Tracking of airway and tissue mechanics during TLC maneuvers in mice

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Hantos, Zoltán, Rachel A. Collins, Debra J. Turner, Tibor Z. Jánosi, and Peter D. Sly. Tracking of airway and tissue mechanics during TLC maneuvers in mice. J Appl Physiol 95: 1695–1705, 2003.—A tracking impedance estimation technique was developed to follow the changes in total respiratory impedance (Zrs) during slow total lung capacity maneuvers in six anesthetized and mechanically ventilated BALB/c mice. Zrs was measured with the wave-tube technique and pseudorandom forced oscillations at nine frequencies between 4 and 38 Hz during inflation from a transrespiratory pressure of 0–20 cmH2O and subsequent deflation, each lasting for ~20 s. Zrs was averaged for 0.125 s and fitted by a model featuring airway resistance (Raw) and inertance, and tissue damping and elastance (H). Lower airway conductance (Glaw) was linearly related to volume above functional residual capacity (V) between 0 and 75–95% maximum V, with a mean slope of dGlaw/dV = 13.6 ± 4.6 cmH2O−1 s−1. The interdependence of Raw and H was characterized by two distinct and closely linear relationships for the low- and high-volume regions, separated at ~40% maximum V. Comparison of Raw with the highest-frequency resistance of the total respiratory system revealed a marked volume-dependent contribution of tissue resistance to total respiratory system resistance, resulting in the overestimation of Raw by 19 ± 8 and 163 ± 40% at functional residual capacity and total lung capacity, respectively, whereas the lowest frequency reactance was proportional to H; these findings indicate that single-frequency resistance values may become inappropriate as surrogates of Raw when tissue impedance is changing.

airway resistance; tissue elastance; forced oscillations; volume dependence; total lung capacity

The increasing use of murine models in the investigation of pulmonary diseases has made it particularly important to establish specific methods of respiratory mechanical measurements in this small species. In a previous study (24), we have documented the values of airway and tissue mechanical parameters at different levels of transrespiratory pressure (Prs) between 0 and 20 cmH2O in anesthetized and mechanically ventilated mice. Although the use of low-frequency oscillations and constant-phase model fitting provides an accurate determination of airway and tissue parameters as functions of Prs (14, 17, 24), the application of successive levels of mean Prs proved time consuming and made this technique unsuitable for evaluation of the short-term volume-dependent changes in these parameters.

Continuous estimation of impedance has been applied in the characterization of transient changes in lung mechanics during a constrictor challenge (2–4); slow partial volume inflations in anesthetized, mechanically ventilated subjects and conscious volunteers (19); and deep inhalation in healthy and asthmatic subjects (5, 18). In these studies, either forced oscillations of a single frequency of 3 Hz (19), 6 Hz (3), or 8 Hz (5, 18) were used to estimate the resistance of the total respiratory system (Rrs) or the lungs (Rl) on a cycle-by-cycle basis, or resistance and elastance values were tracked at two (2) or three frequencies (4) applied simultaneously. The ease of collection of impedance data at a high temporal resolution is nevertheless associated with problems in the interpretation of the mechanical properties manifested at a single frequency, the most important question being to what extent and under what conditions a single-frequency value of Rrs or Rl can be regarded as the resistance of the airways (Raw) (2–5, 18, 19).

The separate assessment of airway and tissue mechanical parameters from low-frequency input impedance data requires a number of simultaneously applied frequency components in an appropriate range that permits the manifestation of the mechanical properties of both the airways and tissues in the particular species, and the use of a validated model in the evaluation of the impedance data (14, 16, 17, 21, 22, 24, 26). In the present report, we employ pseudorandom forced oscillations in anesthetized mice in the frequency range of 4–48 Hz and demonstrate for the first time that the airway and tissue parameters can be obtained reliably by fitting the instantaneous spectra of total respiratory input impedance (Zrs) collected during a slow continuous inflation-deflation maneuver. The multitude of Zrs parameter values obtainable from a single maneuver enables us I) to show the characteristic patterns of pressure and volume dependences derived for the air-
ways and tissues, 2) to explore the relationship between the Newtonian (airway) resistance and tissue elastance, which breaks down into two distinctly different linear segments in the low- and high-volume ranges, and 3) to demonstrate that single-frequency resistance values can seriously overestimate Raw because of the involvement of tissue resistance, which varies systematically with lung volume.

