Respiratory transfer impedance between 8 and 384 Hz in guinea pigs before and after bronchial challenge

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Sobh, Jamil F., Craig M. Lilly, Jeffrey M. Drazen, and Andrew C. Jackson. Respiratory transfer impedance between 8 and 384 Hz in guinea pigs before and after bronchial challenge. J. Appl. Physiol. 82(1): 172–181, 1997.—We report a forced oscillatory technique for noninvasively measuring respiratory transfer impedance (Ztr) between 8 and 384 Hz in guinea pigs. This technique uses a device consisting of two chambers: one surrounding the animal’s head that is used as a plethysmograph to measured flow through the airway opening and the other that surrounds the animal’s body and is used to apply pressure oscillations to the body surface. Ztr was measured in spontaneously breathing awake guinea pigs and while the animals were anesthetized in normal and methacholine-challenged conditions. An eight-element model consisting of an airway compartment separated from a tissue compartment by a shunt gas compression compartment was fit to the data. Anesthesia increased central and peripheral airway resistance and bronchial airway wall compliance by 37% and 44%, respectively, whereas it decreased tissue compliance by 37%. Compared with the unanesthetized condition, the methacholine challenge (20 µg/kg) resulted in an increase in central and peripheral airway resistance (69 and 319%, respectively) and a decrease in bronchial airway wall and tissue compliance (37 and 79%, respectively). This technique is capable of measuring Ztr in anesthetized and awake guinea pigs. Analysis of these data with this eight-element model provides reasonable estimates of airway and tissue parameters.

MATERIALS AND METHODS

Experimental system. The data acquisition and measurement system for Ztr is shown in Fig. 1. The two-compartment HB-P consisted of a cylindrical Plexiglas chamber. The body chamber (1,000 ml) had a sloping front, which permitted the guinea pig to sit with its front feet extended, the only position in which unanesthetized guinea pigs will sit quietly (1). A removable neck plate held the animal in place and provided a seal around the neck. The head chamber (150 ml), joined to the body chamber by adjustable spring clamps, enclosed the head of the animal. This method depends on an airtight seal between the body and the head of the guinea pig, which was achieved by molding Play Dough around the neck of the animal.

Pressure oscillations were generated by a 25-W loudspeaker mounted in the body chamber. The airway opening...
and body surface pressures relative to atmospheric were measured with Microswitch pressure transducers (model 163PC01DW44). The pressure signals were separately bandpass filtered between 8 and 400 Hz using fourth-order Butterworth filters (model 4113, Ithaco) and amplified. The pressure transducers, centered at the top of the body and head chamber, were digitally matched within \( \pm 2\% \) of amplitude and \( \pm 1^\circ \) of phase at all frequencies (27). The effect of the vibration or sound transmission to the reference side of the transducers was tested by completely sealing the head chamber from the body chamber and measuring the head chamber pressure \( (P_{ao}) \), which was \(<0.1\% \) of the applied pressure amplitude. The homogeneity of the applied pressure within the body chamber was measured as the ratio of pressures at the two extreme points inside the body chamber over the entire frequency range. This pressure ratio deviated from unity by more than \( \pm 10\% \) at frequencies >384 Hz, and thus \( Z_{tr} \) measurements were limited to <384 Hz.

Signal processing. All impedance measurements were performed using an optimized pseudorandom noise input signal with a frequency content from 8 to 384 Hz in 8-Hz increments. The amplitude of the input signal was enhanced at frequencies <40 Hz, and the phases were optimized to minimize the crest factor and to improve the signal-to-noise ratio at those frequencies (4). Eight consecutive bursts (\( t = 1 \) s) were output for each data acquisition run. The pressure signals were sampled at 2,049 Hz. At least 24 individual bursts were obtained for each animal. Bursts with excessively noisy data were eliminated by hand. Fourier analysis on blocks of 512 data points was performed, and impedance was computed using the cross-spectral technique (7). Impedance data with a coherence value <0.9 were discarded.

Calibration. \( Z_{tr} \) is defined as the pressure difference across the respiratory system divided by \( V_{ao} \)

\[
Z_{tr} = (P_{bs} - P_{ao})/V_{ao}
\]  

\( P_{ao} \) is related to \( V_{ao} \) by the impedance of the front chamber \( (Ze) \)

\[
P_{ao} = Ze \cdot \dot{V}_{ao}
\]  

Solving for \( \dot{V}_{ao} \) and substituting this into Eq. 1

\[
Z_{tr} = [(P_{bs}/P_{ao}) - 1] \cdot Ze
\]  

Thus \( Z_{tr} \) can be computed from the measured \( P_{bs} \) and \( P_{ao} \) provided \( Ze \) is known.

