Oscillation mechanics of the human lung periphery in asthma

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Submitted 22 March 2004; accepted in final form 21 June 2004

Kaminsky, David A., Charles G. Irvin, Lennart Lundblad, Henrique T. Moriya, Sherburn Lang, Jennifer Allen, Tracey Viola, Mary Lynn, and Jason H. T. Bates. Oscillation mechanics of the human lung periphery in asthma. J Appl Physiol 97: 1849–1858, 2004. First published June 25, 2004; doi:10.1152/japplphysiol.00300.2004.—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 four healthy and eight mildly 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 positive and negative frequency dependence across subjects. After direct instillation of methacholine, R rose in both groups, but compared with healthy subjects, the asthmatic subjects displayed upward, parallel shifts in their dose-response curves. The baseline frequency-response patterns of E were enhanced after methacholine. Frequency dependencies of R and E were well reproduced in two normal subjects by a computational model that employed rigid airways connected to constant-phase tissue units but were better reproduced in the other two normal and three asthmatic subjects when the model employed heterogeneous, peripheral airway narrowing and compliant airways. To capture the frequency dependencies of R and E in the remaining five asthmatic subjects, the model was modified by increasing airway wall stiffness. These results indicate that the lung periphery of mildly asthmatic subjects is not well distinguished from that of healthy subjects by measurement of mechanical impedance at baseline, but group differences are seen after 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.

forced oscillation technique; impedance; airway remodeling

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 fiber-optic 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 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 to the wedged lung segment to determine the mechanical input impedance of the segment (Zseg) (10).

The purpose of the present study was to develop a bronchoscopic method for determining Zseg 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 asthmatic subjects 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 been directly demonstrated.

METHODS

Human subjects and pulmonary function testing. Healthy and asthmatic subjects were recruited to participate in the study. All subjects were screened by medical history, physical examination, and measurement of pulmonary function, including spirometry and airway hyperresponsiveness to methacholine. Spirometry [forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC)] was performed according to American Thoracic Society guidelines (1) with a pneumotachograph (model GS, Collins, Braintree, MA). The provocative concentration of methacholine causing a 20% fall in FEV1 (PC20) was measured according to American Thoracic Society guidelines (2) using the five-deep-breath method and doubling doses of methacholine from 0.03 to 16 mg/ml, or until FEV1 was reduced by ≥20%.

Healthy subjects had no history of smoking or lung disease, no pulmonary complaints, and FVC, FEV1, and FEV1/FVC within normal ranges. In addition, all healthy subjects demonstrated no airway hyperresponsiveness to methacholine, defined as PC20 >16 mg/ml. Asthmatic subjects met the National Institutes of Health definition of asthma (29), were nonsmokers, and had PC20 <8 mg/ml. None of the subjects was tested within 4 wk of any upper respiratory tract infection, and the asthmatic subjects refrained from use of all short-
acting bronchodilators for ≥8 h and long-acting bronchodilators for ≥24 h before 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 four healthy subjects and eight subjects with asthma were studied. There were no significant differences in age, gender, or height between the groups. All healthy subjects had pulmonary function within normal limits (FEV1 = 90 ± 15% predicted) and no evidence of airway hyperresponsiveness. The asthmatic subjects had pulmonary function ranging from normal to moderately reduced (FEV1 = 90 ± 15% predicted) and were hyperresponsive to methacholine [geometric mean PC20 = 1.28 (range 0.13–6.5) mg/ml].

**Bronchoscopy.** Bronchoscopy was performed in accordance with published guidelines (5) and as previously described (21). Subjects had nothing to eat for ≥8 h before testing and then were premedicated with atropine (0.6 mg im) 30 min before testing to prevent excessive secretions. An intravenous line was started, and supplemental O2 was provided at 1–6 l/min by nasal cannula to maintain O2 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 O2 saturation were monitored throughout the procedure. We used a conventional bronchoscope (model BF-XT40, Olympus, 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 the right middle lobe. A plastic catheter connects the piston of the oscillator to the instrument channel of the bronchoscope, through which an oscillatory volume signal is delivered.

