the same manner as was described above and divided in the Fourier domain to yield a closed transfer function $H_C(f)$. Next, the perturbations were delivered through the bronchoscope with its distal end completely open to the atmosphere, and a second, open transfer function $H_O(f)$ was calculated between P_{cyl} and V_{cyl}. Examples of such analyses for the real and imaginary parts of $H_C(f)$ and $H_O(f)$ are shown in Fig. 2.

The real part of $H_C(f)$ reflects the compressibility of the gas in the cylinder-bronchoscope system and is relatively constant over the frequency range investigated, with the slight increase with frequency perhaps due to a progression from isothermal to adiabatic compression conditions. The imaginary part of $H_C(f)$ is close to zero, as expected given that by blocking the end of the bronchoscope we essentially eliminate any flow throughout the system so that resistive losses are negligible. By contrast, the increasing imaginary part of $H_O(f)$ reflects the resistance of the bronchoscope to gas flow, while the decreasing real part reflects the inertance of the gas in the bronchoscope channel.

The next step in processing the data was to remove $H_O(f)$ and $H_C(f)$ from $H(f)$ to yield a transfer function $H_L(f)$ attributable only to the load beyond the distal tip of the bronchoscope (i.e. the subject). This was done according to the formula (9)

$$H_L(f) = \frac{[H_O(f) - H(f)]H_C{}^2(f)}{[H_C(f) - H_O(f)][H(f) - H_C(f)]} \tag{1}$$

Finally, $Z_{seg}(f)$ was calculated from $H_L(f)$ as

$$\begin{aligned} Z_{seg}(f) &= R(f) + iX(f) \\ &= -iH_L(f)/2\pi f \end{aligned} \tag{2}$$

where R is the real part of Z_{seg}, resistance, X is the imaginary part of Z_{seg}, reactance, and i is the imaginary unit. (Note that by performing most of our calculations in the frequency domain in terms of pressure and volume, rather than the more usual use of pressure and flow, we avoided introducing numerical error that would have arisen had we first differentiated volume to get flow. By finally converting a transfer function to an impedance through Eq. 2, we obtained an exact expression that was not affected by the inevitable error occurred in numerical differentiation). Elastance (E) was calculated as

$$E(f) = -2\pi f X(f) \quad (3)$$

In order to validate the ability of the system to make accurate measurements of impedance, we tested the measurement system in an *in vitro* experiment in which the bronchoscope was connected via a narrow tube to a 1 L glass flask. The flask was filled with copper wool to ensure isothermal conditions during compression and expansion of the gas inside. The resistance of the narrow tube was calculated to be approximately 0.08 $cmH_2O.s.ml^{-1}$ and the elastance of the gas in the flask was approximately 1 $cmH_2O.ml^{-1}$. When the impedance of the system was measured directly with the *flexiVent* (i.e. without using the bronchoscope) we obtained a *R* that decreased slightly with frequency, with a mean value of 0.061 $cmH_2O.s.ml^{-1}$. When impedance was measured through the bronchoscope, *R* differed from that obtained without the bronchoscope by an average of 0.004 $cmH_2O.s.ml^{-1}$ (~ 7%) over the same frequency range. We repeated the measurement with the bronchoscope bent at 90 degrees to mimic the shape of the bronchoscope when it would be inserted into a wedged lower airway, and found *R* to differ from its value without the bronchoscope bent by an average of 0.002 $cmH_2O.s.ml^{-1}$ (~ 3%). *E* measured without the bronchoscope was close to 1 $cmH_2O.ml^{-1}$ at low frequencies, as calculated, but became negative by about 12 Hz as the effects of gas inertance in the tubing became dominant. When measured through the bronchoscope, both straight and bent, the values of *E* over this frequency range differed from those obtained without the bronchoscope by an average of 0.11 $cmH_2O.ml^{-1}$ (11%).

Before proceeding with the protocol, we also assessed whether oscillatory flow was likely to be transmitted to the pleural space and hence influenced by chest wall mechanics. In 2 subjects, we measured esophageal pressure using a thin latex balloon on the end of a 100 cm catheter passed into the esophagus via the nares. The balloon was inflated with approximately 1 ml air and the

proximal end of the catheter was attached to a piezoresistive pressure transducer. Placement was confirmed by the occlusion test (3).