\( Ze \) was determined by using the calibration technique described by Hantos et al. (7). A calibration impedance comparable in magnitude to the impedance of the guinea pig, consisting of a cylindrical tube (4.8 cm long, 0.54 cm ID) with a screen added to one end, was placed between the body chamber and the head chamber. The impedance of the tube was calculated using equations provided by Benade (3) and added to the resistance of the screens (174 cmH\(_2\)O·l\(^{-1}\)·s). The \( P_{ao} \)-to-\( P_{bs} \) ratio was measured in the frequency range 8–384 Hz, and the impedance \( Ze \) was calculated from

\[
Ze = Z_{tr} (P_{bs}/P_{ao})^{-1}
\]

A relatively large component of the imaginary part of \( Ze \) indicates that \( Ze \) contains a significant capacitive component. This component is the head chamber volume minus the volume of the guinea pig's head. By trial and error, it was found that a head chamber volume of 150 ml was optimal. The volume of the guinea pig's head was accounted for as follows. The head volumes \( (V_{head}) \) of 22 guinea pigs were measured by water displacement and plotted as a function of the body weight \( (wt) \). A linear regression analysis was performed indicating the following relationship: \( V_{head} = wt \cdot 0.14 + 2.93 \). From this equation, if the body weight of the guinea pig is known, the head volume can be estimated with a
maximum error of 7 ml (maximum residual), which would result in a maximum error in the estimate of Ze of 7%.

Experimental protocol. Measurements were made in 20 guinea pigs weighing 350–500 g. The animals were placed in the body chamber and held in position using the neck plate. The animal’s neck was moistened to remove air from the fur, and Play Dough was placed around the neck to prevent leakage between the head and body chambers. Data acquisition was done while the guinea pig breathed spontaneously. Between measurements the head chamber was flushed with fresh air (25 ml/s) to prevent CO₂ buildup. The bias flow through the front chamber was discontinued during the measurement. After baseline measurement the animal was removed from the chamber and anesthetized with an intraperitoneal injection of 100 mg/ml ketamine hydrochloride and 20 mg/ml of xylazine (Rompun). A 22-gauge catheter was inserted into the jugular vein and securely tied into position, and the animal was repositioned in the chamber. The Ztr was again measured while the animal was anesthetized. The guinea pig was then injected with increasing doses of methacholine (0.1, 0.5, 1, 5, 10, and 20 µg/kg). Ztr was measured 60 s after each dose was delivered. After each dose, the animal was allowed to recover for 15 min before the next dose was delivered.

Modeling analysis. The Ztr data for baseline, anesthetized, and methacholine-challenged conditions were analyzed with two different models. In the six-element model of DuBois (6) (Fig. 2A), the respiratory system was represented by an airway compartment (Zaw) separated from a tissue compartment (Zti) by a shunt gas compression compartment (Zg). Zaw is represented by the airway resistance (Raw) in series with an airway inertance (Iaw). Zti is represented by the series combination of resistance (Rti), inertance (Iti), and compliance (Cti). Finally, the alveolar gas shunt impedance (Zg) is simply gas compressibility (Cg). The second model was a modification of the six-element model, where the airway compartment was separated into a central and a peripheral compartment (Fig. 2B). The airway compartment of the eight-element model consisted of the central airways (with resistance Rcaw and inertance Icaw) and the peripheral airways (with resistance Rpaw) separated by the bronchial airway wall compliance (Cbr).

Parameter estimates were obtained using a gradient optimization technique (2), which adjusted model parameters such that the sum of squares of the difference between the data and the model impedance values was minimized (Eq. 5). The performance index (PI) is defined by

\[
PI = \sum_{i=1}^{n} \left( \frac{|Re_d(i) - Re_m(i)|^2}{Re_d(i)^2} + \frac{|Im_d(i) - Im_m(i)|^2}{Im_d(i)^2} \right)
\]  

where \(Re_d\) is the real part of the impedance data at the \(i\)th frequency and \(Re_m\) is the model-predicted real part of the impedance at the \(i\)th frequency; likewise, \(Im_d\) and \(Im_m\) represent the imaginary parts of the data and model, respectively, and \(n\) is the total number of data points.

Fig. 2. DuBois 6-element model (A) and 8-element model (B) of respiratory system. Raw, airway resistance; law, airway inertance; Cg, gas compressibility; Rti, tissue resistance; Iti, tissue inertance; Cti, tissue compliance; Vao, flow at airway opening; Rcaw, central Raw; Rpaw, peripheral Raw; Icaw, central law; Cbr, bronchial airway wall compliance; Pcw, pressure at chest wall.

