Effects of heterogeneities on the partitioning of airway and tissue properties in normal mice

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Submitted 10 August 2006; accepted in final form 26 November 2006

Impedance data acquired over a low-frequency range covering the normal breathing rates carry information about both airway and tissue properties. Hantos et al. (14) introduced an approach to partition lung impedance to airway and tissue components using a noninvasive method, by fitting a model to the impedance data. This model consists of a single compartment, including airway (Zaw) and tissue impedance elements connected in series. The real part of the Zaw is airway resistance (Raw), which is obtained as the pressure drop across the airway component in phase with and normalized by the flow. The tissue impedance is termed the constant-phase (CP) model, because the phase angle of the tissue impedance is independent of frequency. The tissue resistance (Rti) is calculated as the in-phase component of the pressure drop across the tissues normalized with the flow. This single-compartment model represents a homogeneous lung and has been applied to the mouse lung under various conditions (12, 16, 17, 32, 33, 36, 41).

However, it has been recognized that the partitioning of lung resistance to airway and tissue components based on the single-compartment model can lead to an overestimation of Rti during severe constriction due to airway-related heterogeneities in the lung (6, 26). To account for airway heterogeneities that occur during asthmatic airway constriction, Suki et al. (39) developed a distributed model of airway pathways with different Raw values but identical tissue elements. Alternatively, in other disease conditions, such as emphysema (1, 34) and acute respiratory distress syndrome (7), tissue heterogeneities can develop with significant impact on the pathophysiology of these diseases. Recently, Ito et al. (20) developed a distributed model, which included pathways with different tissue elements (H) but identical Raw values. This model provides an improved partitioning of tissue properties from impedance data in emphysematous mice (20). More recently, Kaczka et al. (22) further developed the tissue heterogeneity model by allowing different distributions for the H. Interestingly, the model that includes airway-related time constant heterogeneity significantly improved the model fit over the single-compartment CP model, even in normal rats (35), suggesting some degree of heterogeneity in normal animals. Indeed, morphological studies have shown that some heterogeneities in the alveolar size exist even in normal mice (20, 37). Thus it is possible that the mouse lung also exhibits some functional heterogeneities, even in baseline conditions.

The purpose of the present study was to carefully characterize the effects of heterogeneities on the partitioning of total respiratory and lung mechanics into airway and tissue properties.

MICE ARE WIDELY UTILIZED AS animal models of lung diseases due to the availability of genetic manipulations (10). Since changes in lung function are important in various diseased conditions, recent studies have attempted to characterize the mechanical properties of the respiratory system and the lung in mice, despite their small size (5, 10, 37, 40). The forced oscillation technique (FOT) is an accurate and powerful method to assess the detailed mechanical properties of the lung from input impedance (Zin) measurement made over a range of frequencies (29). With the use of the FOT, the mechanical properties of the lung have been compared in various rodents (15, 26, 35). Interestingly, the mechanical properties of mouse lungs have been reported to be different from those of larger rodents (12).

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ties in normal mice using the FOT under several conditions. We compared the mechanical parameters obtained by fitting the impedance data to three different models discussed above to reveal how the airway and tissue heterogeneities may affect the estimation of airway and tissue parameters. Besides heterogeneities, various factors, including absolute lung volume, tidal volume ($V_T$), and the presence of the chest walls, may also have an impact on lung function, characterized by the apparent mechanical properties of the respiratory system (2-4, 13, 20, 30, 32, 36). The optimal ventilation waveform (OVW) is a unique method that is able to mimic tidal breathing during measurements (27), and its peak-to-peak oscillatory volume amplitude usually matches the size of a normal VT. Thus we further investigated the effects of the chest wall, positive end-expiratory pressure (PEEP), and $V_T$ of the OVW on the mechanical properties in normal mice.