Figure 3 shows an example of the pressure at the distal end of the bronchoscope during application of a volume perturbation in each of the 2 subjects who had esophageal balloons in place. Pressure was estimated by subtracting the pressure drop along the bronchoscope channel from that measured in the *flexiVent* cylinder. Also shown are the corresponding esophageal pressure (P_{es}) signals. The excursions in P_{es} are small compared to those at the tip of the bronchoscope, indicating that the applied pressure oscillations were not transmitted through to the pleural space, at least as assessed by the esophageal balloon technique. We interpreted this finding to indicate that the pressure oscillations were likely not influenced by changes in pleural pressure that might occur with chest wall mechanical activity. In addition, the steady tracing of esophageal pressure demonstrates that the subjects were able to suspend their breathing at FRC, relax their inspiratory muscles, and maintain a constant lung volume. Interestingly, the oscillator pressure tracing from the asthmatic subject in Fig. 3 has larger high-frequency components than the tracing from the normal subject. This no doubt reflects the greater lung impedance in the asthmatic subject, particularly in terms of its resistive component, which would have amplified the high-frequency response in pressure to a given broad-band flow signal applied to the segment.

Computational Modeling

We constructed an anatomically-based computational model of a segment of the human lung in order to simulate impedance at the same frequencies as used experimentally to determiner Z_{seg}. The computational model employed a subset of the morphometric data used by Gillis and

colleagues (13) in their simulations of the impedance of an entire lung. The conducting airways of the model thus followed the asymmetrical branching scheme of Horsfield (16). The radius of each airway was chosen randomly from a Gaussian distribution having a mean appropriate to the airway order as per the data (16) and with a standard deviation set to various values. As our subjects were supine, we might expect a subtle decrease in radius compared to a vertical subject due to the ensuing decrease in lung volume (23). However, we neglected this in our calculations. Each of the most distal airways of the model terminated in an identical tissue unit having a constant-phase impedance (14). Poiseuille flow through the airways was assumed. In some simulations we assumed the airways to be rigid conduits, while in other simulations we allowed the airways of the model to be distensible to permit central airway shunting of applied oscillations in flow, as has previously been demonstrated to be a significant effect following bronchoconstriction (18). In some simulations, we also allowed the airway walls to have mass, assuming a tissue density of $1 g.ml^{-1}$ and a wall thickness that decreased with the size of the airway. The complete set of equations used for the model are given in the Appendix.

A key consideration in these simulations was deciding which airway should be the most proximal in the model. This is determined to a certain extent by the outside diameter of the bronchoscope, which was about 6 mm. However, the bronchoscope was wedged while the subject breathed, but the measurements of Z_{seg} were made at FRC when the airways would have relaxed around the end of the scope to make a good seal. Therefore, we would expect the normal diameter of the wedged airway to be somewhat smaller than the outside diameter of the scope. Thus, the bronchoscope was wedged in an airway of about the 5^{th} generation, i.e. that was reached after 4 bifurcations. Because the human lung is asymmetrical, however, a 5^{th} generation airway can correspond to a range of airway orders. In the human lung model, upon which we

based our simulations, the trachea was assigned order 35 (24). A 5th generation airway in this model can have an order anywhere from 21 to 31, depending on which sequence of branches is chosen as one proceeds into the lung from the trachea. The smaller the airway order, the smaller is the segment of lung it subtends. We found that starting our model simulations from an airway of order 25 gave baseline segmental impedances that were quantitatively similar to those measured in the normal subjects. The airway tree was continued in the model down to airways of order 7, as beyond that the contribution of bulk flow to gas transport is negligible and so would not be expected to contribute significantly to mechanical impedance (24).

Statistical Analysis

Descriptive data are summarized by mean ± SD, or median ± 25-75 interquartile range (IQR), as determined by data distribution. Outcome data are summarized by mean ± SEM or median ± 25-75 interquartile range, as appropriate . PC20 data are summarized by geometric mean and range. Categorical data were compared using Fischer's exact test, and continuous data compared using t-tests for normally distributed data, and Wilcoxon signed-rank test for skewed data. To determine the effects of methacholine on *R*, mean *R* data for low-frequency (0-5 Hz) and high frequency (5-20 Hz) ranges were compared to baseline mean data in the same range following the 0.01mg dose of methacholine, which was the only overlapping dose at which all subjects had data for comparison. Data on *E* and on *R* and *E* post-albuterol and deep inhalation were not compared because of extreme variability in the results. The overall dose-response to methacholine between groups was compared using unpaired t-tests for the difference in the 95% confidence intervals of the slope and intercept of the linear regression determination of the dose-response curves. Two-tailed p-values ≤ 0.05 were considered statistically significant.