Fig. 3. Transfer impedance (Ztr) data (means ± SD) in baseline, anesthetized, and methacholine-challenged conditions: real [Re(Ztr)] and imaginary [Im(Ztr)] part of Ztr as a function of frequency.
The quality of the model fit to the data \( s^2 \) was determined by

\[
s^2 = \frac{PI}{(n-p)}
\]

where \( n \) is the number of data points fit and \( p \) is the number of parameters.  

Ztr uniquely identifies only five of the six parameters in the six-element model and only seven of the eight parameters in the eight-element model. Therefore, one of the parameters must be obtained by an independent measurement. We estimated \( C_g \) for each guinea pig from the reported values of functional residual capacity (FRC) in guinea pigs as a function of body mass (17.0 \( \pm \) 3.2 ml FRC/kg body wt) (25). With the assumption of isothermal conditions (12), \( C_g \) was calculated and fixed as follows

\[
C_g = \frac{FRC}{P_{\text{atm}} - P_{H_2O}}
\]

where FRC is expressed in milliliters, \( P_{\text{atm}} \) is atmospheric pressure (1.033 cmH\(_2\)O), and \( P_{H_2O} \) is partial pressure of water vapor at 100% saturation and 37°C (64 cmH\(_2\)O).

When applicable, we predicted the 95% joint and regional confidence bounds on the parameter estimates using a theoretical approach modified to incorporate the weighted least square of Eq. 5 (16). Statistical reliability relates to the level of confidence or degree of uncertainty in the parameter estimates (19). The reliability of the estimated parameters is characterized by their 1) confidence intervals (CIs), which reflect the percentage by which an estimated parameter can vary, with the other parameters kept fixed while the same degree of fit is maintained, and 2) their joint confidence regions (CRs), which reflect the percentage by which a parameter can vary, with the other parameters allowed to vary simultaneously while the same degree of fit to the data is maintained (18).

**RESULTS**

Ztr. Ztr data in the baseline, anesthetized, and methacholine-challenged conditions are shown in Fig. 3. The real part of baseline Ztr [Re(Ztr)] decreased with frequency from 8 to 384 Hz, crossing the zero axis at 250 \( \pm \) 31 Hz. The imaginary part of Ztr [Im(Ztr)] first increased from 8 Hz, reaching a maximum at \( \sim \) 224 Hz, and then decreased for higher frequencies. The first resonance frequency \( f_0 \) in the Ztr spectrum occurred at 60 \( \pm \) 7 Hz, while a second resonance occurred at 375 \( \pm \) 35 Hz. This frequency-dependent behavior of the Im(Ztr) and Re(Ztr) are qualitatively similar to the Ztr spectra in humans, except in human data the Re(Ztr) crosses the zero axis at \( \sim \) 32 Hz and the first and the second resonance frequencies occurred at \( \sim \) 8 and 64 Hz, respectively (17).

The reproducibility of Ztr measurements was investigated by measuring Ztr, removing the animal from the chamber, and then returning the animal to the chamber for a second measurement (Fig. 4).

Anesthesia significantly changed the Ztr spectrum compared with the baseline Ztr. Compared with anesthesia, the low doses of methacholine (<1 µg/kg) had little or no effect on the Re(Ztr) or the Im(Ztr) (Fig. 3). Higher doses of methacholine induced significant changes in Ztr compared with anesthesia. Re(Ztr) increased at all frequencies in response to anesthesia and was further increased on challenge with the higher doses of methacholine. The higher the methacholine dose, the higher was the increase in Re(Ztr) at all frequencies. The frequency-dependent drop in Re(Ztr) at low frequencies in the baseline condition increased with anesthesia and with increasing doses of methacholine. The frequency \( f_{\text{real}} \) at which the real part crossed the zero axis [Re(Ztr) = 0] was 250 \( \pm \) 31 Hz in baseline conditions, and it increased significantly to 322 \( \pm \) 26 Hz (P < 0.05) in response to anesthesia, but methacholine did not significantly alter \( f_{\text{real}} \) (P > 0.05; Table 1).

**Table 1.** Mean resonance frequencies in baseline, anesthetized, and methacholine-challenged conditions

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Baseline</th>
<th>Anesthesia</th>
<th>Methacholine Dose, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>( f_0 )</td>
<td>60 ( \pm ) 5</td>
<td>83 ( \pm ) 18*</td>
<td>84 ( \pm ) 18†</td>
</tr>
<tr>
<td>( f_{\text{real}} )</td>
<td>250 ( \pm ) 3</td>
<td>322 ( \pm ) 26*</td>
<td>319 ( \pm ) 27</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SD in Hz. *Significantly different from control, P < 0.05; †significantly different from anesthesia, P < 0.05.
In response to anesthesia, the Im(Ztr) decreased at the lower frequencies and increased at the higher frequencies (Fig. 3). In response to the higher doses of methacholine, Im(Ztr) further decreased at low frequencies and further increased at high frequencies. Similarly, $f_0$ shifted toward higher frequencies in response to anesthesia, and only the higher methacholine doses (>1 µg/kg) caused a further shift in $f_0$ to the right (Table 1).