**MATHEMATICAL MODELS**

**CP model.** In the CP model (14), the linear tissue impedance ($Z_{L_{ti}}$) is calculated as:

$$Z_{L_{ti}}(\omega_n) = (G - jH)/\omega_n^\alpha$$

where $\omega_n$ and $\omega$ are the normalized and absolute circular frequency, respectively; $G$ and $H$ are the coefficients of tissue damping and elastance, respectively; and $j$ is the imaginary unit. The exponent $\alpha$ describes the frequency dependence of tissue resistance [$R_{ti} = G/(\omega_n)^\alpha$] and tissue elastance [$E_{ti} = H/(\omega_n)^{1-\alpha}$]. The normalization factor $\omega_0 = 1 \text{ rad/s}$ is used to obtain meaningful units for the parameters $G$ and $H$. The lung or respiratory system impedance can be obtained by adding the impedance of the airway tree ($Z_{aw}$) in series to the tissue impedance, where $Z_{aw}$ is the series combination of $R_{aw}$ and an airway inertance ($I_{aw}$). The heterogeneity of $R_{aw}$ distribution was characterized by the coefficient of variation (CV) of $R_{aw}$ defined as the ratio of the SD of $n(R)$ to $R_{aw}$.

$$Z_{aw}(\omega_n) = R_{aw} + j\omega I_{aw}$$

In this model, there are four parameters to estimate from the impedance spectra: $R_{aw}$, $I_{aw}$, $G$, and $H$. Hysteresivity $\eta$ (11) was calculated as $G/H$.

**Heterogeneous Raw model.** To account for the presence of airway heterogeneities, the airway tree was represented by a set of parallel pathways, each composed of a Newtonian resistance $R$ and an inertance $I_{aw}$, connected in series with a tissue compartment (39). The values of $G$ and $H$ were the same in each pathway, and the $R$ was varied in each pathway, according to a hyperbolic distribution function $n(R)$, where $n(R)$ is proportional to $1/R$, and $R$ varies between a minimum ($R_{\min}$) and a maximum ($R_{\max}$) value. In this model, five parameters ($G$, $H$, $R_{\min}$, $R_{\max}$, and $I_{aw}$) were determined. Total $R_{aw}$ was obtained from the model as the expected value of $n(R)$. The value of $R_{aw}$ and the standard deviation (SD) of $n(R)$ were calculated from estimates of $R_{\min}$ and $R_{\max}$ as:

$$R_{aw} = \frac{R_{\max} - R_{\min}}{\ln R_{\max} - \ln R_{\min}}$$

SD of $n(R) = \frac{(R_{\max}^2 - R_{\min}^2)}{2(\ln R_{\max} - \ln R_{\min})} - \frac{(R_{\max} - R_{\min})^2}{(\ln R_{\max} - \ln R_{\min})^2}$$

The heterogeneity of $R_{aw}$ distribution was characterized by the coefficient of variation (CV) of $R_{aw}$ defined as the ratio of the SD of $n(R)$ to $R_{aw}$.

**Heterogeneous H model.** To account for the presence of the $H$ heterogeneities, the airway tree was represented by a set of parallel pathways, each composed of $R_{aw}$, $I_{aw}$, and a tissue compartment (20). The values of $R_{aw}$ and $I_{aw}$ were the same in each pathway, but each compartment had a different elastance value denoted by $H'$. Since $H'$ was distributed, $\alpha$ in Eq 1 was also distributed, which would not allow for a simple solution of the network. To avoid this difficulty, Eq. 1 is first written as:

$$Z_{L_{ti}}(\omega_n) = (\eta - jH'/\omega_n^\alpha)$$

Thus, if we assume that each tissue compartment had the same $\eta, \alpha$ would not depend on $H'$ in Eq. 5. Similar to the distributed resistance model, the distribution function $n(H')$ was also hyperbolic, $n(H') = 1/H'$, between a minimum ($H_{\min}$) and maximum ($H_{\max}$) value. The corresponding $Z_{in}$ of the network was then obtained as:

$$Z_{in} = \frac{FZ_{aw}}{F + \ln \left( \frac{Z_{L_{ti}}_{min} + Z_{aw}}{Z_{L_{ti}}_{max} + Z_{aw}} \right)}$$