MATERIALS AND METHODS

Six 8- to 10-wk-old female BALB/c mice (16.7 ± 1.4 g) were studied. Anesthesia was induced intraperitoneally with 0.1 ml/10 g of a mixture containing xylazine (2 mg/ml, Bayer) and ketamine (40 mg/ml, Parnell Laboratories). Supplemental doses were given when the animal was attached to the ventilator, and subsequently at ~40–60-min intervals, as required. After the surgical anesthesia, a tracheostomy was performed and a polyethylene cannula (10 mm long, ID = 0.81 mm) was inserted. Mechanical ventilation (flexiVent, Scieq, Montreal, Canada) was maintained with a tidal volume of 8 ml/kg, a rate of 450 min⁻¹, and a positive end-expiratory pressure of 2 cmH₂O. The animal handling and study protocol conformed to the guidelines of the Australian National Health and Medical Research Council and were approved by the Animal Ethics Committee of the Institute for Child Health Research.

Zrs was determined with pseudorandom forced oscillations in the frequency range of 4–38 Hz, by using the wave-tube technique (12, 27) adapted to small animals (21, 24). The oscillatory signal consisted of nine components, which were relative primes, i.e., noninteger multiples of each other (the 2-Hz fundamental was of zero amplitude), to minimize the effects of nonlinear harmonics production (9, 23). The component amplitudes decreased hyperbolically between 4 and 22 Hz and were then kept constant, in accordance with the expected frequency dependence of the magnitude of Zrs and the signal-to-noise values. The phase angles of the components were optimized to find the minimum peak-to-peak excursion of the composite signal at the specified total energy (11). All these considerations relating to nonlinearity and signal-to-noise ratios were particularly important because the measurements were not made under strictly apneic conditions despite the absence of spontaneous breathing (the mean flow changed according to the volume maneuver), there was interference by the cardiac activity, and moreover Zrs was not in a steady state. Zrs was determined as the load impedance on a wave tube (12, 27)

\[
Z_{rs} = Z_0 \sinh(\gamma L)/(P2/P1 - \cosh(\gamma L))
\]

where \(Z_0\) and \(\gamma\) are the characteristic impedance and wave number of the tube, respectively; \(L\) is the tube length; and \(P1\) and \(P2\) are the lateral pressures at the outlet and inlet of the tube, respectively. \(Z_0\) and \(\gamma\) can be expressed as

\[
Z_0 = (Z/Y)^{1/2} \quad \text{and} \quad \gamma = (Z/Y)^{1/2}
\]

where \(Z\) and \(Y\) are the series impedance and the shunt admittance per unit length of the tube, respectively. The dimensions of the setup were adjusted to the size of the animal: the diameter of the polyethylene wave tube (ID = 1.17 mm) was selected first to match that of the trachea, and the value of \(L\) (100 cm) was then chosen so that the ratio \(P2/P1\) could be recorded reliably in the whole frequency range and at all Zrs values. \(P1\) and \(P2\) were measured through 0.2-mm-ID side arms occupying most of the pressure ports of the transducers (ICS model 33NA002D, ICSensors, Millipas, CA) to minimize the dead space of the sensors.

Before the oscillatory measurements, the mouse was hyperinflated by superimposing three tidal volume inspirations and was then ventilated normally for 1 min. The respirator was next stopped, and the tracheal cannula was switched to the wave tube and the loudspeaker box. The measurement started with an ~2-s baseline recording, during which the mean values of \(P1\) (\(P1_{mean}\)) and \(P2\) (\(P2_{mean}\)) were zero, and was followed by a slow (~20 s), nearly linear inflation of the whole system from an O₂ source up to 20 cmH₂O; the inflation was then stopped and the system was connected to an expiratory line, the resistance of which was chosen to result in quasi-exponential decay in \(P1_{mean}\) and \(P2_{mean}\) to nearly atmospheric pressure in ~20 s. The length of the total recording was 45 s, after which the tracheal tube was switched back to the respirator, and the ventilation was resumed.