**Discussion**

Sources of error. The calibration method does not necessitate any assumptions on the topology of $Z_e$, which represents the impedance of the head chamber, including the impedance of the pneumotachometer screen in parallel with the gas compression (Eq. 4). Because $Z_e$ is complex, we used a reference in which impedances were complex and magnitude was similar to $Z_{tr}$ in guinea pigs. We verified the $Z_e$ values by measuring the $Z_{tr}$ of a second calibration device (4.8-cm-long 0.54-cm-ID tube, adding screen resistance 104 cm$^2$·s$^{-1}$·cm$^{-3}$, and comparing its measured $Z_{tr}$ with the theoretical prediction of its $Z_{tr}$. The measured and the predicted impedances of the second calibration device were within 5% of the expected values over the entire frequency range.

An airtight seal around the animal’s neck is of crucial importance for $Z_{tr}$ measurements, because any leak between the two chambers will act as an impedance in parallel with $Z_{tr}$, and thus $Z_{tr}$ would be underestimated. Leaks were minimized, if not totally eliminated, by a tight neck plate and by molding Play Dough around the animal’s neck. The reproducibility of our results (Fig. 4) is evidence that with care leaks around the neck can be reduced to the point where they are negligible.

Guinea pigs have a relatively high spontaneous breathing frequency (2–3 Hz) that could contain higher-frequency harmonics and, thus, interfere with the $Z_{tr}$ measurements. Measuring the box pressure signal when the guinea pig was breathing spontaneously without forced oscillations revealed that the amplitude of the 8-Hz (the minimum frequency used) component of spontaneous breathing was <10% of the amplitude of the corresponding frequency in the applied signal. Hence, harmonics from spontaneous breathing were not thought to influence the $Z_{tr}$ estimates.

**Modeling analysis.** Baseline $Z_{tr}$ showed a rather sharp frequency-dependent drop in the Re($Z_{tr}$) for frequency <40 Hz that increased and became more significant in anesthetized and challenged animals. The six-element model was unable to fit this low-frequency-dependent drop. It has been suggested that the frequency-dependent decrease in the Re($Z_{tr}$) can be due to increased inhomogeneities in parallel airways (24) or to the effects of airway wall compliance (21).

The model of Otis et al. (24) includes two parallel pathways representing mechanically inhomogeneous parallel lung units, each consisting of a resistance and compliance in series (Fig. 5). If the time constants ($\tau = R \times C$, where $R$ is resistance and $C$ is compliance) of the two pathways are not equal, the effective resistance and compliance will then decrease with increasing frequency. We fitted the Otis model to our $Z_{tr}$ data of baseline, anesthesia, and methacholine-challenged conditions in the frequency range below the resonance frequency. As expected, the time constant ($\tau$) of one compartment was larger than that of the other for all cases (Table 2). However, with increasing levels of methacholine, the apparent degree of parallel inhomogeneity decreased (i.e., the ratio $\tau_1/\tau_2$ decreased), whereas we would have expected it to have increased.

We interpret these results as evidence that this model does not provide a physiologically realistic interpretation of the data obtained during methacholine challenge.