where $F$, minimum $Z_{L_{ti}}$ ($Z_{L_{ti}}_{min}$), and maximum $Z_{L_{ti}}$ ($Z_{L_{ti}}_{max}$) are given by
In this model, five parameters (Raw, Iaw, η, Hmin, and Hmax) were determined. The mean elastance of the network (H) was obtained as the expected value of the distribution function \( \mu(H') \). Both H and the SD of \( \mu(H') \) were calculated from estimates of \( H_{\text{min}} \) and \( H_{\text{max}} \) as:

\[
\text{SD of } \mu(H') = \sqrt{\frac{(H_{\text{max}} - H_{\text{min}})^2}{2F} - \frac{(H_{\text{max}} - H_{\text{min}})^4}{2F^2}}
\]

The heterogeneity of H was characterized by the CV of H, defined as the ratio of the SD of \( \mu(H') \) to H. The parameter G was calculated as \( G = \eta H \). Therefore, G was also distributed between a minimum and a maximum value, and the CV of G, characterizing its heterogeneity, was same as the CV of H.

**Fitting errors.** In each model fitting, by use of a global optimization algorithm (8), the model parameters were estimated by minimizing the following root-mean-square error (RMSE) between the model and the data:

\[
\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} [Z(\omega_i)_{\text{data}} - Z(\omega_i)_{\text{model}}]^2}
\]

where \( \omega_i \) refers to the nonsum nondifference (NSND) frequencies in the OVW (38), and \( N = 5 \) is the number of NSND frequencies.

**METHODS**

**Animal preparation.** Normal male C57BL/6 mice (23–26 g) \((n = 12)\) (Charles River, Cambridge, MA) were studied. The animals were deeply anesthetized by intraperitoneal injection of pentobarbital (70 mg/kg), tracheostomized, and cannulated in the supine position. The cannula was connected to a computer-controlled ventilator (Flexivent, SCIREQ, Montreal, PQ, Canada). All animal procedures were approved by the Animal Care and Use Committees of Boston University.

**Protocol.** Mice were mechanically ventilated with room air using VT of 8 ml/kg at a frequency of 240 breaths/min. After stabilization, respiratory mechanics were measured at four different PEEP levels (0, 3, 6, and 9 cmH2O) in the closed-chest condition, as described previously (20). Following measurements in the closed-chest condition, the chest wall was opened by widely cutting diaphragm, and dynamic respiratory mechanics were assessed again at four different PEEP levels (3, 6, 9, and 15 cmH2O) \((n = 8)\) by measuring forced oscillatory impedance data using the computer-controlled pneumatic ventilator system (Flexivent). In another set of four mice, two different values of VT (4 and 8 ml/kg) were applied during measurements at PEEP levels of 0, 3, 6, and 9 cmH2O in the closed-chest condition. To standardize volume history, each measurement was preceded by two consecutive inflations of the lungs to total lung capacity, defined as a tracheal pressure of 25 cmH2O.

**Impedance measurements.** Impedance data collection was made by interrupting mechanical ventilation for 6 s using the OVW technique, which is a broadband waveform containing energy from 0.5 to 15 Hz (27). The frequencies in the OVW are selected according to a NSND criterion, which eliminates harmonic distortion and minimizes cross
talk among the frequencies that are present in the input flow waveform and hence provides smooth estimates of the $Z_{in}$ of the system (38). In our experiment, we matched the peak-to-peak OVW amplitude to the Vt delivered by the mechanical ventilator. The ventilator displacement and cylinder pressure signals were low-pass filtered at 30 Hz and sampled at 256 Hz. With the use of Fourier analysis, impedance spectra were calculated on overlapping blocks of pressure and flow data as the ratio of the cross-power spectrum of pressure and flow and the autopower spectrum of flow. We also measured the $Z_{in}$ of several bottles with known mechanical properties, with and without the tracheal cannula, using several flow rates that were controlled via the Vt of the OVW. We then separately measured the cannula impedance with similar flow rates. This allowed us to recreate the impedance of the bottle from the bottle-cannula measurement by subtracting the flow-dependent impedance of the cannula. During the measurement of a mouse, we used the appropriate cannula impedance with the corresponding flow rate to eliminate its effect from the total mouse-cannula measurement.