RESULTS

Experimental:

Figure 4 presents baseline Z_{seg} obtained from all subjects. Two of the asthmatics had significantly higher baseline *R* compared to the other asthmatics (Fig. 4, top panel), and showed significant negative dependency on frequency. These two subjects (B2 and B5) also had the most widely varying values of *E* (Fig. 4B, bottom panel), had the lowest FEV1 of the group (68 and 75% predicted), and moderate PC20's (3.0 and 1.0 $mg.ml^{-1}$). Baseline *R* at low frequency between healthy subjects and subjects with asthma (including subjects B2 and B5) was not statistically different (0.05 (0.03-0.07) vs. 0.08 (0.05-0.57) cm H20.s.ml $^{-1}$, median (IQR), p= 0.11, respectively), nor was it different at high frequency (0.04 (0.02-0.06) vs. 0.06 (0.04-0.35) cm H20.s.ml $^{-1}$, p=0.15, respectively).

Methacholine instillation caused *R* to increase in all subjects, although to varying degrees that depended on the dose, as indicated in Fig. 5. Both healthy subjects and subjects with asthma had significant increases in *R* following methacholine at both low (percent change from baseline = 1631 ± 514%, p = 0.050; 1655 ± 814%, p=0.01, respectively) and high (percent change from baseline = 1048 ± 322%, p= 0.05; 737 ± 326%, p=0.01, respectively) frequencies. Post-methacholine *R* was not statistically different between healthy control subjects and subjects with asthma at low (0.68 (0.36-0.95) vs. 0.52 (0.40-3.14) cm H20.s.ml $^{-1}$, p=0.93, respectively) or high (0.36 (0.19-0.46) vs. 0.33 (0.28-1.22) cm H20.s.ml $^{-1}$, p=0.55) frequencies. However, when straight lines were fit to both the low and high frequency dose-response data from healthy and asthmatic subjects, the 95% confidence intervals around the slopes of the lines overlapped, while the confidence intervals either side of the intercepts did not overlap. Furthermore, in both

asthmatic and healthy subjects, the increases in the lower frequency portions of *R* (Fig. 5, top panel) were greater than the increases in the higher frequency portions (Fig. 5, bottom panel).

Because of extreme variability in *E*, which we suspect was due to the variable and opposing contributions of gas inertance and lung elastance, especially at frequencies > 5 Hz, summary statistical analysis was not performed on this parameter. Instead, we considered the generalized patterns of response. *E* exhibited two distinct among the different healthy and asthmatic subjects. The left panel of Fig. 6 shows an example of one such pattern, which begins with an increase in *E* with frequency followed by a descent below zero at the higher end of the frequency spectrum. The right panel of Fig. 6 shows the other pattern, a progressive increase in *E* with frequency.

The responses to albuterol and deep inhalation were also highly variable between subjects, precluding summary statistical analysis. Again, we focused on the general patterns of response. At baseline, a deep breath did not alter *R* or *E*, but after the highest dose of methacholine, a deep inhalation brought *R* and *E* closer to baseline in many individuals. Some subjects, however, had increases in *R* and *E*. In most cases, the effects of deep inhalation were seen across the frequency range, suggesting both proximal and distal effects. Albuterol, likewise, resulted in reductions in *R* and *E* toward baseline in almost all subjects, but in some subjects increases in *R* and *E* were observed. Finally, some subjects did not respond to albuterol alone, but did show reductions in *R* and *E* after further deep inhalations were taken following adminstration of albuterol. We believe these variable responses may reflect the distribution of albuterol having been affected by differing levels of constriction caused by prior administration of methacholine. Also, methacholine causes secretion of mucus, which could be redistributed with a deep inflation, again leading to unpredictable variations in Z_{seg}.

Computational:

Figure 7 shows impedances generated by the computer model with rigid airway walls under baseline (unconstricted) conditions together with the mean data obtained under control conditions from the normal subjects. The simulated data were generated with the airway dimensions used by Gillis and Lutchen (13) beginning with airway order 25, using a 16-run Monte-Carlo simulation in which the airway radii were randomly varied about their nominal mean values with a coefficient of variation of 25%. The latter value was chosen empirically to impose a moderate degree of heterogeneity of airway narrowing. The baseline simulations of R and E are similar in magnitude and shape to those observed experimentally for the normal subjects (Fig. 4), supporting the notion that our measurements of Z_{seg} are indeed reflective of the mechanical properties of a sub-segment of the lung. Addition of compliant airway walls and airway wall inertance (see Appendix) made no observable difference to the simulations.