**Mead** (21) proposed an alternative mechanism that could result in frequency dependence of Re($Z_{tr}$) on the...

**Table 2.** Mean parameters of model of Otis et al. (24) for control, anesthetized, and methacholine-challenged conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$R_1$ (10$^{-2}$)</th>
<th>$C_1$ (10$^{-3}$)</th>
<th>$R_2$</th>
<th>$C_2$ (10$^{-3}$)</th>
<th>$\tau_1 - R_1 C_1$</th>
<th>$\tau_2 - R_2 C_2$</th>
<th>$\tau_1/\tau_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>595 ± 130</td>
<td>4.40 ± 2.66</td>
<td>522 ± 116</td>
<td>0.17 ± 0.05</td>
<td>348 ± 130</td>
<td>8 ± 2</td>
<td>45 ± 18</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>715 ± 259</td>
<td>1.86 ± 0.13</td>
<td>514 ± 63</td>
<td>0.11 ± 0.04</td>
<td>132 + 43</td>
<td>5 ± 2</td>
<td>45 ± 30</td>
</tr>
<tr>
<td>Methacholine, µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>760 ± 265</td>
<td>1.46 ± 0.42</td>
<td>457 ± 90</td>
<td>0.11 ± 0.07</td>
<td>107 ± 35</td>
<td>5 ± 2</td>
<td>25 ± 12</td>
</tr>
<tr>
<td>0.5</td>
<td>829 ± 296</td>
<td>2.05 ± 0.68</td>
<td>473 ± 80</td>
<td>0.09 ± 0.03</td>
<td>179 ± 97</td>
<td>4 ± 1</td>
<td>51 ± 37</td>
</tr>
<tr>
<td>1</td>
<td>918 ± 379</td>
<td>1.54 ± 0.57</td>
<td>482 ± 78</td>
<td>0.11 ± 0.06</td>
<td>145 ± 86</td>
<td>5 ± 2</td>
<td>36 ± 30</td>
</tr>
<tr>
<td>5</td>
<td>1436 ± 897</td>
<td>0.73 ± 0.54</td>
<td>431 ± 121</td>
<td>0.08 ± 0.03</td>
<td>70 ± 35</td>
<td>3 ± 1</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>10</td>
<td>2352 ± 1372</td>
<td>0.25 ± 0.17</td>
<td>505 ± 152</td>
<td>0.07 ± 0.04</td>
<td>44 ± 13</td>
<td>3 ± 1</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>20</td>
<td>2617 ± 1414</td>
<td>0.24 ± 0.18</td>
<td>540 ± 123</td>
<td>0.10 ± 0.09</td>
<td>54 ± 28</td>
<td>4 ± 2</td>
<td>14 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SD. $R_1$ and $R_2$, resistance (cm$^2$·s$^{-1}$·cm$^{-3}$); $C_1$ and $C_2$, compliance (ml/cmH$2O$); $\tau_1$ and $\tau_2$, time constant (10$^{-3}$ s).
basis of the nonrigid behavior of the airway walls. We implemented compliant airway walls by using the model in Fig. 2A. This model resulted in more realistic parameter values. For example, Rpaw and Rcaw increased while Cti decreased with increased levels of methacholine.

System identification. The six-element model described the frequency-dependent decrease of $\text{Re}(Z_{tr})$ at frequencies $>40$ Hz and the curvilinear increase followed by a decrease of $\text{Im}(Z_{tr})$ throughout the entire frequency range (Fig. 6). However, this model was unable to follow the frequency-dependent decrease in $\text{Re}(Z_{tr})$ at the low frequencies ($<40$ Hz). The mean values of the six-element model parameter estimates are presented in Table 3. The resistance was partitioned into airway and tissue components, where $\text{Raw}$ ($172 \pm 25$ cmH$_2$O·l$^{-1}$·s) was 74% of the total respiratory resistance ($\text{Rrs}$) and $\text{Rti}$ ($62 \pm 25$ cmH$_2$O·l$^{-1}$·s$^{-2}$) was 26% of $\text{Rrs}$. $\text{Iaw}$ ($0.094 \pm 0.016$ cmH$_2$O·l$^{-1}$·s$^{-2}$) and $\text{Iti}$ ($0.048 \pm 0.021$ cmH$_2$O·l$^{-1}$·s$^{-2}$) were 66 and 34% of the total respiratory inductance ($\text{Irs}$), respectively. $\text{Cti}$ was $0.033 \pm 0.005$ ml/cmH$_2$O.

We found that an eight-element model (Fig. 7) was able to follow the frequency-dependent behavior of $\text{Re}(Z_{tr})$ and $\text{Im}(Z_{tr})$ in the whole frequency range. The fit, especially at the low frequencies, was improved by 45% compared with the fit with the six-element model. The mean parameter estimates of the eight-element model are given in Table 4. $\text{Rcaw}$ was partitioned into central and peripheral $\text{Rpaw}$, where $\text{Rcaw}$ ($158 \pm 36$ cmH$_2$O·l$^{-1}$·s$^{-1}$) and $\text{Rpaw}$ ($224 \pm 48$ cmH$_2$O·l$^{-1}$·s$^{-1}$) were 41 and 59% of $\text{Rrs}$, respectively. $\text{Icaw}$ ($0.128 \pm 0.036$ cmH$_2$O·l$^{-1}$·s$^{-2}$) and $\text{Ipaw}$ ($0.039 \pm 0.021$ cmH$_2$O·l$^{-1}$·s$^{-2}$) were 77 and 23% of $\text{Irs}$, respectively, and were significantly different from the values obtained with the six-element model.

Table 3. Mean six-element model parameters at baseline conditions

<table>
<thead>
<tr>
<th>Raw</th>
<th>Iaw</th>
<th>Rti</th>
<th>Iti</th>
<th>Cti</th>
<th>Cg</th>
<th>$s^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>172</td>
<td>0.094</td>
<td>62</td>
<td>0.048</td>
<td>0.033</td>
<td>0.0070</td>
</tr>
<tr>
<td>±SD</td>
<td>25</td>
<td>0.016</td>
<td>25</td>
<td>0.021</td>
<td>0.005</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Values represent results for 20 guinea pigs. Raw and Rti, airway and tissue resistance (cmH$_2$O·l$^{-1}$·s$^{-1}$); Iaw and Iti, airway and tissue inductance (cmH$_2$O·l$^{-1}$·s$^{-2}$); Cti, tissue compliance (ml/cmH$_2$O). $s^2 = P^2/(n-p)$, where $P^2$ is performance index, $n$ is no. of points, and $p$ is no. of parameters estimated. By use of Boyle's law (assuming isothermal conditions, Eq. 7), $\text{Cg}$ was computed from functional residual capacity which was estimated from body weight (25).