**Statistical analysis.** All data were expressed as means ± SD. Student’s $t$-test, $F$-test, and two-way repeated-measures ANOVA followed by Tukey test for post hoc analysis were used to evaluate the significance of differences between means and variances, with $P < 0.05$ as the level of significance (SigmaStat3.0, SPSS). Statistical analysis was performed according to guidelines for reporting statistics in journals published by the American Physiological Society (8a).

**RESULTS**

**Comparison of fitting errors among the three models.** All three models, the CP model, the heterogeneous airway resistance (HAR) model, and the heterogeneous tissue elastance (HTE) model, fit the respiratory impedance data at all PEEP levels reasonably well in both the closed- and the open-chest conditions. The fitting errors, expressed as RMSE (Eq. 9), significantly depended on PEEP in all models, both in the closed- and open-chest conditions by repeated-measures two-way ANOVA ($n = 8$) ($P < 0.001$) (Fig. 1). Both in the closed- and open-chest conditions, the fitting errors significantly depended on the model type by ANOVA ($P < 0.001$), and the fitting errors of the HAR ($P < 0.001$) and HTE models ($P < 0.01$ and $P = 0.01$), respectively, were significantly lower than those of the CP model, suggesting that some sort of heterogeneity affected the respiratory mechanical parameters in normal mice. The fitting errors of the HAR model were also significantly smaller than those of the HTE model, both in the closed- ($P < 0.001$) and and open-chest ($P < 0.001$) conditions. The differences are the largest at PEEP = 0 cmH$_2$O in the closed-chest and at PEEP = 15 cmH$_2$O in the open-chest conditions. By post hoc analysis with Tukey test, there were no significant differences in the errors at PEEP = 3 cmH$_2$O in the closed-chest and at PEEP = 3 and 6 cmH$_2$O in the open-chest conditions. Because the number of the parameters were different between CP ($n = 4$) and HAR ($n = 5$) or HTE ($n = 5$) models (see **MATHEMATICAL MODELS** section), the difference in fitting errors was also analyzed by $F$-test on an individual basis. Even though the heterogeneous models always gave lower RMSE, there was no significant difference between CP and HAR models or between CP and HTE models at an equivalent PEEP by $F$-test.

![Fig. 3](http://jap.physiology.org/)

**Fig. 3.** Means ± SD are shown of G (A), Raw (B), H (C), and hysteresivity (D) in the open-chest condition obtained by the CP (●), HAR (■), and HTE (□) models as a function of PEEP (3–15 cmH$_2$O) ($n = 8$). *$P < 0.05$ between the *CP and HAR models, the #CP and HTE models, and the $\#$HAR and HTE models.
Comparison of respiratory mechanics among the three models. There were statistically significant differences between the four mechanical parameters (G, H, Raw, and hysteresivity η) obtained by three models as a function of PEEP in the closed-chest condition (Fig. 2). The values of Raw and H represent the expected values of the resistance distribution in the HAR model and the elastance distribution in the HTE model, respectively. All four parameters significantly depended on the model type and PEEP (P < 0.001) (Fig. 2). The values of G, H, and Raw were the highest and η was the lowest at PEEP = 0. The G was the highest in the HTE model. The values of G and η were the lowest and Raw was the highest in the HAR model. There were no differences in η or in Raw between the CP and HTE models or in H between the CP and HAR models. The H was the same among three models, except at PEEP = 0.

In the open-chest condition, the four parameters also significantly depended on the model type and PEEP (P < 0.001) (Fig. 3). The values of G, H, and Raw were the highest and η was the lowest at PEEP = 15 cmH2O. Similar to the closed-chest condition, G and H were the highest in the HTE model, and η was the lowest while Raw was the highest in the HAR model. There were no differences in η or in Raw between the CP and HTE models or in H between the CP and HAR models.