Figure 8 shows the results of further Monte-Carlo simulations demonstrating how Z_{mod} is affected by mild bronchoconstriction. Keeping the airways rigid and reducing their diameters by a mean factor of 2, using a 25 % coefficient of variation, increases R at all frequencies but increases E only up to about 5 Hz. Above 5 Hz E starts to decrease and becomes substantially negative by 20 Hz. Making the airways compliant using A = 5 (A = constant of proportionality between airway E and radius, see Appendix) lessens the negativity of E at high frequencies. Increasing the compliance 5-fold (A = 1) prevents E from becoming negative at all frequencies below 20 Hz. Changing to a distal airway constriction pattern in which the terminal bronchioles narrow by a factor of 4 while all other airways narrow by a factor of 1.5 further elevates E at high frequencies. This is only marginally affected by the inclusion of airway wall inertance (see Appendix).

DISCUSSION

We have collected lung segmental impedance data from 0.50 to 19.75 Hz in human volunteers by applying forced oscillations in flow through a bronchoscope wedged in a 5th-generation bronchus. A major advantage of employing the forced oscillation technique directly to a peripheral lung segment is that the upper airway shunting that corrupts the technique when applied at the mouth (30) is avoided. In addition, unlike the global response measured when the forced oscillations are applied at the airway opening, our segmental technique allows a more precise localization of the response. In particular, the Z_{seg} measured reflects the mechanical properties of the wedged segment downstream from the tip of the bronchoscope (15).

Two important technical issues that must be addressed include the role of the chest wall, and the influence of collateral flow. We determined that esophageal pressure is virtually unaffected by the applied oscillations in volume. This implies that the volume changes induced in the wedged segment were not transmitted through to the chest wall. We surmise that these oscillations were instead accommodated by lateral distortions in the remaining un-wedged lung. This is understandable on the basis of the bulk modulus of lung parenchyma being much greater than its shear modulus (32). We conclude, therefore, that the Z_{seg} we obtain with our technique corresponds to the impedance of a lung segment alone.

Another important consideration is the influence of collateral ventilation. Our previous studies applying steady state flow of gas through the wedged segment were a direct measure of the resistance to flow leaking out of the segment (20). This resistance was due to the small airways that accommodate the collateral ventilation of the segment. We found that collateral resistance at baseline in normal subjects was approximately 3 cmH20.s.ml^{-1}, compared to 5 cmH20.s.ml^{-1} in

asthmatics. These values are 2 orders of magnitude higher than the baseline R we measured in our current subjects. This order of magnitude difference is similar to that found in the study by Hantos and colleagues (15). In addition, our previous modeling study on collateral resistance (20) determined that the time constant for decay of collateral flow was on the order of 2 sec, which is too long to have a significant effect at the frequencies involved in the present study (> 0.50 Hz); hence, there would be insufficient time to establish significant flow through the collateral channels during the forced oscillation manuever. In addition, if collateral flow were contributing to our measurement of Z_{seg}, we would have expected to see E reverse direction and approach zero as frequency decreased, which we did not observe. Thus, we believe that the contribution of collateral ventilation to our results was negligible, and hence we ignored it in our modeling analysis.

To our knowledge, only two other studies in the literature have used forced oscillation through a conduit to explore peripheral lung mechanics. In 1990, Csete and colleagues (8) applied a single frequency oscillation through a bronchoscope wedged into a segmental bronchus of ewes to measure the response of peripheral resistance to drugs altering blood flow to the lung periphery. The investigators demonstrated that vasodilation increases peripheral resistance, presumably because of vascular congestion. More germane to our interests, Hantos and colleagues (15) used forced oscillations through a wedged 2mm diameter catheter in dog lung segments to measure impedance by a wave tube method. They found that the peripheral lung behaved in a qualitatively similar to the whole lung, similar to our findings, with the impedance scaling to the volume of lung subtended.

We have shown that, among the small group of asthmatics and healthy individuals we studied, there is substantial overlap of baseline lung Z_{seg}. We did not expect this finding, as we presumed

that Z_{seg} would be sensitive to subtle changes in peripheral lung mechanics and thus reveal differences in healthy and asthmatic subjects. Interestingly, previous studies on collateral resistance, including our own, did demonstrate significant differences in peripheral resistance between healthy and asthmatic subjects at baseline (21, 22, 40). This suggests that changes in collateral airways may be a much better discriminator of functional differences between asthmatic and healthy subjects than changes in mechanical impedance of the lung. If so, this supports the view that subtle, early changes in lung function in asthma occur first in the most peripheral airways, particularly in the respiratory bronchioles and alveolar ducts that comprise the pathways for collateral flow (28) .

