Nonlinearity of respiratory mechanics during bronchoconstriction in mice with airway inflammation

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1Vermont Lung Center, Department of Medicine, University of Vermont, Burlington, Vermont 05405; 2Department of Clinical Physiology, Malmö University Hospital, Lund University, SE-205 02 Malmö, Sweden; and 3Department of Biomedical Engineering, University of Sao Paulo, Sao Paulo, Brazil, CNPQ, Brazil

METHODS

Wagers, Scott, Lennart Lundblad, Henrique T. Moriya, Jason H. T. Bates, and Charles G. Irvin. Nonlinearity of respiratory mechanics during bronchoconstriction in mice with airway inflammation. J Appl Physiol 92: 1802–1807, 2002.; 10.1152/japplphysiol.00883.2001.—Respiratory system resistance (R) and elastance (E) are commonly estimated by fitting the linear equation of motion $P = EV + RV + P_0$ (Eq. 1) to measurements of respiratory pressure ($P$), lung volume ($V$), and flow ($V^\prime$). However, the respiratory system is unlikely to behave linearly under many circumstances. We determined the importance of respiratory system nonlinearities in two groups of mechanically ventilated Balb/c mice [controls (E2V2)] and mice with allergically inflamed airways [ova/ova], by assessing the impact of the addition of nonlinear terms ($E_2V^2$ and $R_2V^3|V|$) on the goodness of model fit seen with Eq. 1. Significant improvement in fit ($51.85 \pm 4.19\%$) was only seen in the ova/ova mice during bronchoconstriction when the $E_2V^2$ alone was added. An improvement was also observed with addition of the $E_2V^2$ term in mice with both low and high lung volumes ventilated at baseline, suggesting a volume-dependent nonlinearity of E. We speculate that airway closure in the constricted ova/ova mice accentuated the volume-dependent nonlinearity by decreasing lung volume and overdistending the remaining lung.

Respiratory system resistance; elastance; airway closure; hysteresis; asthma; pulmonary mechanics

RESPIRATORY SYSTEM RESISTANCE (R) and elastance (E) are commonly estimated by fitting the linear equation of motion

$$P = EV + RV + P_0 \quad (I)$$

to measurements of pressure ($P$), flow ($V$), and volume ($V$), where $P_0$ is a constant the function of which is to absorb any errors in estimating functional residual capacity ($FRC$) (15). The single-compartment linear model corresponding to this equation provides a reasonable description of respiratory system mechanics under most circumstances. However, the lung can exhibit nonlinear mechanical behavior under many circumstances, such as when airflow in the airways becomes turbulent ($RV$ becomes nonlinear) or when the lung parenchyma becomes overdistended ($EV$ becomes nonlinear). Under such circumstances, adding additional terms to Eq. 1 to account for these nonlinear phenomena would be expected to improve the description of the data, as has been previously shown (2, 10, 18, 23).

Improving the goodness of fit with the addition of nonlinear terms is of more than purely mathematical interest. If one can ascribe to the additional terms a plausible physiological mechanism or explanation, our understanding of the link between lung structure and function will improve. For example, if the addition of nonlinear terms improves model fit in some circumstances, e.g., bronchoconstriction, but not in others, one can make inferences about the importance or unimportance that certain nonlinear phenomena such as turbulence or overdistention have in a given pathophysiological setting.

We conducted the current study to examine the utility of adding nonlinear terms to the standard equation of motion (Eq. 1). Assessments were made at baseline and during bronchoconstriction in untreated mice as well as mice with antigen-induced airway inflammation to determine utility of such an approach in detecting physiological and pathological alterations. We studied mice because of the obvious potential they provide for conducting mechanistic studies.

METHODS

Study animals. Female Balb/c mice (Jackson Laboratories) 6–8 wk of age free of known murine pathogens were acclimatized in an animal facility for 1 wk with the provision of adequate food and water. The protocol was approved by the institutional animal care and use committee of the University of Vermont.

Allergen sensitization and challenge. We studied two groups of animals: 1) a group in which allergic airway inflammation was produced by sensitization and challenge with chicken egg albumin (ovalbumin grade V, Sigma Chemical) and 2) a control group age matched by using techniques as

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previously described (22). Mice in the sensitized and challenged group (ova/ova) received an intraperitoneal injection of 400 µg of ovalbumin mixed with an equal volume of aluminum hydroxide solution on day 1 and day 14. On day 21, the mice were placed in a Plexiglas chamber and exposed to an aerosolized solution of ovalbumin mixed with Dulbecco’s phosphate-balanced saline (PBS) to achieve a 1% concentration. Aerosol was delivered with a jet nebulizer (PARI LC PLUS, PARI Respiratory Equipment, Midlothian, VA) for 30 min a day for a total of 3 days. Forty-eight hours after the last exposure, a time point previously shown to coincide with maximal airway inflammation and responsiveness, the measurements described below were made. Inflammation was confirmed by counting cells by using a hemocytometer in fluid obtained by bronchoalveolar lavage with 0.8 ml of cold PBS.