Next, the heterogeneity of Raw (CV of Raw) obtained by the HAR model and heterogeneity of H (CV of H) obtained by the HTE model were compared (Fig. 4). Both in the closed- and the open-chest conditions, the CV of Raw was significantly higher than the CV of H at any PEEP (P < 0.001) (Fig. 4). The CV of Raw significantly depended on PEEP (P < 0.001), but the CV of H did not.

Effects of chest walls on respiratory mechanics. The values in Fig. 5 were obtained by reploting data in Figs. 2 and 3. The values of G, H, Raw, and η obtained by the CP model in the closed- and open-chest conditions were compared at equivalent PEEP levels (3, 6, and 9 cmH2O) in Fig. 5. The G (P = 0.001), η (P < 0.001), and Raw (P < 0.01) were significantly lower in the open-chest condition. There was no significant difference in H between the two conditions by ANOVA, and H was lower in the open-chest condition only at PEEP = 3 cmH2O by Tukey test (P < 0.05). The relative contributions of the chest wall on the values of G, H, η, and Raw obtained by the distributed models (the HAR and HTE models) were similar to those obtained by the single-compartment CP model (data not shown). When the values of CVs shown in Fig. 4 are compared, there was no significant difference in the CVs of Raw and H between the two conditions at equivalent PEEPs.

Effects of lower VT on the mechanical properties. Figure 6 shows the values of the mechanical parameters obtained by the three models as a function of PEEP in the closed-chest condition (n = 4). In this case, VT was lowered to 4 ml/kg during the OVW measurement. The results were qualitatively similar, but the values of the parameters were different from those obtained with VT of 8 ml/kg in Fig. 2. All four parameters significantly depended on the model and PEEP (P < 0.001) (Fig. 6). The values of G, H, and Raw were the highest and η was the lowest at PEEP = 0. The G was the highest in the HTE model, and G and η were the lowest and Raw was the highest in the HAR model. There were no differences in Raw and η between the CP and HTE models or in H between the CP and HAR models.

The mechanical parameters (G, H, Raw, and η) obtained by applying different VT values (4 or 8 ml/kg) during the OVW at equivalent PEEP levels (0–9 cmH2O) were compared in Fig. 7, A–D (n = 4). The values obtained by the CP model using VT = 4 ml/kg in the closed-chest condition were compared and redrawn from Fig. 6. The G (P < 0.01) and η (P < 0.001) were significantly higher when measured using VT = 4 ml/kg. There were no significant differences in H or Raw corresponding to the two VT values when all PEEP levels were included in the statistics. However, H was higher using VT = 4 ml/kg at PEEP = 0 (P = 0.01) and 3 cmH2O (P < 0.05) by Tukey test.

The effects of VT on the values of G, H, η, and Raw obtained by HAR or HTE models were similar to those by the CP model (data not shown). There was no significant difference in the CV of Raw (Fig. 7E) but the CV of H was significantly higher at PEEP = 0 using the 8 ml/kg VT (P < 0.01) (Fig. 7F).

Airway-tissue partitioning by the three models. The values of Rti at 1 Hz of frequency were obtained as Rti = G/ωn with ωn = 2π (see Mathematical models). The Rti normalized with Raw (Rti/Raw) was compared among three models as a function of PEEP in the closed-chest (Fig. 8A) and the open-chest conditions (Fig. 8B). The Rti/Raw values significantly depended on the model type and PEEP, both in the closed- and open-chest conditions (P < 0.001) (Fig. 8, A and B). The values of Rti/Raw were the highest in the HTE model and the lowest in the HAR model in both conditions.
The Rti/Raw obtained by applying different VT values (4 or 8 ml/kg) during the OVW at equivalent PEEP levels (0 – 9 cmH2O) were compared in Fig. 8C (n = 4). The data were obtained by the CP model in the closed-chest condition. The Rti/Raw values were significantly higher when measured using VT = 4 ml/kg (P < 0.05). These results imply a significant effect of model-based heterogeneity, PEEP, and VT on the airway-tissue partitioning in mice.