The differences in the nature of the lung periphery between healthy subjects and those with asthma become apparent upon stimulation with methacholine. Our findings clearly show that the lung periphery of asthmatics is hyperresponsive to methacholine. Hyperesponsiveness refers to increased sensitivity (leftward shift in dose-response curve), increased reactivity (increased slope of dose-response curve) or increased maximal response for a given dose (33). Our data support there being hyperresponsiveness of the lung periphery on the basis of increased maximal response, but less so on the basis of sensitivity and reactivity, all of which are features that may vary independently. Mechanisms that may be responsible for the increased maximal response in our subjects include increased contractility of smooth muscle, increased smooth muscle shortening, and geometric amplification of the response due to airway wall thickening (33), but we can not discriminate between these possibilities. In any case, our findings are in accord with previous work demonstrating that collateral resistance rises more in response to bronchocontrictive stimuli in asthmatic compared to healthy subjects (4, 21, 39, 40).

The pattern of hyperresponsiveness appears to be more operative at low than at high frequencies, implying a substantial contribution from the lung periphery and not just the larger airways immediately distal to the wedged bronchoscope. This is despite the fact that the methacholine was physically instilled just beyond the tip of the scope. Either the methacholine was able to move by bulk flow into the lung periphery due the fact that the subjects were supine and freely ventilating the segment during the 2 min wait period before measurements were made, or there were direct, proximal effects that had secondary, distal effects on lung mechanics. Such distal effects may be due to release of mediators, changes in blood flow or activation of neural pathways. This phenomenon of proximal stimulation with distal effect may be important in asthma pathogenesis, since many irritants and other stimulating factors, especially of large particle size, would be most likely to impinge directly on the central airways (7).

The observation that there was substantial increase in low frequency resistance following methacholine suggests that significant peripheral airway heterogeneous narrowing or closure was involved (13, 35). Kaczka and colleagues have shown that in both asthmatic (18) and healthy subjects (19) most of the increase in R (70%) is due to airway resistance, not tissue resistance. The concurrent positive frequency dependence of E in subjects in both their study and in ours suggests that a substantial component of the changes in low frequency R was due to heterogeneous and severe peripheral airway narrowing (18). Previous work suggests that little, if any, direct change in tissue rheological properties need occur to explain the frequency response of R and E (26, 35).

Computational modeling suggests that under baseline conditions, the lung behaves predominantly as a series of rigid airways attached to viscoelastic tissue units. However, under conditions of bronchoconstriction, two different patterns of behavior became apparent. Two of

our healthy subjects continue to behave as if they have rigid airways that constrict to methacholine, resulting in slight increases in *R* and very little change in *E*. The other two healthy subjects, and three of the asthmatics, appear to have an increase of *E* with frequency, indicating shunting of flow into central airways. This may be due either to more severe peripheral airway narrowing or to increased airway compliance. Our methods cannot distinguish these two possibilities. However, while the flow oscillations were being applied to our subjects we did observe lateral movement of the airway wall through the bronchoscope, particularly following methacholine, so the airways were certainly not completely rigid. The remaining five asthmatic subjects in whom *E* did not increase with frequency thus probably had relatively stiffer airway walls or a lesser degree of peripheral airway narrowing. Increased stiffness of asthmatic airways has been documented by other studies (6, 41), a condition that may be due to the effects of airway inflammation and remodeling. Alternatively, methacholine-induced smooth muscle constriction of the larger airways just distal to the wedged bronchoscope may have caused an increase in their stiffness (36) .

Our separation of asthmatic subjects into two distinctive groups is similar to the finding of Kaczka and colleagues who used whole-lung forced oscillation in asthmatic subjects (18). They found that asthmatics were distinguished at baseline by the degree of peripheral constriction, and subsequent central airway shunting (i.e. positive frequency dependence of *E*), with Type A having less peripheral constriction and no central shunting, and Type B having more peripheral constriction and the presence of central shunting. In their study, Type A asthmatic subjects appeared to have less severe asthma than Type B subjects on the basis of FEV1. Our results are similar in that asthmatic subjects either had or did not have evidence of central airway shunting, although the correlation with the degree of peripheral constriction was not found. We suspect

that this discrepancy between the two studies primarily stems from the fact that the Type B asthmatic subjects studied by Kaczka and colleagues were likely more severe (mean FEV1 = 70% predicted) than our group of asthmatic subjects (mean FEV1 = 90% predicted), in addition to other factors such as technique (whole-lung vs. peripheral oscillation), sample size (21 vs. 8 asthmatics), and study design (effect of albuterol vs. effect of methacholine). Importantly, we also suspect that the discrepancy arises because of the variable and unpredictable net effect of airway remodeling on airway constriction, with airway remodeling having the potential to both enhance airway narrowing by increasing wall thickness, as well as reduce airway narrowing by stiffening the airway wall (27).