The signals were filtered with fifth-order Butterworth anti-aliasing low-pass filters at 50 Hz and sampled at a rate of 256 s⁻¹. \(Z_{rs}\) was determined after digital high-pass filtering of \(P1\) and \(P2\) at 3.5 Hz, which eliminated the slow pressure components of the total lung capacity (TLC) maneuver. To further suppress the effects of nonoscillatory pressure components, \(P2/P1\) was computed from the cross spectra between \(P1\) and \(U\), and \(P2\) and \(U\), where \(U\) is the noise-free driving signal of the loudspeaker (10). The time window of fast Fourier transformation was 0.5 s, and the spectra estimated by moving sample-by-sample along the recording were averaged for 0.125 s; hence, these \(Z_{rs}\) data represented the average impedance for a 0.625-s interval, and the successive overlapping estimates were obtained every 0.125 s. These \(Z_{rs}\) spectra were then fitted to a four-parameter model (16) to estimate the Raw and airway inertance (Iaw) and the constant-phase tissue damping (\(G\)) and elastance (\(H\))

\[
Z_{rs} = \text{Raw} + j\omega \text{Iaw} + (G - jH)/\omega^2
\]

where \(\omega\) is angular frequency and \(\alpha = (2/\pi) \cdot \text{arctan}(H/G)\). Strictly speaking, the parameters Raw and Iaw, respectively, include the Newtonian components of tissue resistance and tissue inerance. However, measurements in intact and open-chest rats (17, 22) demonstrate that the contributions of the tissues to Raw and Iaw can be neglected. The model fitting was accomplished by minimizing the sum of squared differences, normalized by \(Z_{rs}\), between the measured and model data. A smoothing procedure (a Gaussian weight function and quadratic fit on 5% blocks of the entire data sequence) was then applied to the time sequences of the parameters, which performed a resampling reduction of each parameter set to 100 data points per maneuver.

\(P1_{mean}\) and \(P2_{mean}\) were obtained by low-pass filtering \(P1\) and \(P2\), respectively, at 3.5 Hz, which removed the oscillatory components from the pressure signals. \(P2_{mean}\) was used as the estimate of \(P_{rs}\) in the pressure-volume relationship. The volume above functional residual capacity (FRC) (\(V\)) was obtained by integrating the nonoscillatory flow computed as the ratio of the pressure difference \(P1_{mean} - P2_{mean}\) and the flow resistance of the wave tube. The setup was regularly checked by applying the usual inflation-deflation protocol to measure a mechanical lung model which consisted of a 20-ml syringe attached to the end of the tracheal tube used in the mouse experiments.

RESULTS

The changes in Zrs during the TLC maneuver, together with those in P2mean, are illustrated in Fig. 1.
The high-frequency values of Rrs decrease and increase during inflation and deflation, respectively, which suggests that these values are determined primarily by the airway caliber. Rrs at low frequencies and Xrs at all frequencies exhibit a similar biphasic change: the initial decrease in these values is replaced by a temporary elevation in the high P2mean range, which can be attributed to the stiffening of the respiratory tissues at high lung volumes.

Typical recordings of P2 and V, and the temporal changes in the model parameters during the inflation-deflation maneuver, are depicted in Fig. 2. The oscillatory excursions of P2 ranged from \( \pm 2 \) cmH\(_2\)O (at mid-inspiration) to \( \pm 3.5 \) cmH\(_2\)O (at TLC), depending on the actual value of Zrs. The change in V was mirrored by those in Raw and Iaw, although the courses of the latter were noisy and sometimes inconsequential. Both tissue parameters decreased initially, then increased steeply until the maximum in V, and displayed the opposite changes with decreasing V. The volume-dependent changes in H exceeded those in G. These temporal patterns are in accordance with the qualitative changes in Zrs illustrated in Fig. 1. The fitting error was on average \( \pm 5\% \), its variations following the rate of change in Zrs, the major determinant of Zrs, changed rapidly in either direction (Fig. 2).