Table 4. Mean eight-element model parameters at baseline conditions

<table>
<thead>
<tr>
<th>Rcaw</th>
<th>Icaw</th>
<th>Cbr</th>
<th>Rpaw</th>
<th>Rti</th>
<th>Iti</th>
<th>Cti</th>
<th>$s^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>158</td>
<td>0.128</td>
<td>0.028</td>
<td>224</td>
<td>61</td>
<td>0.039</td>
<td>0.147</td>
</tr>
<tr>
<td>±SD</td>
<td>36</td>
<td>0.036</td>
<td>0.110</td>
<td>48</td>
<td>34</td>
<td>0.021</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Values represent results for 20 guinea pigs. Rcaw and Rpaw, central and peripheral Raw (cmH$_2$O·l$^{-1}$·s$^{-1}$); Icaw and Ipaw, central and peripheral inductance (cmH$_2$O·l$^{-1}$·s$^{-2}$); Cbr, bronchial airway wall compliance (ml/cmH$_2$O). See Table 3 footnote for definition of other abbreviations. By use of Boyle's law (assuming isothermal conditions Eq. 7), Cg was computed from functional residual capacity, which was estimated from body weight (25).
The mean $C_{br}$ was 0.028 ± 0.011 ml/cmH$_2$O, whereas the mean $C_{ti}$ was 0.15 ± 0.05 ml/cmH$_2$O.

For anesthetized and methacholine-challenged conditions, the $Z_{tr}$ data were only fitted with the eight-element model, since at low frequencies the frequency-dependent drop, which was not fit with the six-element model, increased and became more significant. The eight-element model provided a very good fit to the baseline, anesthetized, and methacholine-challenged $Z_{tr}$ data. The mean parameter estimates of the eight-element model for baseline, anesthetized, and methacholine-challenged conditions are presented in Table 5.

For the baseline state, $R_{ca}$ was partitioned into upper and lower components, where $R_{ca}$ was 45% of $R_{rs}$. $I_{ca}$ accounted for 88% of $R_{rs}$. In baseline conditions the mean $C_{ti}$ (0.139 ml/cmH$_2$O) was much higher than the mean $C_{br}$ (0.027 ml/cmH$_2$O). This implies that the airways are much stiffer than the lungs and chest wall. $I_{ti}$ accounted for 29% of $I_{rs}$.

During anesthesia, total $R_{ca}$ ($R_{ca} + R_{pa}$) increased by 22%, with most of the increase occurring in the peripheral airways. Total $R_{ca}$ accounted for 93% of $R_{rs}$ (Table 5). $I_{ca}$ increased by 13%, whereas $I_{ti}$ decreased by 60%. In response to anesthesia, $C_{br}$ increased by 45% and $C_{ti}$ decreased by 35%, implying that the airway walls became less stiff while the tissues became stiffer.

In response to increasing doses of methacholine, $R_{pa}$ drastically increased compared with $R_{ca}$ and $R_{ti}$ (Fig. 8). Compared with the anesthesia values, with the highest methacholine dose (20 µg/kg), $R_{ca}$, $R_{pa}$, and $R_{ti}$ increased by 50, 220, and 20%, respectively, which resulted in a 140% increase in $R_{rs}$. Some of the increase in $R_{ca}$ relative to $R_{pa}$ might be due to the fact that the $C_{br}$ shunt was moved mouthward, and thus less of the airways was included in the central airways. Methacholine affected $I_{ca}$ and $I_{ti}$ only at the very high doses (>1 µg/kg), where central airway inertance ($I_{ca}$) was decreased by 20% and $I_{ti}$ was increased by 76%. During the maximum methacholine challenge, $C_{br}$ and $C_{ti}$ decreased by 48 and 65%, respectively, compared with the anesthesia values, indicating that with methacholine the airways and tissues became stiffer.

Sensitivity analysis. Sensitivity analysis was performed on the eight-element model for the control animals to evaluate how $Z_{tr}$ data are influenced by its separate parameters. The percent change in the impedance modules $|Z| = (R_e^2 + I_m^2)^{1/2}$ was computed when...