Our model simulations support the notion that airway wall stiffness is an important determinant of Z_{seg}, in addition to the obvious contributions from airway caliber. Other studies have also incorporated airway wall mechanical properties into modeling of lung impedance (12, 25, 34), although ours is the first to apply this approach to data derived from living human subjects. Lutchen and colleagues presented the closest approach to our method by incorporating nonrigid airway walls with mass into a morphometrically accurate computational model (25). These authors found similar patterns of frequency response of R and E when varying the extent and degree of peripheral constriction as well as the compliance of the airway walls. Of course,we made a number of assumptions about exactly how wall stiffness should vary throughout the airway tree, and we did not incorporate the effects of smooth muscle constriction on airway wall stiffness. The effect of these assumptions was to make intrinsic stiffness decrease with airway size, as is readily justified on the basis of airway anatomy. The precise details of how it varies with airway size, however, is open to question. We do not claim that our formula is the only one possible, and indeed others have been used (e.g., (13, 25)), including a recent approach

incorporating heterogeneous tissue properties into the model (17). Our formula was chosen because it is mathematically straightforward, biologically plausible, and caused the model to reproduce the essential features of the experimental data.

In summary, we have shown that measurements of Z_{seg} made through a wedged bronchoscope in human subjects are comparable to those calculated from an anatomically accurate computer model, supporting the usefulness of the technique as an investigative tool in human lung disease. Similar to other studies (18, 24, 25, 35), our model analysis suggests that heterogeneous airway narrowing as well as airway wall stiffness are important factors in determining the mechanical impedance of the peripheral lung in asthma. Nevertheless, such changes are not readily apparent when comparing mild asthmatic subjects to normal individuals at baseline, unlike collateral resistance which is able to distinguish the two groups (21, 22, 40). However, when challenged with methacholine, separation of the two groups by Z_{seg} becomes apparent, with the asthmatic subjects reacting more to the methacholine than the healthy subjects. Given the increasing body of data defining inflammation and remodeling in the peripheral lung in asthma (37), we now have a method for determining the relative contributions of airway narrowing and wall properties to the functional effects of airway remodeling in asthma.

APPENDIX

***Computational model with rigid airways*:** If the airways of the computational model are assumed to be rigid conduits, the impedance of each airway branch is

$$Z_{aw}(f) = \frac{8\mu l}{\pi r^4} + i\frac{2\pi f l \rho}{r^2} \qquad \text{(A-1)}$$

where μ is the viscosity of air (1.8 x 10^{-7} g.cm $^{-1}$.s^{-1}), ρ is the density of air (1.2 x 10^{-6} g.cm $^{-3}$), r is the radius of the airway, and l is airway length. The first term in Eq. A-1 describes Poiseuille flow while the second term accounts for the mass of the gas in the airways.

The impedance of the entire model was determined at each value of f using a recursive subroutine that calculated the combined impedance of the current airway together with the impedances subtended by its two daughter airways (35). The two daughter impedances in turn had to be determined using the same subroutine. The subroutine thus kept calling itself as it worked its way from the segmental bronchus down to the terminal bronchioles. Once a terminal bronchiole was reached, the algorithm terminated its self-calling sequence and simply added the serial impedance of the subtended tissue unit to the already accumulated airway tree impedance.

Each of the most distal airways terminated in an identical tissue unit having an impedance Z_{ti} given by

$$Z_{ti}(f) = \frac{G_{ti} - iH_{ti}}{\omega^{\alpha}} \qquad \text{(A-2)}$$

where $\omega = 2\pi f$, G_{ti} and H_{ti} are parameters that characterize, respectively, the dissipative and elastic properties of the lung tissues, and