**Determination of respiratory mechanics.** Anesthesia was achieved with 90 mg/kg of pentobarbital sodium injected intraperitoneally and confirmed by the absence of response to paw pinch. Mice were then tracheostomized, and an 18-gauge metal IV adapter, the tip of which had been beveled and ground smooth, was inserted into the trachea and firmly tied in place. Mice were then connected to a computer-controlled small animal ventilator (flexiVent, SCIREQ, Montreal, PQ, Canada), which allowed the application of specifically tailored volume perturbations to the lungs (8, 9, 21). Positive end-expiratory pressure (PEEP) of 3 cmH2O was generated by submerging the expiratory line 3 cm into a water trap.

P and V data were generated by applying a 2-s sine wave volume perturbation (SW) with an amplitude of 0.2 ml and a frequency of 2.5 Hz (simulating typical mechanical ventilation with the flexiVent piston) (18). V (i.e., volume delivered to the animal) was determined by correcting the volume displacement of the ventilator piston for gas compression in the ventilator cylinder as previously described (2, 9). P (i.e., pressure at the tracheal opening) was obtained by subtracting the resistive pressure across the flexiVent tubing from the pressure in the flexiVent cylinder. The E of gas in the cylinder and the R of the tubing were determined in separate calibration experiments. V was determined by numerically differentiating volume. After 5 min of regular mechanical ventilation, the SW perturbation was applied three times and the average was taken to generate a baseline measurement.

The standard linear equation of motion (Eq. 1) was extended in two ways, by the addition of a nonlinear E term

\[
P = E_1V + E_2V^2 + RV + P_o
\]  

(2)

and alternatively by the addition of a nonlinear R term

\[
P = EV + R_1V + R_2V|V| + P_o
\]  

(3)

All three models were fit to each set of P-V-V data. Goodness of model fit of each model was quantified in terms of the estimated noise variance (ENV)

\[
ENV = \left( \frac{\sum P_i - \bar{P}}{n - m} \right) \times 100
\]  

(4)

where \( P_i \) = measured pressure, \( \bar{P} \) = pressure by the model, \( n \) = sample size, and \( m \) = number of model parameters. A change in ENV occurring with the addition of either nonlinear term was quantified as

\[
\Delta ENV = \left( \frac{ENV_1 - ENV_2}{ENV_1} \right) \times 100
\]  

(5)

where ENV1 = ENV obtained when Eq. 1 was used and ENV2 = ENV obtained when either Eq. 2 or 3 was used. An F test, where \( F = ENV_1/ENV_2 \), was used to judge goodness of model fit. The F-distribution is driven by the sample size and the number of model parameters in the two equations being compared. The \( P < 0.05 \) value of the F-distribution pertaining to our situation is 1.3. Therefore, we took a value of \( \Delta ENV \) of 30% or greater as signifying a statistically significant improvement in model fit in going from the linear model (Eq. 1) to the nonlinear model (Eq. 2 or 3, as the case may be).

**Methacholine challenge.** Bronchospasm was induced with methacholine in PBS at a concentration of 10 mg/ml aerosolized by an ultrasonic nebulizer (Devilbuss AeroSonic 5000D, Somerset, PA). The aerosol was delivered to the airway opening by diverting the inspiratory ventilator flow through the aerosol chamber of the nebulizer for a total of 20 breaths at a tidal volume of 0.4 ml (allowing for gas compression in the nebulizer chamber) at a rate of 30 breaths/min. After the methacholine, the SW perturbation was applied every 30 s for a period of 10 min.

**PEEP challenge.** PEEP was randomly changed from 0 to 9 cmH2O in 3-cm increments in each mouse. Three successive SW perturbations were applied at each PEEP level, and the average \( \Delta ENV \) was determined.

**Static P-V curve measurements.** Starting at FRC, the flexiVent was programmed to deliver seven inspiratory volume steps for a total volume of 1 ml followed by seven expiratory steps, pausing at each step for at least 1 s. Plateau P at each step was recorded and related to the total V delivered. For each mouse, two successive P-V curves were measured before the methacholine challenge, and the values were averaged.