DISCUSSION

Noninvasive, model-based partitioning of airway and tissue mechanical properties has been the topic of many studies in many species (12, 14–16, 18, 20, 21, 26, 32, 33, 35, 36, 39, 41). Often important biological conclusions are drawn about certain molecular mechanisms based on the effects of various drugs on the airways alone (e.g., Refs. 23, 31). Thus accurate and reliable partitioning of airway and tissue mechanical properties using noninvasive tools is of crucial importance. The primary objectives of the present study were to investigate how this partitioning is influenced by a variety of factors in normal mice and to determine which model is suitable for the partitioning under those conditions. Our main findings are that 1) both in the closed- and the open-chest conditions, heterogeneity was present in the respiratory system of the normal mouse with consequences on airway-tissue partitioning, as assessed from the mechanical parameters of three impedance models; 2) the chest wall significantly affected all viscous parameters, including the G and η as well as R (Raw), but its contribution to H was small; and 3) both PEEP and the VT of the OVW significantly affected the mechanical properties and the airway-tissue partitioning.

Comparison of three different models. In the present study, we compared the fitting errors, expressed as RMSE, and the values of the mechanical properties of mouse lungs obtained by fitting the impedance data to models based on the heterogeneous distributions of Raw (39) or H (20) and compared them to those obtained by the single-compartment CP model (14). The CP model is useful to analyze respiratory mechanics and widely applied to various species, including mice (12, 16, 17, 30, 36). One of the most important factors that affects the values of the mechanical parameters is the heterogeneity of the airway tree and the parenchyma because of the complex structure of the respiratory system (6, 20, 26, 39). Small but significant improvement of the errors (Fig. 1) suggests that both airway and tissue heterogeneities may exist, even in normal mice. The errors of the HAR model were significantly smaller than those of the HTE model (Fig. 1). Since these two models contain the same numbers of parameters, the HAR model could provide better estimation of the mechanical properties than the HTE model in normal mice. Interestingly, the CV of H from the HTE model was significantly less than the CV of Raw from the HAR model, both in the closed- and open-chest conditions (Fig. 4), implying a reasonably homogeneous alveolar ventilation. Moreover, the improvement of
the errors by the HAR model depended on PEEP and was larger at PEEP levels of 0 and 9 cmH\textsubscript{2}O in the closed-chest condition and at 9 and 15 cmH\textsubscript{2}O in the open-chest condition (Fig. 1). Furthermore, this improvement tends to correlate with airway heterogeneity (the CV of Raw shown in Fig. 4). Hence, in normal mice, the contribution of airway heterogeneity to respiratory mechanics appears to be greater than that of tissue heterogeneity, so that the HAR model can be more reliable to estimate the tissue mechanical properties of normal mice.

Sakai et al. (35) reported that the HAR model caused significantly large (20–60%) reduction in the fitting errors compared with the CP model, both in the closed- and the open-chest conditions in normal rats. Therefore, in normal rats without bronchoconstriction, heterogeneity can influence the estimation of mechanical parameters. In the present study, improvement in the errors by the HAR model over the CP model was much less than in rats (35) and was not significant by F-test, indicating that the effects of airway heterogeneities are less in mice than in rats. Nevertheless, as the results in Fig. 8, A and B, suggest, the type of heterogeneity built into a model does have a significant influence on the airway-tissue partitioning.

Mechanical properties obtained by three different models. The HAR and HTE models include some form of heterogeneities of Raw and H, respectively, so that the estimation of Raw by the HAR model and of H by the HTE model may be more accurate than by the CP model. Compared with the HAR model, the CP and HTE models reduced Raw, both in the closed- and the open-chest conditions (Figs. 2 and 3). This would indicate that the CP and HTE models underestimate Raw. Moreover, the HTE model slightly but significantly increased H compared with the CP and HAR models (Figs. 2 and 3). Therefore, it is likely that H may be somewhat underestimated by the CP and HAR model.

Recently, Kaczka et al. (22) introduced several newer versions of the HTE model by implementing a uniform or a linearly increasing distribution of H\textsuperscript{11032}/H\textsubscript{11032} and applied the model in dogs before and after injury. It is possible that these models would provide a slightly better partitioning by the HTE model. However, it is unlikely that the results of Kaczka et al. (22) could be directly applied to our case because of the significant differences between the chest wall and lung structures of the dog and the mouse.