**Application of oscillatory flow.** Oscillatory flow was generated by a computer-controlled ventilator (flexiVent, SCIREQ, Montreal, PQ, Canada). 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 of 0.50–19.75 Hz. The amplitudes of the sine waves were roughly hyperbolic with frequency, and the duration of the signal was 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-to-peak amplitudes of 3–20 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 ~30 cm (Fig. 1). The instrument channel of the bronchoscope was cleared of any secretions with a flexible brush before 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 (Vcyl) and the pressure inside the flexiVent cylinder (Pcyl) were measured. All signals were low-pass filtered at 30 Hz and sampled at 128 Hz before 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.** Vcyl and Pcyl in response to the forced oscillations were measured 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 the bronchoscope was in a properly wedged position, whereas little if any pressure oscillations were recorded with the bronchoscope in an unwedged situation (because the pressure was quickly dissipated through the leak).

After breathing was resumed for three to five breaths, measurements were repeated until two stable patterns of Vcyl and Pcyl were observed. This measurement sequence was used at all times throughout the protocol.

Baseline measurements were obtained first. Then, after the wedge was released, subjects took three deep breaths to establish volume history, and baseline measurements were repeated. Methacholine solution was then instilled directly through the bronchoscope into the wedged segment. The wedge was held for 30 s and then released, allowing the subjects to ventilate the segment freely. The wedge was reestablished after 2 min, and measurements were repeated to assess the response of the segment to methacholine. Asthmatic subjects initially received 0.001 mg of methacholine in a total volume of 1 ml of normal saline and, if Pcyl did not exceed 20 cmH2O, the instillation-and-measurement procedure was repeated with 0.01 mg of methacholine. A further, maximal dose of 0.1 mg of methacholine was used, again if Pcyl was <20 cmH2O. In healthy subjects, the dose sequence was 0.01, 0.1, and 1.0 mg, with the same dose-measurement sequence that was used for the asthmatic subjects. These doses represented the optimal ranges we determined from earlier experiments in other healthy and asthmatic subjects. Measurements were repeated after another series of three deep inhalations, during which the bronchoscope wedge was again released to allow ventilation of the segment.

![Fig. 1. Experimental setup. 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 an oscillatory volume signal is delivered.](image-url)
Lastly, measurements were repeated in all subjects 2 min after instillation of 0.25 mg of albuterol in a total volume of 1 ml of normal saline directly into the wedged segment and then again after a final series of three deep inhalations. The bronchoscope was then removed, and the subjects were placed under direct medical supervision for the next 1–2 h before their discharge from the research suite.

Data analysis. \( Z_{\text{sys}} \) was calculated as follows. First, the ratio of cross-power spectrum of \( P_{\text{cyl}} \) with \( V_{\text{cyl}} \) to the auto-power spectrum of \( V_{\text{cyl}} \) 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-s 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 before they were 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 by use of the approach described previously (31). Specifically, we first obtained dynamic calibration signals of \( P_{\text{cyl}} \) and \( V_{\text{cyl}} \) from the flexiVent by applying the volume perturbation through the bronchoscope when its distal end was completely closed. These data were processed in the manner described above and divided in the Fourier domain to yield a closed transfer function \( H_{\text{c}}(f) \). Next, the perturbations were delivered through the bronchoscope, with its distal end completely open to the atmosphere, and a second, open, transfer function \( H_{\text{o}}(f) \) was calculated between \( P_{\text{cyl}} \) and \( V_{\text{cyl}} \). Examples of such analyses for the real and imaginary parts of \( H_{\text{c}}(f) \) and \( H_{\text{o}}(f) \) are shown in Fig. 2.

The real part of \( H_{\text{c}}(f) \) 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 \( H_{\text{c}}(f) \) 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 \( H_{\text{c}}(f) \) reflects the resistance of the bronchoscope to gas flow, whereas the decreasing real part reflects the invariance of the gas in the bronchoscope channel.