The shape factor (k) of the expiratory limb of each P-V curve was determined by fitting the Salazar-Knowles equation

\[
V = A - Be^{-4P}
\]  

(6)

where A and B are parameters (6). The area enclosed by each P-V curve was determined by subtracting the area under the inspiratory limb (obtained by numerical integration) from the area under the expiratory limb (10).

**Statistics.** The values of k and the P-V loop areas were compared by use of paired two-tailed t-tests.

**RESULTS**

Cell counts confirmed that the ova/ova mice were inflamed compared with the controls (cell numbers: 37.12 ± 10.10 × 106/ml vs. 7.0 ± 0.655 × 106/ml, \( P < 0.05 \)).

We first compared linear and nonlinear model fits in all mice at 3 cmH2O PEEP. When the model with the \( R_2V|V| \) term (Eq. 3) was compared with the linear model (Eq. 1) at baseline and during bronchoconstriction in both ova/ova and control mice, \( \Delta ENV \) did not exceed the 30% significance level (max = 16.06 ± 2.60% SE) (Fig. 1). The 30% significance level was also not exceeded with addition of the \( E_2V^2 \) term (Eq. 2) in the control mice; at baseline \( \Delta ENV \) was 9.56 ± 1.87% (n = 8), whereas 2 min after methacholine delivery the \( \Delta ENV \) was 9.23 ± 2.8%. In contrast, in the ova/ova mice (n = 8), after methacholine the \( \Delta ENV \) rose significantly to 51.85 ± 4.19% from a baseline value of 4.28 ± 2.27% (Fig. 2). Thus a significant improvement in the goodness of model fit was observed only in the inflamed mice during bronchoconstriction when a nonlinear E term was added to the linear model. Table 1 gives the parameter values of all the models in the
control and ova/ova mice both at baseline and after bronchoconstriction.

Next, we assessed whether the improvement in the goodness of model fit was affected by increases in mean lung volume. ΔENV was assessed in both control and ova/ova mice under baseline conditions at various lung volumes achieved by varying PEEP. At PEEP levels of 0, 6, and 9 cmH$_2$O, ΔENV was 36.47 ± 5.11, 72.9 ± 2.34, and 81.44 ± 0.74, respectively (Fig. 3). Table 2 gives the parameter values of all the models at the various PEEP levels in the control and ova/ova mice.

Static P-V curves of control and ova/ova mice were similar during expiration (Fig. 4), confirmed by a lack of significant difference between values of $k$ from the two groups (Table 3). (The difference between the mean $k$ values was 0.005; at a power level of 70% with our sample size of 6, we would be able to detect a difference in $k$ between the two groups of 0.028, whereas at a power level of 80% we could detect a difference of 0.031.) However, the area of the static P-V curves was significantly increased in the ova/ova mice compared with the control animals (Table 3). This matches the observation of a difference in the inspiratory limbs of the P-V curves from the two groups (Fig. 4).

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Table 1. Parameter values at baseline and during bronchoconstriction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Baseline</th>
<th>Control Bronchoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$, cmH$_2$O/s</td>
<td>0.649 ± 0.04</td>
<td>0.867 ± 0.06</td>
</tr>
<tr>
<td>$E$, cmH$_2$O</td>
<td>25.28 ± 1.75</td>
<td>28.06 ± 1.55</td>
</tr>
<tr>
<td>Nonlinear model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$, cmH$_2$O/s</td>
<td>0.649 ± 0.04</td>
<td>0.868 ± 0.06</td>
</tr>
<tr>
<td>$E_1$, cmH$_2$O</td>
<td>26.13 ± 1.77</td>
<td>28.89 ± 1.83</td>
</tr>
<tr>
<td>$E_2$, cmH$_2$O</td>
<td>-7.41 ± 1.19</td>
<td>-7.42 ± 4.91</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Parameter values obtained from the fitting of the linear (Eq. 1) and nonlinear elastance ($E$) model (Eq. 2) at baseline with and without bronchoconstriction induced by methacholine where positive end-expiratory pressure (PEEP) = 3 cmH$_2$O. $R$, resistance; $E_1$, linear elastance; $E_2$, nonlinear elastance. Values of all parameters increase during bronchoconstriction.