As expected, the parameters obtained in the mechanical model were fairly time invariant, i.e., independent of the applied pressure (Fig. 2). The “tissue” parameters of the model are related to the gas compressibility in the syringe, which produced a value of \( H \) comparable with the average \( H \) during the maneu-

![Fig. 1. Impedance vs. frequency data displayed at 0.5-s intervals during the total lung capacity (TLC) maneuver in a mouse, with the temporal change in mean transrespiratory pressure (P2mean). Note the different scales in the resistance (Rrs) and reactance (Xrs) plots. The Xrs axis is inverted for clarity.](image-url)
ver in a mouse, and a small value of G resulting from the polytropic gas compression process; accordingly, their estimations serve only as the validation and quality check of the measurement and evaluation techniques. However, as the values of Raw estimated in the mechanical model (i.e., the resistance of the tracheal cannula with connections) comprised a constant part of the measured in the animals, it can be subtracted from the latter to obtain the lower airway resistance (Rlaw) of the mice, which varied as a function of P2 or V. Although the same applies for the inertance values, Iaw was a minor parameter in determining Zrs at the relatively low oscillation frequencies (which were well below the resonant frequency) and was easily biased by other parameters in the model fitting; its correction for the tracheal tube component (0.00056 cmH2O s^-2 ml^-1) would have resulted in unrealistic values, and hence this was not done.

In one animal, the tracking estimates of model parameters were compared with those obtained at constant levels of Prs. After the recordings of two TLC maneuvers, P2mean was set successively to 0, 2, 5, 10, 15, and 20 cmH2O, four 6-s measurements were col-
lected at each level, and then another two TLC maneuvers were performed. The parameter values from the individual tracking estimations were interpolated to a common sequence of Prs data and then averaged separately for inflation and deflation (Fig. 3). Although slight but systematic loopings were formed by the inspiratory and expiratory limbs in the Raw, G, and H vs. Prs graphs, the agreement between the tracking estimates and the steady-state parameter values was good, and the variabilities in the courses of the parameters were comparable to those in the steady-state recordings at most Prs levels.

Examples of the estimates of Raw, G, H, and tissue hysteresivity, η = G/H (13), plotted against Prs and V, are presented in Fig. 4. The dependences of the parameters on Prs and V exhibit similar patterns, although the inspiratory and expiratory limbs of the courses of Raw, H, and η are closer to each other if plotted as functions of V. The values of the parameters at end expiration remained slightly below the corresponding values at the beginning of the maneuver in the case of G (3.9 ± 1.9%, P < 0.001) and H (6.6 ± 4.2%, P < 0.02), whereas this decrease was more marked in Raw (18.1 ± 8.1%, P < 0.005).

The lower airway conductance (Glaw = 1/Rlaw) is plotted against V for each animal in Fig. 5. The Glaw vs. V relationships are fairly linear and similar for the inspiratory and expiratory limbs up to 75–95% of the maximum V and become much steeper above those levels. From the regression Glaw = a + bV calculated for the visually selected linear ranges, fairly uniform conductance values at FRC (a = 2.53 ± 0.21 ml·cmH2O⁻¹·s⁻¹) and high correlation coefficients (r² = 0.93 ± 0.01) and somewhat more variable coefficients of volume dependence (b = dGlaw/dV = 13.6 ± 4.6 cmH2O⁻¹·s⁻¹) were obtained. When Glaw is plotted against Prs, the relationships become more linear, and the regression Glaw = c + dPrs applied to the whole data range in each mouse results in values with similar variability as in the volume dependence of Glaw (c = 2.32 ± 0.23 ml·cmH2O⁻¹·s⁻¹, d = 0.41 ± 0.14 ml·cmH2O⁻²·s⁻¹, and r² = 0.91 ± 0.03).

When Raw is plotted against H during a TLC maneuver, a characteristic pattern of interdependence between the airway and tissue properties is disclosed (Fig. 6). In the early phase of the maneuver, both parameters decrease, but H later begins to rise whereas Raw continues to decrease. During expiration, the reverse changes occur in the Raw vs. H data, which closely follow the same trajectories as in inspiration, although the end-expiratory slope of the relationship is slightly but systematically lower than the early inspiratory slope. The two distinct phases form a low-volume and a high-volume region, separated by the minima of H in the inspiratory and expiratory directions, which take place at a mean V of 0.175 ± 0.038 and 0.174 ± 0.028 ml, respectively. These on average identical values of V correspond to 40% of the maximum V and to values of Raw amounting to 44% of
those at FRC. The Raw vs. H relationships are characterized by high correlation coefficients of 0.95 ± 0.03 and 0.84 ± 0.06, respectively, for the low-volume and high-volume regions.

