Hyperoxia-induced changes in mouse lung mechanics: forced oscillations vs. barometric plethysmography

FERENC PETÁK,1,2 WALID HABRE,3 YVES R. DONATI,4,5 ZOLTÁN HANTOS,4 AND CONSTANCE BARAZZONE-ARGIROFFO4,5
1Division of Anesthesiologic Investigations, Departments of 2Pathology and 5Pediatrics, University of Geneva, 1211 Geneva; 3Division of Anesthesia, Geneva Children’s Hospital, 1205 Geneva, Switzerland; and 2Department of Medical Informatics and Engineering, University of Szeged, 6720 Szeged, Hungary

Received 23 October 2000; accepted in final form 1 February 2001

Peták, Ferenc, Walid Habre, Yves R. Donati, Zoltán Hantos, and Constance Barazzone-Argiroffo. Hyperoxia-induced changes in mouse lung mechanics: forced oscillations vs. barometric plethysmography. J Appl Physiol 90: 2221–2230, 2001.—Hyperoxia-induced lung damage was investigated via airway and respiratory tissue mechanics measurements with low-frequency forced oscillations (LFOT) and analysis of spontaneous breathing indexes by barometric whole body plethysmography (WBP). WBP was performed in the unrestrained awake mice kept in room air (n = 12) or in 100% oxygen for 24 (n = 9), 48 (n = 8), or 60 (n = 9) h, and the indexes, including enhanced pause (Penh) and peak inspiratory and expiratory flows, were determined. The mice were then anesthetized, paralyzed, and mechanically ventilated. Airway resistance, respiratory system resistance at breathing frequency, and tissue damping and elastance were identified from the LFOT impedance data by model fitting. The monotonous decrease in airway resistance during hyperoxia correlated best with the increasing peak expiratory flow. Respiratory system resistance and tissue damping and elastance were unchanged up to 48 h of exposure but were markedly elevated at 60 h, with associated decreases in peak inspiratory flow. Penh was increased at 24 h and sharply elevated at 60 h. These results indicate no adverse effect of hyperoxia on the airway mechanics in mice, whereas marked parenchymal damage develops by 60 h. The inconsistent relationships between LFOT parameters and WBP indexes suggest that the changes in the latter reflect alterations in the breathing pattern rather than in the mechanical properties. It is concluded that, in the presence of diffuse lung disease, Penh is inadequate for characterization of the mechanical status of the respiratory system.

respiratory mechanics; airway resistance; lung elastance; enhanced pause

OXYGEN IS USED TO TREAT a variety of lung injuries, such as respiratory distress syndrome, although long exposure to high oxygen concentrations has been shown to be deleterious. The lung damage that develops during hyperoxia consists of lung tissue and alveolar edema (2, 3, 8, 14, 15, 34), surfactant dysfunction (10, 11, 13, 14, 21), lung inflammation (2–5, 10, 11), and subsequent deterioration of the lung function (2, 8, 10, 11, 13, 14, 15, 25, 26, 31, 34, 35). Studies on oxygen toxicity have primarily focused on parenchymal damage (2–5, 10, 11, 25), whereas the potential adverse effects of prolonged oxygen exposure on the airways have not been characterized. Numerous studies have demonstrated decreases in the compliance of the lungs (2, 8, 10, 11, 13, 14, 25) or of the total respiratory system (26, 35) and in the vital capacity (13) after oxygen exposure. We argue, however, that the previously reported marked increases in the resistance of the total respiratory system (Rrs) or of the lungs (Rl) (10, 11, 13, 14, 26, 31, 34, 35) do not necessarily reflect alterations in the airway function because these global resistive parameters incorporate a significant component related to the respiratory tissues (Rti) (1, 16, 18, 19, 23, 29, 30).

Because of the technical difficulties in the measurement of air flow in small animals, lung functions have mostly been evaluated in large mammalian species. Although the study of mouse lung pathophysiology has gained ground in asthma models and other pathological lung conditions, including hyperoxia (2–5, 25), very few techniques are available to estimate the lung function in mice. The low-frequency forced oscillation technique (LFOT) and model-based evaluation of respiratory system impedance (Zrs) data have been used in various mammals to partition Rt into airway and parenchymal components under control conditions and during lung constriction (1, 16, 19, 23, 29, 30). Small laboratory animals also have been investigated extensively by the wave-tube version of this technique, and the results were successfully validated in rats (23, 29). Therefore, adaptation of the LFOT for mice appears to be appropriate to characterize the hyperoxia-induced changes in the lungs because it provides direct and separate assessment of the airway and the tissue mechanics.