The η has been introduced as a mechanical property determined by the material itself (11). Surprisingly, we found different η values estimated by the three models. Compared with the CP model, the HAR model reduced G without changing H, both in the closed- and the open-chest conditions (Figs. 2 and 3). As a result, η became significantly lower in the HAR model. These observations are similar to those observed in normal rats, although the differences are less than those in rats (35). On the other hand, the HTE model did not change η compared with the CP model (Figs. 2 and 3). In the HTE model, η is defined to be constant in each pathway (20). Since each pathway consists of CP tissue impedances, it appears that the HTE model provides estimates of η that are similar to those of the CP model. However, in light of the better performance...
of the HAR model, η may be somewhat overestimated by the CP and HTE models.

In summary, the present results suggest that the HAR model has the advantage of providing the most appropriate airway-tissue partitioning in normal mice. Nevertheless, the smaller improvement of the fitting errors by the heterogeneous models suggests that the estimation of the mechanical properties and airway-tissue partitioning by the CP model is suitable in normal mice. Specifically, the partitioning by the CP model is in between that of the HAR and the HTE models (Fig. 8) and appears more accurate when the airway heterogeneity (the CV of Raw) and the improvement of RMSE were the lowest at mild PEEP levels (3–6 cmH2O). However, following treatment or in transgenic animals, the single-compartment CP model may not be able to provide appropriate partitioning or detect airway or tissue abnormalities. Indeed, recently, we found evidence that Hmax differed in normal mice and Pallid mice, a model of α1-antitrypsin deficiency (28), as a function of PEEP that was not detected by the CP model at the early age of 7 wk (19). This is important, since previously the Pallid mice was thought to develop emphysema only by ~10 mo of age (28).

Effects of chest walls. It is known that the chest wall significantly contributes to respiratory mechanics in humans and various animals (2–4, 9, 13, 15, 35, 36). Therefore, since
the esophageal balloon technique may not be feasible in the mouse, it is the invasive surgery of opening the chest walls that has been the only method to obtain direct information on lung mechanics (15, 35, 36). Hirai et al. (15) reported that the contribution of the chest walls to $G$ and $H$ values was ~60 and 50%, respectively, of the total respiratory system in rats. In normal rats, $\eta$ was also significantly lower in the open-chest condition than in the closed-chest condition; however, there was little contribution of the chest wall to Raw (15). Compared with the results in rat lungs, the present findings demonstrate that the contribution of the chest wall to $H$ and Raw is much less in mice. In the mice, the average contributions of the chest wall to $G$ and Raw were 19 and 31%, respectively, of the total respiratory system at PEEP = 3–9 cmH2O (Fig. 5). However, when PEEP was increased up to 6 cmH2O, the difference in $H$ values between the open- and closed-chest conditions disappeared, consistent with a recent study in mice, in which lower peak-to-peak oscillatory amplitude oscillations were used to obtain mechanical parameters (36). Although the chest wall in the mouse significantly contributes to the mechanical properties (Fig. 5), this contribution is less than in other larger species (12, 13). The reason may be that the chest wall tissue is somewhat smaller, resulting in higher surface forces (25). We also note that, despite the fact that in the present study the open-chest condition was obtained by opening the abdomen-diaphragm pathway, the relative contribution of the chest wall to the mechanical parameters is consistent with a previous report in mice, in which the chest wall was widely retracted using sternotomy (36). Taken together, under the conditions of mild PEEP levels (3–6 cmH2O) that are adequate to prevent alveolar collapse, noninvasive measurements in the closed-chest condition are appropriate for the characterization of lung tissue elasticity in normal mice. Thus further invasive surgery, which may alter other physiological processes in the body, may not be necessary in mice.