Model parameters and single-frequency impedance values during the TLC maneuver were compared to establish whether the values of the highest frequency Rrs (Rrs38) and the magnitude of the lowest-frequency Xrs (Xrs4) followed the changes in Raw and H, respectively. As illustrated in Fig. 7, a very high correlation coefficient was obtained for both relationships. Raw and Rrs38 displayed a similar dependence on V, with an upward shift for Rrs38, indicating a tissue resistance component in the latter. However, with decreasing Raw, the change in Rrs38 gradually became smaller as a result of an increased contribution of the tissue resistance to Rrs38 with increasing V (cf. the change in G in Fig. 4). For all animals, Rrs38 overestimated Raw by 19 ± 8 and 163 ± 40% at FRC and TLC, respectively. By contrast, the proportionality between Xrs4 and H was much better; the mild curvilinearity apparent at high values of H can be explained on the basis of the change in η with V (see DISCUSSION).
DISCUSSION

Methodological considerations. The purpose of this study was to develop a method by which Zrs can be tracked continuously during a TLC maneuver, in a sufficiently wide frequency range that allows the model-based separate estimation of the airway and tissue mechanical parameters at any instant of the maneuver. The key issue associated with this task was to find the optimum match of the spectral content of the oscillatory signal and the speed of the inspiration-expiration maneuver. On one hand, the time window used in the computation of the impedance spectra had to be kept short enough to fulfill the criterion of the time invariance of the measured system within the window; on the other hand, it had to last long enough to include low-frequency oscillations that allow manifestation of the tissue properties in Zrs. In consequence of the relatively high baseline setting of minute ventilation and the high O2 concentration of the inspired air, the animals remained apneic for the 45 s of the maneuver, although the possible alterations in blood gases were not checked. At this slow rate of deformation, the time window could even be expanded from 0.25 to 0.5 s, thereby making possible to use the non-integer multiples of the 4-Hz fundamental and a raised signal amplitude and to benefit from the increased signal-to-noise ratio at a decreased nonlinear distortion (9, 23). Although neither Prs nor V had symmetrical changes in inflation and deflation, each phase was long enough for this small species to be regarded as a quasi-static, almost rate-independent deformation.

The reproducibility of the Zrs parameters in subsequent TLC maneuvers was good (see Fig. 3) and was comparable with that obtained from the more time-consuming measurements at a sequence of constant Prs levels in one animal in the present study and in the previous investigation (24). The errors of model fitting to Zrs data ranged from 2 to 10% during the maneuvers and averaged ~5%, roughly twice as high as the values of F% obtained invariably at all Prs levels (24). The reasons for this difference in fitting quality are 1) the involvement of cardiac artifacts, which were detectable via the increased scatter in the Zrs data at certain frequencies in the repeated measurements at the same Prs and which were omitted from the fitting, and 2) the increase in F% in phases of the TLC maneuver in which the change in Zrs was relatively fast. In these phases, however, the impairment in fitting quality was caused by increased fluctuations in the Zrs vs. frequency data (this is also apparent in Fig. 1) rather than systematic differences in the fitting.

The impedance parameters obtained from the tracking and constant-Prs estimations were in good agreement and exhibited comparable variabilities in subsequent measurements; the patterns of the dependences on Prs were also similar to those observed previously (24). Raw decreased monotonically with Prs, covering a wide range between 0 and 20 cmH2O, especially if the

Fig. 5. Lower airway conductance (Glaw) vs. V in the 6 mice. • and ○, Inspiratory and expiratory values, respectively, during the TLC maneuver. Lengths of the regression lines indicate the volume range considered for the linear Glaw vs. V relationship.
Fig. 6. “Elbow plots”: the relationships between Raw and $H$ during inflation (●) and deflation (○) in each animal (1–6). Regression lines correspond to 2 distinct phases of the Raw vs. $H$ relationship: the “low-volume” region, including early inflation until the inspiratory minimum of $H$ and late deflation starting with the expiratory minimum of $H$, and the “high-volume” region, covering the rest of the data.