Barometric whole body plethysmography (WBP) has been used in unrestrained mice to detect changes in lung function indirectly from the characteristic times...
and flows of spontaneous breathing (17). The empirically derived parameter enhanced pause (Penh) determined by means of WBP has been demonstrated to increase during a constrictor challenge (9, 12, 17, 22, 32), and the increases paralleled those in Rt. in mice during a methacholine challenge (17). Consequently, it has been concluded that the use of Penh is suitable for detection of a transient response of the respiratory system after a constrictor challenge. However, the relevance of Penh for characterization of the mechanical status of the respiratory system afflicted by a generalized lung disease has not been validated. Accordingly, it remains unclear whether Penh is an appropriate index for characterization of the mechanical properties of the lungs, i.e., whether it provides an objective estimation of the altered Rt. or merely reflects changes in the ventilatory pattern.

In the present study, we adapted the LFOT for mice to investigate how hyperoxia-induced lung damage is manifested in the airways and the respiratory tissues. We additionally aimed to determine separately the time courses of the lung damage for the airways and the respiratory tissues. Finally, we related the parameters obtained by means of WBP to direct measures of airway, tissue, and total respiratory mechanics within individual mice, to validate the usefulness of WBP in the assessment of the respiratory function in a diseased lung.

METHODS

Mice

C57BL/6 mice were purchased from IFFA Credo (Labrisle, France) and were kept in the animal facility before the experiments. The measurements were performed on 6- to 8-wk-old female mice weighing 20–23 g.

Oxygen Exposure

The protocol was approved by the institutional ethics committee for animal experiments (Office Vétérinaire Cantonal de Genève). Mice were randomly assigned to one or other of the following four protocol groups: the mice in the control group were kept in room air (n = 12), and the mice in the other three groups were exposed to 100% oxygen for 24 (n = 9), 48 (n = 8), or 60 (n = 9) h. Because preliminary experiments revealed that anesthesia and mechanical ventilation eventually led to mortality in mice exposed to hyperoxia for 72 h, the length of oxygen exposure was limited to 60 h. The animals were exposed to oxygen in a sealed (8-liter) Plexiglas chamber under minimal oxygen inflow and outflow (0.5 l/min). The CO2 level in the box was maintained at 1% by using a CO2 absorber (Drägersorb 800, Dräger Medizintechnik, Lübeck, Germany). Food and water were available ad libitum.

Barometric WBP

The characteristic parameters of spontaneous breathing were recorded with a whole body plethysmograph (Buxco, Troy, NY), as described in detail previously (17). Briefly, the unrestrained, spontaneously breathing mice were placed in the main chamber of the plethysmograph. The changes caused by the pressure difference between the main chamber and the reference chamber (Pb) by spontaneous breathing were recorded for 5 min. The whole body plethysmograph with its accessories was placed in a large Plexiglas cylinder. Inspiratory (Ti), expiratory (Te), and relaxation (TR, defined as the time until 36% of the total box pressure decay during expiration) times, peak inspiratory and expiratory pressures, tidal volume (VT), minute ventilation (VE), and respiratory rate (RR) were extracted from the Pb recordings. To estimate the flow and volume units from the Pb signal, the plethysmograph was calibrated by injecting 0.5 ml of air before the measurements. Accordingly, peak expiratory (PEF) and inspiratory (PIF) flows were calculated from peak inspiratory and expiratory pressures, respectively (17). Pause (= [TE – TR/2]/[TR + TR]) and Penh (= pause PEF/PIF) parameters were also calculated (17).

Forced Oscillatory Measurements

Animal preparations. After the WBP measurements, the mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Tracheostomy was performed with a polyethylene cannula (30 mm long, 1.17 mm ID) was inserted into the trachea. The mice were then mechanically ventilated (model 683, Harvard Apparatus, South Natick, MA) with a VT of 10 μl/kg at a frequency of 180/min. Muscle paralysis was accomplished through the intraperitoneal administration of pancuronium bromide (1 mg/kg). To avoid acute hypoxia in the hyperoxic mice, the animals were kept in 100% oxygen during the surgical preparation and mechanical ventilation.