The next step in processing the data was to remove \( H_{\text{o}}(f) \) and \( H_{\text{c}}(f) \) from \( H(f) \) to yield a transfer function \( H_{\text{ref}}(f) \), attributable only to the load beyond the distal tip of the bronchoscope (i.e., the subject). This was done according to the formula (9)

\[
H_{\text{ref}}(f) = \frac{[H_{\text{o}}(f) - H(f)]H_{\text{c}}^{-1}(f)}{[H_{\text{o}}(f) - H_{\text{c}}(f)]H(f) - H_{\text{c}}(f)}
\]

Finally, \( Z_{\text{sys}}(f) \) was calculated from \( H_{\text{ref}}(f) \) as follows

\[
Z_{\text{sys}}(f) = R(f) + iX(f) = -\frac{1}{H_{\text{ref}}(f)}/2\pi
\]

where \( R \) is the real part of \( Z_{\text{sys}} \) (i.e., resistance), \( X \) is the imaginary part of \( Z_{\text{sys}} \) (i.e., 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, which would have arisen had we first differentiated volume to obtain 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 in numerical differentiation.) Elastance (E) was calculated as

\[
E(f) = -2\pi X(f)
\]

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-liter 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 \( ~0.08 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{ml}^{-1} \), and the elastance of the gas in the flask was \( ~1 \text{ cmH}_2\text{O/ml} \). When the impedance of the system was measured directly with the flexiVent (i.e., without using the bronchoscope), we obtained an \( R \) that decreased slightly with frequency, with a mean value of 0.061 cmH2O/ml. When impedance was measured through the bronchoscope, \( R \) differed from that obtained without the bronchoscope by an average of 0.11 cmH2O/ml, and the elastance of the gas in the system \( \sim 0.08 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{ml}^{-1} \) and the elastance of the gas in the system \( \sim 0.08 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{ml}^{-1} \).

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 two subjects, we measured esophageal pressure (Pes) 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 \( ~0.1 \text{ ml} \) of 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 two subjects with 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 Pes signals. The excursions in Pes are small compared with those at the tip of the bronchoscope, indicating that the applied pressure oscillations were not transmitted 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 trace of Pes demonstrates that the subjects were able to suspend their breathing at the ERCP, relax their inspiratory muscles, and maintain a constant lung volume. Interestingly, the oscillator pressure trace from the asthmatic subject in Fig. 3 has larger high-frequency components than the trace from the normal subject. This no doubt reflects the greater lung

Fig. 2. Transfer functions between cylinder pressure (\( P_{\text{cyl}} \)) and cylinder volume (\( V_{\text{cyl}} \)) obtained with the distal end of the bronchoscope closed and open to atmosphere.

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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 to simulate impedance at the same frequencies used experimentally to determine $Z_{seg}$. The computational model employed a subset of the morphometric data used by Gillis and Lutchen (13) in their simulations of the impedance of an entire lung. The conducting airways of the model thus followed the asymmetric branching scheme of Horsfield et al. (16). The radius of each airway was chosen randomly from a Gaussian distribution with a mean appropriate to the airway order as per the data used by Gillis and Lutchen (13) in their simulations of the human lung model, on which we based our simulations, the trachea was assigned order 35 (24). A fifth-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 to 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, because beyond that the contribution of bulk flow to gas transport is negligible and, therefore, would not be expected to contribute significantly to mechanical impedance (24).

Statistical analysis. Descriptive data are summarized by means ± SD or medians and 25th–75th interquartile range (IQR), as determined by data distribution. Outcome data are summarized by means ± SE or median and 25th–75th IQR, as appropriate. PC$_{20}$ data are summarized by geometric mean and range. Categorical data were compared using Fisher’s exact test, and continuous data were compared using t-tests for normally distributed data and Wilcoxon’s 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 with baseline mean data in the same range after the 0.01-mg 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 after 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 $P \leq 0.05$ was considered statistically significant.