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### Table 5. Mean eight-element model parameters for baseline, anesthetized, and methacholine-challenged conditions

<table>
<thead>
<tr>
<th></th>
<th>$R_{ca}$</th>
<th>$I_{ca}$</th>
<th>$C_{br}$</th>
<th>$R_{pa}$</th>
<th>$R_{ti}$</th>
<th>$I_{ti}$</th>
<th>$C_{ti}$</th>
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<td>Methacholine, µg/kg</td>
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<td>0.825</td>
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Values represent results for 5 animals. See Tables 3 and 4 footnotes for definition of abbreviations.

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**Fig. 8.** Mean data from methacholine-challenged dose-response curves for 5 guinea pigs. Methacholine doses ([MCh]) are plotted on a logarithmic scale and percent change of parameters on an arithmetic scale.
the value of each parameter in the eight-element model was increased by 20% (Fig. 9). The reference values of the parameters were calculated using their mean values. For the first one-half of the impedance spectrum, Ztr was very sensitive to Rcaw and slightly sensitive to Icaw. As frequency increased, the sensitivity to Rcaw decreased slightly and Ztr became increasingly sensitive to Icaw. Conversely, Ztr was insensitive to Rpaw and Cbr except at frequencies <64 Hz. At low frequencies (<192 Hz), Ztr was slightly sensitive to Rti and relatively insensitive to Iti, but the sensitivity to Rti and Iti increased drastically at higher frequencies. This is consistent with the findings of Lutchen and Jackson (18) in humans, in that one needs to go to higher frequencies to reliably estimate the separate tissue and airway properties. Furthermore, Ztr is insensitive to Cti, except at frequencies <64 Hz. Conversely, Ztr was sensitive to Cg only at frequencies >256 Hz, which indicates the importance of Cg to the reliable estimation of the tissue properties.

Airway parameters (Raw and Iaw) largely influenced the data from 8 to 192 Hz, and tissue parameters (Rti and Iti) largely influenced Ztr at frequencies >192 Hz. To reliably separate airway from tissue properties, it is necessary to measure Ztr to high enough frequencies that include the zero crossing in the real part and a significant drop in the imaginary part. The increasing sensitivity of Ztr to Cg at high frequencies raised the issue of how the accuracy of the Cg value influenced the airway-tissue separation.

Impact of Cg accuracy. To study the effect of errors in the assigned value for Cg on the estimates of the airway and tissue properties, Cg was varied over ±40%, Ztr was predicted, and the other parameters were estimated from the predicted Ztr. For the eight-element model, Rpaw, Iaw, and Cbr estimates were little affected by errors in Cg of this magnitude, and Cti was independent of Cg (<1% Fig. 10). However, Rcaw, Rti, and Iti were greatly affected by errors in Cg. With a ±40% error in Cg, Rcaw, Rti, and Iti were underestimated by <30%. With a ±40% error in Cg, there were larger errors in Rcaw, Rpaw, and Iti (≈60, ≈150, and ≈70%, respectively). It is important to note that Cg might decrease with anesthesia; thus if an error in estimating Cg is made, it is most likely to be overestimated. Because of the asymmetry of the influence of Cg on the other parameter estimates (Fig. 10), an overestimation in Cg results in rather small errors in the mechanical parameter of interest (i.e., Rcaw, Rpaw, and Cti). It is interesting to note that $s^2$ was unchanged as Cg was varied. These results and those from the previous sensitivity test (Fig. 9) indicate the importance of Cg for the reliable estimate of Rti and Iti, since Ztr is most sensitive to Cg for frequencies >224 Hz, which is also where the parameters Rti and Iti have their greatest influence. Similarly, for the eight-element model, errors in the estimated Cg had the

![Fig. 9. Simulated sensitivity of Ztr to 8-element model parameters. Percent change in magnitude of Ztr due to a 20% increase in respective parameter (other parameters held fixed) is plotted vs. frequency.](image)

![Fig. 10. Impact of errors in fixed value of Cg on airway and tissue parameters of 8-element model. Percent change in parameters estimated is shown vs. percent change in Cg.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>CR</th>
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<tr>
<td>Rcaw</td>
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<td>14±9</td>
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<tr>
<td>Icaw</td>
<td>10±4</td>
<td>17±8</td>
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<tr>
<td>Cbr</td>
<td>16±7</td>
<td>20±8</td>
</tr>
<tr>
<td>Rpaw</td>
<td>20±7</td>
<td>39±15</td>
</tr>
<tr>
<td>Rti</td>
<td>13±5</td>
<td>23±11</td>
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<tr>
<td>Iti</td>
<td>7±4</td>
<td>11±6</td>
</tr>
<tr>
<td>Cti</td>
<td>37±15</td>
<td>57±25</td>
</tr>
</tbody>
</table>

Values are 8-element model means ± SD of 95% individual confidence interval (CI) and 95% joint confidence region (CR) when transfer impedance data are fitted from 8 to 384 Hz. CI and CR reflect percent change by which a parameter can vary and maintain same fit. For CI, other parameters are fixed; for CR, other parameters are allowed to vary.
greatest impact on Rt and It and negligible impact on the other parameters. Thus inaccuracies in the assigned Cg will distort the estimated tissue properties (Rt and It) but will have little effect on the estimated airway properties (Rcaw, Icw, Rpaw, and Cb).