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Fig. 1. Improvement in fit (ΔENV) with the addition of a nonlinear resistance term ($R_2V/|V|$, where $R$ is resistance and $V$ is flow) in ova/ova mice ($n = 8$) and control mice ($n = 8$) at baseline and after 10 mg/ml methacholine aerosol.

Fig. 2. Improvement in fit (ΔENV) with the addition of a nonlinear elastance term ($E_2V^2$, where $E$ is elastance and $V$ is volume) in ova/ova mice ($n = 8$) and control mice ($n = 8$) at baseline and after delivery of a 10 mg/ml aerosol of methacholine. ΔENV >30 represents a significant improvement in fit ($P < 0.05$).

Fig. 3. ΔENV obtained with the addition of an $E_2V^2$ term in ova/ova ($n = 8$) and control mice ($n = 8$) under baseline conditions as a function of positive end-expiratory pressure (PEEP).
DISCUSSION

We have shown that the respiratory mechanics of bronchoconstricted mice with airway inflammation are significantly better described by a model including a $E_2V^2$ term than by the conventional linear equation of motion (Eq. 2). This phenomenon has also been demonstrated in adult humans (3), neonates and dogs (11), infants (20), and neonatal lambs (25). The EV term, as used in the conventional equation of motion (Eq. 1), assumes that the dynamic pressure-volume relationship of the respiratory system is linear. Addition of a $E_2V^2$ term herein enables the model to significantly better describe nonlinear pressure-volume behavior. Therefore, the improvement we observed with the addition of the $E_2V^2$ term indicates that during bronchoconstriction the dynamic pressure-volume curve of the inflamed ova/ova mouse lung becomes significantly curvilinear (or nonlinear). In contrast, addition of an $R_vV^2$ term did not result in a significant improvement in fit in either control or inflamed mice (Fig. 1), suggesting that turbulent gas flow in the airways was not occurring to a significant degree.

Bronchoconstriction resulted in an increase in all model parameters over their baseline values in both the control and ova/ova mice, but the increases were substantially greater in the ova/ova mice (Table 1), which is consistent with previously published data (13).

The necessity for a nonlinear volume-dependent $E$ term in the constricted inflamed airways can potentially be explained by three mechanisms. First, the intrinsic properties of the lung parenchyma may have changed. Changes in the intrinsic properties of the parenchyma as seen in pulmonary fibrosis have been correlated with changes in the shape of the expiratory limb of the static P-V curve quantified by the shape factor (6). We did not find a difference in the values of the shape factor obtained from the expiratory limbs of the static P-V curves of the ova/ova mice compared with controls (Table 3). Therefore, we conclude that at baseline the intrinsic properties of the ova/ova mouse lung are not much different from untreated controls. We did see differences in the inspiratory limbs of the P-V curves from the two groups (Fig. 4) and in the areas circumscribed by the inspiratory and expiratory limbs (Table 3). This area is significantly increased in the ova/ova mice.

Table 2. Parameter values during PEEP challenge

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEEP 3</th>
<th>PEEP 6</th>
<th>PEEP 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R$, cmH$_2$O/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear model</td>
<td>0.838 ± 0.03</td>
<td>0.649 ± 0.04</td>
<td>0.645 ± 0.04</td>
<td>1.06 ± 0.07</td>
</tr>
<tr>
<td>Nonlinear model</td>
<td>32.64 ± 1.59</td>
<td>25.28 ± 1.75</td>
<td>26.87 ± 1.19</td>
<td>66.40 ± 3.37</td>
</tr>
<tr>
<td></td>
<td>$E_1$, cmH$_2$O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear model</td>
<td>39.17 ± 2.14</td>
<td>26.13 ± 1.77</td>
<td>24.11 ± 1.25</td>
<td>61.48 ± 4.76</td>
</tr>
<tr>
<td>Nonlinear model</td>
<td>-43.41 ± 4.16</td>
<td>-7.41 ± 1.19</td>
<td>34.30 ± 5.31</td>
<td>295.26 ± 26.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. At lower PEEP, $E_2$ is negative (PEEP = 0) or near zero (PEEP = 3), paralleling the upward concavity of the pressure-volume (P-V) curve at low lung volume (Fig. 5). At the higher PEEP levels, $E_2$ increases substantially.

Table 3. Static P-V curve comparisons

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 7$)</th>
<th>ova/ova ($n = 6$)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape factor ($k$)</td>
<td>0.995 ± 0.009</td>
<td>0.090 ± 0.006</td>
<td>0.63</td>
</tr>
<tr>
<td>Curve area</td>
<td>2.54 ± 0.52</td>
<td>4.24 ± 0.35</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. Comparison of P-V curves of control and ova/ova mice in terms of the averaged shape factor as determined by the Salazar-Knowles equation and P-V curve area.