Forced oscillatory setup. The wave-tube technique (36) was adapted to measure forced oscillatory Zrs in mice. This technique was specially designed to measure the input impedance of rats (29, 30) and guinea pigs (1) without the need for estimation of the oscillatory flow. We miniaturized the setup to reduce the ventilatory dead space (40 μl) and to adjust its impedance to the expected high load impedance of the mouse. The forced oscillatory setup was described in detail previously (29). Briefly, a three-way tap (Becton-Dickinson, model 394600, Helsingborg, Sweden) was used to switch the tracheal cannula from the respirator to a loudspeaker-in-box system at end expiration. A 28-cm-diameter woofer (250 W) enclosed in a 16-liter plastic box served as the pressure generator. The loudspeaker generated a small-amplitude pseudorandom signal (25 integer-multiple frequency components between 1 and 25 Hz) through a 100-cm-long 1.17-mm ID polyethylene tube (Becton-Dickinson, model 6253, Rutherford, NJ). Two identical pressure transducers (model 33NA002D, IC Sensors, Milipitas, CA) were used to measure the lateral pressures at the loudspeaker (P1) and at the tracheal end (P2) of the wave tube. The P1 and P2 signals were low-pass filtered (5th-order Butterworth, 25-Hz corner frequency) and sampled with an analog-digital board of a computer at a rate of 256 Hz. Fast Fourier transformation with 1-s time windows and 90% overlapping was used to compute the pressure transfer functions (P1/P2) from the 3-s recordings. The P1/P2 spectra were used to calculate Zrs as the load impedance of the wave tube (36). Four to six Zrs values were collected in each mouse for averaging. To avoid possible bias in the impedance calculation due to the accumulated oxygen in the wave tube, the oxygen administration was suspended and the lungs were washed with room air for 30 s before each recording. Intervals of at least 2 min were interposed between each two Zrs measurements.

Separation of airway and parenchymal mechanics. On the basis of the different frequency dependencies of the two compartments, the airway and parenchymal properties were quantified by fitting a model incorporating an airway resis-
tance (Raw) and inerterance in series with a constant-phase tissue model (19), including damping (G) and elastance (H), to the Zrs spectra by minimizing the differences between the measured and modeled impedance values. Impedance data at frequencies coinciding with the heart rate and its harmonics were omitted from the fitting if the cardiac activity caused a low signal-to-noise ratio at these frequencies.

Rti was calculated from the model parameters at the rate of spontaneous breathing (RR), recorded by WBP, as Rti = G/(2πRR/60)°, where α = (2π)arctan(H/G). Rrs at RR was calculated as Rrs(RR) = Raw + Rti(RR). The contribution of the measurement apparatus including the tracheal cannula was 0.118 cmH2O·s·ml−1 to the reported Raw and Rrs values.

**Lung Weight and Microscopy**

To estimate pulmonary edema and morphological changes after hyperoxia, C57BL/6 mice with similar body weights were investigated in control conditions (n = 5) and when exposed to 48 (n = 3) and 60 h (n = 3) of hyperoxia. Mice were bled by opening the abdominal aorta under anesthesia, and the thorax was opened. The lungs were dissected and weighed. The lungs were then inflated and fixed by instilling 5% formalin (in 80% ethanol) into the trachea with hydrostatic pressure of 20 cm. Transhilar horizontal sections were embedded in paraffin and processed for light microscopy (3). The sections were stained with hematoxylin and eosin.

**Statistical Analysis**

Scatters in the parameters were expressed in SE values. Kruskal-Wallis one-way ANOVA on ranks with Dunn’s multiple comparisons was applied to compare the mechanical parameters between the protocol groups. The Spearman rank-order correlation test was used to assess the strengths of associations between parameters derived from LFOT and WBP. Statistical tests were performed with a significance level of P < 0.05.