Parameter estimation issues. The reliability of the estimated values of the parameters is characterized by their CIs and joint CRs. We calculated the 95% CIs and the 95% joint CRs for each parameter of the eight-element models for baseline conditions (Table 6). The mean CIs and the CRs include the biological variability, model analysis, and any random noise. All parameters were reliably estimated (low CIs and CRs), except for Cti, which showed unacceptably high CIs and CRs (>30%). Therefore, with these low values of CIs and CRs, changes in these parameters, e.g., induced by pharmacological or toxicological drugs, should be detected by this approach.

Comparison with previous studies. To our knowledge, only two previous studies have reported Ztr in small laboratory animals (8, 23). Oostveen et al. (23), using the forced oscillation technique, measured Ztr in conscious rats from 16 to 208 Hz and fitted various models to the Ztr data. Because their Ztr data did not include a significant drop in the Im(Ztr) and the zero crossing of the Re(Ztr), they were unable to obtain statistically reliable estimates of tissue parameters (Rt and Iti). Furthermore, Oostveen's data included only two data points below the resonance frequency, which were not enough to reliably estimate Cti.

In mice, f0 was estimated between 32 and 48 Hz (8). In rats, f0 ranged from 38 to 48 Hz (23). Hiett (9) reported f0 of 32 Hz in guinea pigs. These values are about one-half of the resonance frequency in guinea pigs as measured by us (60 Hz).

Pulmonary resistance (Rl) values ranging from 339 to 690 cmH2O·l·s−1 have been reported for conscious guinea pigs (1, 4, 9, 15, 29). Hiett (9) reported mean Rl including the upper airways in conscious guinea pigs to be 400 cmH2O·l·s−1, and Skornik et al. (29) reported Rl to be 390 cmH2O·l·s−1; these values are in good agreement with the mean Rs in guinea pigs studied here (443 cmH2O·l·s−1). Our values for Rs were less than the value reported by Amdur and Mead (1) (880 cmH2O·l·s−1). Some previously reported values of Rl were measured using a variety of techniques and were not always corrected for instrument impedance.

Reported values of the total dynamic compliance in awake spontaneously breathing guinea pigs have ranged from 0.16 to 0.22 ml/cmH2O (4, 15), which is in close agreement with Cti from the eight-element model.

Oostveen and Zwart (22) found a 150 and 70% increase in Raw and Iaw, respectively, due to pentobarbital anesthesia compared with our results of a 22 and 13% increase in Raw and Iaw, respectively. Oostveen and Zwart also found that anesthesia caused a 122 and 40% decrease in Rt and Cti, respectively, and a 73% increase in Iti in rats. In our study, Rt and Cti decreased by 25 and 37%, respectively, and Iti increased by 59% in response to anesthesia. The effect of anesthesia on the respiratory parameters is time and dose dependent, and it differs from one type of anesthesia to another, which could account for the differences between our results and those of Oostveen and Zwart. Hulbert et al. (10) reported histamine dose-response curves on anesthetized tracheotomized guinea pigs and found that the Raw increased fivefold and the dynamic compliance fell to 20% of its initial value. In our study, with use of the maximum dose of methacholine (20 μg/kg), the Raw increased only threefold and the Cti decreased to 20% of its initial value.

Conclusion. Analysis of Ztr provided a noninvasive method of assessing the mechanical properties of the respiratory system. However, this method is dependent on the frequency range over which Ztr is measured and the modeling interpretation of the data in terms of the lumped elements representing the respiratory system (17). We have described a method of measuring total Ztr of guinea pigs in baseline (unanesthetized), anesthetized, and methacholine-challenged conditions. This study increased the frequency range over which Ztr can be measured compared with the previous respiratory impedance measurements in laboratory animals. Increasing the frequency range increased the reliability of the tissue parameter estimates. This effect of frequency range was due to the drastic increase in sensitivity of Ztr to Rt and Iti from 192 to 384 Hz.

Ztr provided sensible parameter estimates when fit with the eight-element model, which reflects the lumped-element properties of the respiratory system (i.e., airway and tissue properties). By model analysis of the data, an increase of 30% in Raw was found in response to anesthesia. After methacholine challenge, Raw and total compliance were 250 and 41%, respectively, of the corresponding anesthesiavalues.

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REFERENCES