![Graph](http://example.com/graph.png)
mice, yet the expiratory limbs in the two groups are virtually congruent (Fig. 4). This suggests that there was increased airway recruitment during inspiration in the ova/ova mice, not surprisingly because the inflamed epithelium of these animals would likely have encroached more than normal on the airway lumen and so predisposed them to experiencing closure at the end of the preceding expiration. An alteration in the amount or effectiveness of surfactant could also lead to enhancement of airway closure.

Second, the lung parenchyma could have stiffened during bronchoconstriction. Lung parenchyma has been shown to respond to exogenous constrictors (5, 16, 17). This response, which leads to increased tissue R, has been attributed to either contraction of parenchymal contractile elements or airway-parenchymal interdependence (1, 19). It has also been speculated that contractile elements are present in the alveoli and the alveolar ducts (12, 14) and in parenchymal blood vessels (4), although it is difficult to separate these elements experimentally from those of the airway. The difficulty of separating these two components functionally is exemplified by the fact that, in most studies, the increase in tissue resistance has been linked with increases in airway R (16, 17). It has also been suggested (2, 7) that this apparent increase in tissue resistance is the result of the emergence of heterogeneity of lung ventilation, thereby leading to overdistention of some units that is being observed as increased tissue resistance, which suggests the third explanation of our findings.

We were able to increase ΔENV in both control and ova/ova animals by changing lung volume through an increase or decrease in PEEP from its initial value of 3 cmH₂O. These new values of PEEP would have likely placed the lung on curvilinear portions of its P-V curve, as opposed to the relatively linear portion around 3 cmH₂O (Fig. 5). P-V curves have accentuated curvilinearity (concave upward) at low lung volumes, at which air space closure tends to occur close to FRC (Fig. 5, PEEP = 0). At high lung volumes, the elastic limit of the lung parenchyma is approached (Fig. 5, PEEP = 9). Lung parenchyma contains both extensible (elastin) and very stiff (collagen) fibers (24) and is structured so that at low volumes the extensible elements bear most of the stress whereas at high volumes the relatively stiff elements take over. This results in a progressive increase in lung stiffness with increasing volume. These conclusions are supported by the values on the nonlinear model parameters (Table 2). Specifically at PEEP 3, E₂ is close to zero, corresponding to the straight portion of the P-V curve (Fig. 5). At PEEP 0, E₂ is negative, matching the upward concavity of the lower portion of the PV curve. At PEEP 6 and 9, E₂ becomes progressively more positive, corresponding to the downward concavity of the upper end of the PV curve.

The increases in ΔENV we observed in the bronchoconstricted ova/ova mice were similar to those we saw under baseline conditions at increased PEEP levels. One explanation is that the combination of airway inflammation and bronchoconstriction changed lung volume, thereby placing the lung on a curvilinear portion of its static P-V curve. There are two mechanisms that could be responsible. One possibility is that lung volume could have increased as the result of dynamic hyperinflation, as occurs when lung emptying is diminished because of airflow limitation (23). This would have had the effect of pushing the lung toward the curvilinear upper portion of its P-V curve (Fig. 5). Alternatively, airway closure could have decreased the amount of lung parenchyma available to receive the imposed tidal volume. When mechanically ventilated, mice receive a constant volume, so partial closure of the lung pushes the remaining lung parenchyma closer to its elastic limit, accentuating the curvilinearity of the dynamic P-V curve. We cannot distinguish between these two possibilities from our present data. However, we have evidence, as discussed (Fig. 4) above, for greater airway closure in the ova/ova mice. Consequently, we speculate that partial closure of the lung in the constricted ova/ova mice was the primary mechanism accounting for the dynamic P-V nonlinearity indicated by the large values of ΔENV that were obtained.

Improving our ability to measure features of the pathophysiology of asthma is crucial to the continuing investigation of the mechanisms of physiological alterations in this disease. We have shown that, by assessing the impact of adding terms to the equation of motion of the single-compartment linear model, one can infer the significance or insignificance of the physiological process described by the additional terms, in this case, airway closure leading to overdistention of the remaining patent parenchyma.

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Fig. 5. Idealized static pressure-volume curve showing how variations in PEEP place the lung at different positions on the curve with different degrees of curvilinearity. Loop proceeds in a counterclockwise direction through inspiration and expiration. See text for discussion.
REFERENCES


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