$$\alpha = \frac{2}{\pi}\arctan\frac{H_{ti}}{G_{ti}} \qquad \text{(A-3)}$$

This model of tissue impedance, proposed by Hantos et al (14), is frequently referred to as the constant-phase model, and is recognized as being an accurate description of tissue mechanics. We will use the elegant step of Ito et al. (17) who showed that the rather awkward units of G_{ti} and H_{ti} of $cmH_2O.s^{1-\alpha}ml^{-1}$ can be replaced with units of $cmH_2O.s.ml^{-1}$ if Eq. A-2 is defined in terms of ω/ω_0, where ω_0 is defined as having a value of 1. This does not change the numerical values of G_{ti} and H_{ti}. The ratio G_{ti}/H_{ti} was assigned a value of 0.1, which is typical of measured values in lung tissue strips (11) and was recently used by our group in a mouse model of asthma (38). The value of H_{ti} for each unit was chosen empirically to be 500 $cmH_2O.s^{1-\alpha}ml^{-1}$. As this model has 106,448 acini (13), this choice for H_{ti} gives a value of H_{ti} for the entire lung of 4.7 $cmH2O.l^{-1}$ which is similar to that of a normal adult human.

***Inclusion of compliant airway walls*:** Distensibility of airway walls was incorporated into the computational model by assuming that the tissues of the wall have the same constant-phase form of impedance as parenchyma (Eq. A-2). That is

$$Z_{wall}(\omega) = (\eta - i)\frac{H_{wall}}{(\omega/\omega_0)^{\alpha}} \qquad \text{(A-4)}$$

where H_{wall} is an elastic parameter. In order to determine how H_{wall} should depend on airway size, we will first determine an expression for static airway elastance from first principles. Assume that an airway accommodates increased volume only by radial expansion thus

$$\begin{aligned} dV &= \pi(r+dr)^2 l - \pi r^2 l \\ &\approx 2\pi r l dr \end{aligned} \qquad \text{(A-5)}$$

The resulting circumferential strain in the airway wall, assuming it to be a thin membrane, is dr/r. This causes an increase in tangential wall tension equal to $E_{wall}dr/r$ where E_{wall} is intrinsic wall tissue stiffness. The resulting increase in intra-luminal pressure (P) is given by Laplace's law:

$$\begin{aligned} dP &= \frac{2dT}{r} \\ &= \frac{2E_{wall}}{r^2}dr \\ &= \frac{E_{wall}}{\pi dr^3}dV \end{aligned} \tag{A-6}$$

where T is wall tension and E_{wall} is intrinsic wall tissue stiffness. However, airway walls get thinner as airways get smaller, so we make the additional assumption that E_{wall}, is proportional to r, with constant of proportionality A. This gives the following expression for the static elastance of the airway:

$$\frac{dP}{dV} = \frac{A}{\pi l r^2} \tag{A-7}$$

Substituting for dV from Eq. A-5 into Eq. A-7 and rearranging gives

$$\frac{dP}{(dr/r)} = 2A \tag{A-8}$$

showing that, in this model, a given increase in pressure across the airway wall produces the same fractional increase in airway radius for all airways regardless of size. If, for example, a dP of 1 cmH2O produced a 10% increase in r then A in Eq. A-8 would have a numerical value of 5. We used values for A of both 5 and 1 (Fig. 8).

H_{wall} is not exactly an elastance like dP/dV because its units are $cmH_2O.s^{1-\alpha}.ml^{-1}$ rather than $cmH_2O.ml^{-1}$. However, α is close to 1 and H_{wall} is numerically equal to elastance at a frequency of $1/2\pi$ Hz, so we can approximate H_{wall} in Eq. A-4 by the expression in Eq. A-7, to obtain

$$Z_{wall}(\omega) = (\eta - i)\frac{A}{\pi d r^2 \omega^{\alpha}} \tag{A-9}$$

***Inclusion of airway wall mass*:** If the thickness of the airway wall is h and its density is ρ (assumed equal to that of water), then its mass M is approximately $2\pi rlh\rho$. When the volume of the airway changes from $2\pi r^2 l$ to $2\pi(r + dr)^2 l$ the change in volume is approximately $2\pi rldr$. M is thus displaced by a distance dr. The force F producing this displacement is pressure P acting over an area of $2\pi rl$. Invoking Newton's second law gives

$$\begin{aligned} F &= Ma \\ &= 2\pi rlh\rho \frac{d^2 r}{dt^2} \\ &= \frac{2\pi rlh\rho}{2\pi rl}\frac{d^2 V}{dt^2} \\ &= h\rho \frac{d^2 V}{dt^2} \end{aligned} \tag{A-10}$$

But,

$$\begin{aligned} F &= 2\pi rlP \\ &= h\rho \frac{d^2 V}{dt^2} \end{aligned} \tag{A-11}$$

So,

$$P = \frac{h\rho}{2\pi rl}\frac{d^2 V}{dt^2} \tag{A-12}$$

If we assume that h is proportional to r, with constant of proportionality B, then

$$P = \frac{Br\rho}{2\pi rl}\frac{d^2V}{dt^2} = \frac{B\rho}{2\pi l}\frac{d^2V}{dt^2} \tag{A-13}$$

In the frequency domain this gives

$$P(\omega) = i\frac{fB\rho}{l}\dot{V}(\omega) \tag{A-14}$$

It remains to choose a value for B. According to the computational data of Gillis and Lutchen (13), h/r varies from 0.27-0.67 as one progresses from order 25 down to order 8; we chose the midrange value of 0.40.