RESULTS

Experimental. Figure 4 presents baseline $Z_{seg}$ from all subjects. Two of the asthmatic subjects had significantly higher baseline R than the other asthmatic subjects (Fig. 4, top) and showed significant negative dependence on frequency. These two subjects (B2 and B5) also had the most widely varying values of E (Fig. 4, bottom), the lowest FEV$_1$ (68 and 75% predicted), and moderate PC$_{20}$ values (3.0 and 1.0 mg/ml). Baseline R at low frequency between healthy subjects and subjects with asthma (including subjects B2 and B5) was not statistically different [median (IQR) = 0.05 (0.03–0.07) and 0.08 (0.05–0.57) cmH$_2$O·s·ml$^{-1}$, respectively, $P = 0.11$], nor was it different at high frequency [0.04 (0.02–0.06) and 0.06 (0.04–0.35) cmH$_2$O·s·ml$^{-1}$, respectively, $P = 0.15$].

Methacholine instillation caused R to increase in all subjects, although to various degrees that depended on the dose (Fig. 5). Healthy subjects and subjects with asthma had significant increases in R after methacholine at low [1,631 ± 514 (P = 0.050) and 1,655 ± 814 (P = 0.01) %change from baseline, respectively] and high [1,048 ± 322 (P = 0.05) and 737 ± 326 (P = 0.01) %change from baseline, respectively] frequencies. Postmethacholine R was not statistically different between healthy control subjects and subjects with asthma at low [0.68 (0.36–0.95) and 0.52 (0.40–3.14) cmH$_2$O·s·ml$^{-1}$ (P = 0.93), respectively] and high [0.36 (0.19–0.46) and 0.33 (0.28–1.22) cmH$_2$O·s·ml$^{-1}$ (P = 0.55) frequencies. However, when straight lines were fit to the low- and high-frequency dose-response data from healthy and asthmatic subjects, the 95% confidence intervals around the slopes of the
lines overlapped, whereas the confidence intervals on either side of the intercepts did not overlap. Furthermore, in asthmatic and healthy subjects, the increases were greater in the lower-than in the higher-frequency portions (Fig. 5).

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 patterns among the different healthy and asthmatic subjects (Fig. 6): 1) an increase in E with frequency, followed by a descent below zero at the higher end of the frequency spectrum, and 2) 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 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 after administration of albuterol. We believe these variable responses may reflect the distribution of albuterol, having been affected by different 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 Zseg.

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 healthy subjects (Fig. 4), supporting the notion that our measurements of $Z_{seg}$ are indeed reflective of the mechanical properties of a subsegment of the lung. Addition of compliant airway walls and airway wall inertance (see appendix) did not affect the simulations.

Figure 8 shows the results of further Monte-Carlo simulations demonstrating how model impedance is affected by mild bronchoconstriction. Keeping the airways rigid and reducing their diameters by a mean factor of 2 by use of a 25% coefficient of variation increases $R$ at all frequencies but increases $E$ only to $\sim 5$ Hz. At $>5$ Hz, $E$ starts to decrease and...
becomes substantially negative by 20 Hz. Making the airways compliant using $A = 5$ (where $A$ is the constant of proportionality between airway E and radius; see appendix) lessens the negativity of E at high frequencies. Increasing the compliance fivefold ($A = 1$) prevents E from becoming negative at all frequencies <20 Hz. Changing to a distal airway constriction pattern, in which the terminal bronchiolos 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 $Z_{seg}$ data from 0.50 to 19.75 Hz in human volunteers by applying forced oscillations in flow through a bronchoscope wedged in a fifth-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 PEs is virtually unaffected by the applied oscillations in volume. This implies that the volume changes induced in the wedged segment were not transmitted to the chest wall. We surmise that these oscillations were instead accommodated by lateral distortions in the remaining unwedged 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 $\sim 3$ cmH2O s cm-1, compared with 5 cmH2O s cm-1 in asthmatic subjects. These values are two orders of magnitude higher than the baseline R we measured in our 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 s, 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 maneuver. 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 was not the case. Thus we believe that the contribution of collateral ventilation to our results was negligible; 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. These 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 2-mm-diameter catheter in dog lung segments to measure impedance by a wave-tube method. They found that the peripheral lung behaved in a manner 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 asthmatic and healthy individuals we studied, there is substantial overlap of baseline lung $Z_{seg}$. We did not expect this finding, inasmuch 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, demonstrated 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 and asthmatic subjects become apparent on stimulation with methacholine. Our findings clearly show that the lung periphery of asthmatic subjects is hyperresponsive to methacholine. Hyperresponsiveness refers to increased sensitivity (leftward shift in the dose-response curve), increased reactivity (increased slope of the dose-response curve), or increased maximal response for a given dose (33). Our data support 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 cannot discriminate among these possibilities. In any case, our findings are in accord with previous work demonstrating a greater rise in collateral resistance in response to bronchoconstrictive stimuli in asthmatic than in 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 from the larger airways immediately distal to the wedged bronchoscope, despite the fact that 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 because the subjects were supine and freely ventilating the segment during the 2 min 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, because many irritants and other stimulating factors, especially of large particle size, would be most likely to impinge directly on the central airways (7).