Fig. 7. Left: changes in model parameters (●) and single-frequency impedance values (▲) as functions of $V$ during the TLC maneuver in a mouse. Top: Raw and total respiratory resistance at 38 Hz (Rrs38). Bottom: $H$ and magnitude of total respiratory reactance at 4 Hz ($X_{rs4}$). Right: regression diagrams with 2nd-order polynomial fitting (dashed lines) between Raw and Rrs38 (top) and between $H$ and $X_{rs4}$ (bottom).
Patterns of volume dependence. The dependences of the mechanical parameters on Prs [or the transpulmonary pressure (Ptp)] or V were qualitatively similar to those obtained in previous investigations in rats (17, 22), and in rodents including rats and mice (14), although the ranges of the pressure levels used in those studies were narrower than the span of Prs in the present measurements: 0–9 cmH2O (17), 0–7 cmH2O (14), and 3–11 cmH2O (22). Accordingly, the fall in Raw with increasing Prs, Ptp, or V was generally milder, and the elevations in G and H after the medium-pressure minima of these parameters were less apparent (14, 17, 22, 25) than in the present study; a clear decrease in H was also observed at the highest Ptp levels (17, 22). Among these patterns of pressure dependence, the decreases in Raw with increasing Prs, Ptp, or V obviously reflect the changes in airway diameters, and the increases in G and H at higher lung volume are consistent with the stiffening of the respiratory tissues and particularly that of the lungs (1, 17). It is less straightforward, however, to interpret the increases in G and H toward low values of Prs: whereas the elevations in pulmonary G and H with decreasing Ptp can readily be explained by the progressive closure of lung units (17, 22), perplexingly similar patterns of chest wall G and H have been reported in dogs (1) and rats (17) and have been attributed to the complexity of the chest wall tissues (1). At low lung volumes, artificial increases in tissue parameters resulting from inhomogeneous constriction of the peripheral airways, such as those observed during constrictor challenge (20, 21), might also have been involved.

Airway resistance vs. lung volume. As concerns the mechanical parameters of the respiratory system, the volume dependence of the airway resistance has been of primary interest. The inverse relationship between Raw and V (7, 8) reflects an interdependence between the tissue and bronchial systems and can be regarded as one of the principal determinants of the airway tone. Jordan et al. (19) applied 3-Hz forced oscillations in mechanically ventilated patients during slow inflations and calculated the specific lower airway conductance (sGlaw = dGlaw/dV) from the Rrs data as a function of volume. After the removal of a resistance component, which was assumed to be independent of volume, by fitting a hyperbolic model between Rrs and V, those authors obtained linear relationships between Glaw and V, which were different in normal subjects and in those with chronic pulmonary disease and which changed after bronchodilator treatment. They argued that sGlaw reflects changes in airway distensibility due to a change in bronchomotor tone, and hence it provides an index of airway tone that is independent of V. In the present study, we also established fairly linear Glaw vs. V relationships for most of the covered volume range; however, unlike the relationships in the above-mentioned work (19), the range did not decrease below FRC but included much larger inflation volumes relative to the end-expiratory volume in this species. As TLC was approached, a sudden rise in Glaw was observed in all mice. Although it may be possible that at extreme lung volumes the Glaw vs. V relationship is governed by mechanisms different from those acting at moderate distensions of the respiratory system, it is also possible that measurement inaccuracies were involved. When very low values of Raw are reached, even small errors in the estimation of the frequency-independent resistance, which includes those that do not change with V (e.g., the endotracheal tube) or depend on it in a different manner (such as the Newtonian component of tissue resistance), may distort the linear relationship. However, it should be pointed out that the linear Gaw (1/Raw) vs. V relationship is a simplified concept, and it was never a uniform finding in the earliest work on the volume dependence of plethysmographic Raw (6, 8, 15, 28). In fact, Butler et al. (8) obtained sigmoid-shape Gaw vs. V curves with an upward swing in Gaw at the highest values of V, a pattern similar to that found in the present study (Fig. 5). Those authors also observed that the Gaw vs. Ptp relationships remained fairly linear and, on the basis of measurements obtained during chest strapping, they concluded that a greater pull on the airway wall results in an additional airway enlargement at the same high volume level.