We note that the same PEEP levels in the closed- and open-chest conditions are generally considered to correspond to different transpulmonary pressures. Thus the effect of elastic recoil, a difference of perhaps ~2 cmH2O, should be accounted for when comparing the open- and closed-chest data. We did not measure impedance at PEEP = 0 in the open-chest condition, since the lung would have collapsed at end expiration. While we did not measure the in situ transpulmonary pressure, we believe the 3 cmH2O of PEEP in the open-chest condition are much closer to the 3 cmH2O of PEEP than the 0 PEEP in the closed-chest condition. The reason is that, in Fig. 5, the difference between parameters in the open- and closed-chest conditions is less than ~25% at 3 cmH2O of PEEP, whereas, if we compare the values at 3 cmH2O of PEEP in the open-chest condition with those with no PEEP in the closed-chest condition (Fig. 2), the difference would be very large, maybe even double. Given that the difference is decreasing with PEEP as well as data from the literature (36), our comparison in Fig. 5 appears reasonable based on the available data.

Effects of lung volume. In general, the mechanical parameters are affected by lung volume, as shown in Figs. 2 and 3, in which the volume was changed by adding different PEEPs. Column (A) shows the comparison of tissue resistance ($R_{ti}$) at 1 Hz normalized with Raw ($R_{ti}/Raw$). Values are means ± SD of $R_{ti}/Raw$ in the closed-chest (A) and open-chest conditions (B) obtained by the CP (●), HAR (■), and HTE (○) models as a function of PEEP ($n = 8$). $P < 0.05$ between the *CP and HAR models, the #CP and HTE models, and the HAR and HTE models. C effects are shown of changes of $V_t$, 4 ml/kg (●) and 8 ml/kg (○), on $R_{ti}/Raw$ values obtained by the CP model in the closed-chest condition as a function of PEEP ($n = 4$). *$P < 0.05$.
airway length is increased, and it is possible that lengthening the airway in fact leads to a reduction of the cross-sectional area somewhere in the middle of the airway segment, similar to the reduction of cross section of an elastic bar under uniaxial strain. If this is the case, then, at some sufficiently high lung volume, we might see an increase in Raw, because the resistance is much more sensitive to small reduction in diameter than to small increase in length.

The pressure-volume curves in previous studies have shown that the lung volume keeps increasing with increasing inflation pressures up to nonphysiological levels (>30 cmH2O) in mice (24, 37). We note that, in the open-chest condition, when VT (8 ml/kg, ~0.2 ml) was applied on the highest (15-cmH2O) PEEP during the impedance measurement with the OVW, the peak pressures were <25 cmH2O.

Effects of VT in OVW technique. The peak-to-peak oscillatory amplitude in volume applied in the OVW is matched to the VT of conventional ventilation; therefore, the OVW method is useful to mimic tidal breathing during dynamic measurements (25). This technique has been applied to various species (7, 18, 35), as well as to mice (17, 19–21). Since the applied VT during measurement is larger than that used in many other FOT measurements (12–14, 32, 36), the amplitude of the OVW is expected to affect the values of the mechanical parameter in mice (30). Indeed, the present results reveal that the mechanical properties, specifically G and η, were significantly affected by the VT (Fig. 7). Additionally, Fig. 8 shows evidence that the airway-tissue partitioning is also affected by the VT. The smaller Rti fraction at the larger VT is a result of the tissues being softer, and, due to possible airflow nonlinearities, the Raw becoming larger with increasing VT. As PEEP increases, however, H and consequently G increase, which leads to an increasing Rti fraction.

In summary, respiratory mechanical parameters of mice were different among several mechanical models, including airway and tissue properties, possibly due to the contribution of airway and/or tissue heterogeneity. The contribution of the chest wall to the mechanical parameters was much less in mice than in larger rodents. Finally, we note that a more complex model that features simultaneous airway and tissue heterogeneities may be beneficial for partitioning of airway and tissue properties with potential implications to future studies in which the effects of drugs on separate components of the lung are explored.

GRANT

This study was funded by National Heart, Lung, and Blood Institute Grant HL059215 (B. Suki) and Ministry of Education Science, Sports, and Culture of Japan Grant 17790531 (S. Ito).

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