The substantial increase in low-frequency resistance after methacholine suggests that significant peripheral airway heterogeneous narrowing or closure was involved (13, 35). Kaczka and colleagues showed that in asthmatic (18) and healthy subjects (19) most (70%) of the increase in R is due to airway, not tissue, resistance. The concurrent positive frequency dependence of E in subjects in their study and our study 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 is needed 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 asthmatic subjects appear to have an increase of E with frequency, indicating shunting of flow into central airways. This may be due to more severe peripheral airway narrowing or increased airway compliance. Our methods cannot distinguish between these two possibilities. However, while the flow oscillations were being applied to our subjects, we observed lateral movement of the airway wall through the bronchoscope, particularly after 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 stiffer airway walls or less peripheral airway narrowing. Increased stiffness of asthmatic airways, a condition that may be due to the effects of airway inflammation and remodeling, has been documented by other studies (6, 41). 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 (18), who used whole lung forced oscillation in asthmatic subjects. They found that asthmatic subjects 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 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 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 asthma in type B subjects studied by Kaczka and colleagues was likely more severe (mean FEV1 = 70% predicted) than in 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 asthmatic subjects), 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 enhance airway narrowing by increasing wall thickness and to reduce airway narrowing by stiffening the airway wall (27).

Our model simulations support the notion that airway wall stiffness is an important determinant of Zseg, in addition to the obvious contributions from airway caliper. 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 (25) presented the closest approach to our method by incorporating nonrigid airway walls with mass into a morphometrically accurate computational model. 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, are open to question. We do not claim that our formula is the only one possible; indeed, others have been used (13, 25), including a recent approach incorporating heterogeneous tissue properties into the model (17). Our formula was chosen because it is mathematically straightforward, is biologically plausible, and caused the model to reproduce the essential features of the experimental data.

In summary, we have shown that measurements of Zseg 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 and 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 mildly asthmatic subjects are compared with normal individuals at baseline, in contrast to collateral resistance, which is able to distinguish the two groups (21, 22, 40). However, when the airways are challenged with methacholine, separation of the two groups by Zseg 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

\[ Z_{aw}(f) = \frac{8\mu f}{\pi r^2} + i \frac{2\pi f p}{r^2} \]  

(A1)

where \( \mu \) is the viscosity of air (\( 1.8 \times 10^{-5} \text{ g cm}^{-1} \text{s}^{-1} \)), \( p \) is the density of air (\( 1.2 \times 10^{-6} \text{ g cm}^{-3} \)), \( r \) is the radius of the airway, and \( l \) is airway length. The first term in Eq. A1 describes Poiseuille flow, and 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 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

\[ Z_{ti}(f) = \frac{G_{ti} - iH_{ti}}{\omega} \]  

(A2)