Single- vs. multiple-frequency tracking. The continuous monitoring of respiratory impedance is the most appropriate tool with which to explore the effects of changing lung volume (5, 18, 19) or the temporal dynamics of constrictor responses (2–4). Although such single-frequency measurements are relatively easy to implement, the choice of the oscillatory frequency and the interpretation of the impedance values at that rate are not straightforward. The use of high frequencies offers a good temporal resolution and is usually assumed to provide an estimate of Raw (2, 3, 5, 18), although it is important to assess the contributions of the (negatively) frequency-dependent and Newtonian tissue resistance components to the measured resistance, especially if they are expected to change via volume-dependent stretching or tissue constriction. The values of Rl at 8 and 6 Hz, used in previous studies in humans (5, 18) and dogs (2, 3), respectively, were probably acceptable surrogates for Raw, whereas the selection of the 3-Hz values of Rrs in humans was clearly more critical, despite the relatively small inflation volume (<1 liter) applied above FRC (19).
results show that, at a higher frequency, although in a much smaller species, Rrs includes only an ~20% contribution from the tissue resistance at low lung volumes, whereas this component increases progressively with increasing V and results in an overestimation of Raw by 160% at TLC. Because the magnitude of the constant-phase tissue component decreases with increasing frequency (proportionally to $\omega^{-\alpha}$), an increase in oscillation frequency by factors of 2 and 10 would result in overestimations of Raw at TLC by 87 and 21%, respectively, on the assumption of $\eta = 0.2$ (which corresponds to $\alpha = 0.875$) for simplicity and provided that Raw remains the same at all frequencies. This simple calculation indicates the need for a much higher tracking frequency for a single-frequency estimation of Raw in the mouse, which has been demonstrated to have a relatively low Raw for its size (14). By contrast, Raw in the mouse, which has been demonstrated to remain the same at all frequencies. This simple calculation indicates the need for a much higher tracking frequency for a single-frequency estimation of Raw in the mouse, which has been demonstrated to have a relatively low Raw for its size (14). By contrast, Raw in the mouse, which has been demonstrated to remain the same at all frequencies.

Raw vs. $H$: the elbow plot. The display of the data pairs of two parameters obtained from the TLC maneuver that characterize the overall bronchial geometry and the tissue elasticity, respectively, reveals a biphasic mechanical relationship not explicitly containing Prs or V in this volume region, and in the usual presentation of the data the biphasic relationship between Raw and $H$ therefore remains concealed. Although this pattern could have been observed in previous investigations in which the Newtonian resistance and the tissue elastance were estimated at different levels of Prs or transpulmonary pressure (14, 22, 24), in consequence of both the wide pressure range and the multitude of Zrs estimates the present study is the first to document it. The utility of these plots nevertheless depends on the interpretation of the relationships displayed. In the high-volume region, the Raw vs. $H$ relationship expresses the coupling between the stiffness of the tissues of the chest wall and the lungs and the effective cross-sectional area of the airways embedded in the latter, and it is therefore connected with the relative distensibility of these compartments. The underlying mechanisms are more complex in the low-volume range, where both continuous processes (changes in airway diameters and tissue nonlinearities) and discrete events (closure and reopening of airways and alveoli) may be involved in the parallel changes in Raw and $H$. Extension of the deflation part of the maneuver to negative mean values of Prs, such as those maintained in other studies (1, 19), and separate measurement of the pulmonary and chest wall compartments (1, 17) may help clarify the contributing processes. All these concurrent mechanisms result in remarkably linear Raw vs. $H$ relationships in this region, which is separated from the “high-volume” set of data by the points of minimum elastance. It is also interesting to note that these points, corresponding to 40% of maximum V, are located around the maximum slope, i.e., the inflexion points of the pressure-volume loops (data not shown); thus they represent the joint minima of the dynamically measured (oscillatory) and quasi-static elastance values.

In conclusion, the continuous estimation of respiratory mechanical parameters from the tracking of multiple-frequency Zrs spectra during slow TLC maneuvers is feasible in anesthetized and intubated, but nonparalyzed mice. This method allows the fast revelation of the dependences of the mechanical parameters on Prs and V, and their patterns of interdependence, and hence it is well suited to study the alterations in respiratory mechanics during challenges of the bronchopulmonary system.

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DISCLOSURES

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