The final complete expression for airway impedance thus becomes the parallel addition of Eq. A-1 to the sum of Eqs. A-9 and A-14.

ACKNOWLEDGEMENTS

This work was supported by a Center of Biomedical Research Excellence Grant from the National Center for Research Resources P20RR15557, grants from the National Heart Lung and blood Institute R01 HL-62746 and R01 HL-67273, the General Clinical Research Center of the University of Vermont (RR00109), and a grant from the Whitaker Foundation.

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TABLE 1 – SUBJECT CHARACTERISTICS

		AGE (yrs)	*SEX (m/f)*	*HEIGHT (cm)*	*WEIGHT (kg)*	*FEV1 (L,(%))*	*FEV1/FVC (%)*	*PC20 (mg/ml)*
NORMALS	B1	33	f	167	54	3.02 (97)	0.84 (107)	> 16
	B3	27	m	185	89	3.99 (80)	0.63 (75)	> 16
	B4	30	m	185	72	5.24 (107)	0.93 (112)	> 16
	B12	23	m	179	95	5.04 (109)	0.87 (105)	>16
ASTHMATICS	B2	41	m	175	82	3.03 (75)	0.56 (68)	3.0
	B5	28	m	184	116	3.05 (68)	0.55 (70)	1.0
	B6	23	m	183	76	5.08 (105)	0.79 (95)	0.34
	B7	49	m	178	78	3.39 (85)	0.66 (82)	0.48
	B8	29	f	174	88	3.66 (106)	0.80 (101)	6.5
	B9	24	f	174	73	2.94 (83)	0.69 (87)	0.13
	B10	20	f	161	68	3.77 (110)	0.78 (87)	4.6
	B11	42	f	157	59	2.34 (86)	0.78 (93)	3.78

% indicates percentage of predicted value
FEV_1 forced expiratory volume in one second
FVC forced vital capacity
PC20 provocative concentration of methacholine causing a 20% fall in FEV1

FIGURE CAPTIONS

Figure 1: Experimental set-up. The subject lies supine while the bronchoscope is wedged by the investigator into a subsegment of the right middle lobe. A plastic catheter connects the piston of the oscillator to the instrument channel of the bronchoscope, through which is delivered an oscillatory volume signal.

Figure 2: Example transfer functions between cylinder pressure (P_{cyl}) and cylinder volume (V_{cyl}) obtained both with the distal end of the bronchoscope closed (circles) and with it open to atmosphere (triangles). Closed symbols show real parts of the transfer function, open symbols are imaginary parts.

Figure 3: Pressure at the distal end of the bronchoscope (thin line) together with esophageal pressure (thick line) in the two subjects who had esophageal balloons.

Figure 4: Baseline resistance (R) and elastance (E) obtained from 8 subjects with asthma (open circles) and 4 healthy subjects (closed circles). Notice the R and E of the two subjects with asthma (B2 and B5) who were not in the same range as the other subjects.

Figure 5: Dose-response of resistance (R) to methacholine at low frequencies (0-5 Hz) and high frequencies (5-20 Hz) in healthy subjects (closed circles) and subjects with asthma (open circles).

Figure 6: Patterns of frequency response of resistance and elastance to methacholine at baseline (closed circles) and at two doses of methacholine (0.001 mg, open circles; 0.01 mg, closed triangles) in 2 representative asthmatic subjects.

Figure 7: Frequency responses of resistance and elastance simulated with a computational lung model in which airway radii were randomly varied around their mean value with a coefficient of variation of 25% and airway walls are rigid.

Figure 8: Frequency responses of resistance and elastance simulated with a computational lung model under 5 separate conditions: 1) rigid airways, with airway radii halved; 2) airways with low compliance, with airway radii halved; 3) airways with high compliance, with airway radii halved; 4) airways with high compliance, with central airway radii reduced by factor of 1.5, and peripheral airways reduced by factor of 4; and 5) same airway conditions as previous set, with addition of airway wall inertance.