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

\[ \alpha = \frac{2}{\pi} \arctan \left( \frac{H_{ti}}{G_{ti}} \right) \]  

(A3)

This model of \( Z_{ti} \), 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 \( \text{cmH}_2\text{O s}^{-1} \text{ ml}^{-1} \) can be replaced with \( \text{cmH}_2\text{O s}^{-1} \text{ ml}^{-1} \) if Eq. A2 is defined in terms of \( \omega \omega_0 \), where \( \omega_0 \) 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 \( \text{cmH}_2\text{O s}^{-1} \text{ ml}^{-1} \). Inasmuch 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 \( \text{cmH}_2\text{O} \), 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. A2). That is

\[ Z_{wall}(\omega) = (\eta - i) \frac{H_{wall}}{(\omega/\omega_0)^4} \]  

(A4)

where \( H_{wall} \) is an elastic parameter. 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

\[ dV = \pi(r + dr)^2 - \pi r^2 l = 2\pi rl dr \]  

(A5)

The resulting circumferential strain in the airway wall, if it is assumed to be a thin membrane, is \( d\sigma \). This causes an increase in tangential wall tension equal to \( E_{wall} d\sigma dl \), where \( E_{wall} \) is intrinsic wall tissue stiffness. The resulting increase in intraluminal pressure (\( P \)) is given by Laplace’s law

\[ dP = \frac{2E_{wall}}{r} - \frac{2E_{wall}}{r} = \frac{E_{wall}}{\pi r^2} dV \]  

(A6)

where \( T \) is wall tension and \( E_{wall} \) is intrinsic wall tissue stiffness. However, airway walls become thinner as airways become 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

\[ \frac{dP}{dV} = \frac{A}{\pi r^2} \]  

(A7)

Substituting for \( dV \) from Eq. A5 into Eq. A7 and rearranging gives

\[ \frac{dP}{(dr)} = 2A \]  

(A8)

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 \( \text{cmH}_2\text{O} \) produced a 10% increase in \( r \), then \( A \) in Eq. A8 would have a numerical value of 5. We used values for \( A \) of 5 and 1 (Fig. 8).

\( H_{wall} \) is not exactly an elastance similar to \( dp/dV \), because its units are \( \text{cmH}_2\text{O s}^{-1} \text{ ml}^{-1} \), rather than \( \text{cmH}_2\text{O/ml} \). However, \( \alpha \) 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. A4 by the expression in Eq. A7 to obtain

\[ Z_{wall}(\omega) = (\eta - i) \frac{A}{\pi r^2} \frac{H_{wall}}{(\omega/\omega_0)^4} \]  

(A9)

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

\[ F = Ma = 2\pi rlh \frac{d^2r}{dr^2} = \frac{2\pi rh}{2\pi r} \frac{d^2V}{dr^2} = h \frac{d^2V}{dr^2} \]  

(A10)

However

\[ F = 2\pi rp \]  

(A11)

So

\[ P = \frac{h}{2\pi rl} \frac{d^2V}{dr^2} \]  

(A12)

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

\[ P = \frac{Bp}{2\pi rl} \frac{d^2V}{dr^2} = \frac{Bp}{2\pi rl} \frac{d^2V}{dr^2} \]  

(A13)

In the frequency domain, this gives

\[ P(\omega) = i \frac{Bp}{l} \overline{V}(\omega) \]  

(A14)

It remains to choose a value for \( B \). According to the computational data of Gillis and Lutchen (13), \( h/r \) varies from 0.27 to 0.67 as one progresses from order 25 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. A1 to the sum of Eqs. A9 and A14.

J Appl Physiol • VOL 97 • NOVEMBER 2004 • www.jap.org
GRANTS

This work was supported by National Center for Research Resources Center of Biomedical Research Excellence Grant P20 RR-15557, National Heart, Lung, and Blood Institute Grants R01 HL-62746 and R01 HL-67273, General Clinical Research Center of the University of Vermont Grant RR00109, and a grant from the Whitaker Foundation.

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