**RESULTS**

**WBP Data During Hyperoxia**

Table 1 lists the hyperoxia-induced changes in the parameters obtained by WBP. Significant changes already occurred in RR, Penh, VT, and the parameters relating to the expiratory phase of the spontaneous breathing (TR, PIF, and PEF) by 24 h of hyperoxia. The significant increases observed in RR and VE at oxygen exposures of 24 and 48 h were followed by sharp and statistically significant decreases when hyperoxia was maintained for 60 h. The increases in Penh were relatively moderate but statistically significant in the mice exposed to hyperoxia for 24 and 48 h, whereas the elevation was more than 30-fold at 60 h. Significant changes in the parameters characterizing inspiration (PIF and TI) were observed only at 60 h. Although there were tendencies to increase at 24 and 48 h and a decrease at 60 h, no statistically significant changes occurred in VT.

**Respiratory System Impedances**

The real (Rrs) and imaginary (Xrs) parts of Zrs obtained in three representative mice, one each from the control group, the 24-h hyperoxia group, and 60-h hyperoxia group, together with the corresponding model fits, are shown in Fig. 1. The Rs curves fall with increasing frequency as a result of the decreasing contribution of Rti, and at higher rates they reach plateaus corresponding to Raw. The quasi-hyperbolic rise in Xrs reflects the elastic behavior of the respiratory tissues. The 24-h hyperoxia caused a minor change in Zrs. The marked increases in the frequency dependences of Rrs and Xrs in the mouse exposed to hyperoxia for 60 h suggest significantly elevated values of G and H. Although the changes in the Rrs spectra at high frequencies are far smaller, the Rrs values for the hyperoxia-exposed mice tend to a lower level. Apart from data points corrupted by cardiac artifacts, the model fitted well to all Zrs spectra without detectable systematic fitting errors.

**LFOT Data During Hyperoxia**

The hyperoxia-induced changes in the forced oscillatory airway and tissue parameters are demonstrated in Fig. 2. A mild but statistically significant monotonous decrease was observed in Raw at 24 h (~39%, P < 0.001). Because the inertive contribution of the intrathoracic gas to Xrs was negligible (the resonant frequency in Zrs was above the frequency range measured), the airway inerterance values are fairly scattered and exhibit no obvious change during hyperoxia (P =

| Table 1. Effects of hyperoxia on parameters determined by barometric whole body plethysmography |
|-----------------------------------------------|----------------|----------------|----------------|
|                               | Control    | 24-h Hyperoxia | 48-h Hyperoxia | 60-h Hyperoxia |
| Tt, ms                        | 57.0 ± 1.7 | 58.1 ± 1.9     | 57.5 ± 3.2     | 145.4 ± 16.2*  |
| Te, ms                        | 196 ± 27   | 135 ± 12*      | 124 ± 11*      | 239 ± 15       |
| TR, ms                        | 138 ± 24   | 77.4 ± 5.1*    | 58.5 ± 2.4*    | 40.0 ± 2.1*    |
| PIF, ml/s                     | 8.79 ± 0.40| 9.90 ± 0.44    | 10.0 ± 0.35    | 4.76 ± 0.32*   |
| PEF, ml/s                     | 5.42 ± 0.38| 7.71 ± 0.31*   | 8.31 ± 0.37*   | 8.36 ± 0.36*   |
| Penh                          | 0.387 ± 0.026| 0.805 ± 0.10* | 1.74 ± 0.85*   | 13.0 ± 2.0*    |
| VT, ml                        | 0.294 ± 0.020| 0.326 ± 0.008 | 0.306 ± 0.012  | 0.253 ± 0.0059 |
| VE, ml/min                    | 93.5 ± 5.5 | 127 ± 8.5*     | 126 ± 7.9*     | 48.1 ± 3.6*    |
| RR, l/min                     | 325 ± 22   | 381 ± 19*      | 406 ± 11*      | 183 ± 11*      |

Values are means ± SE; Tt, inspiratory time; Te, expiratory time; TR, relaxation time, defined as the time of the box pressure decay to 36% of the total box pressure during expiration; pause = (Te − TR)/TR; PIF, peak inspiratory flow; PEF, peak expiratory flow; Penh, enhanced pause defined as pause × PEF/PIF; VT, tidal volume; VE, minute ventilation; RR, respiratory rate. *Significant change from control (P < 0.05).
Hyperoxia for 24 or 48 h caused no change in G, H, Rti, or Rrs, whereas marked and statistically significant increases were noted after 60 h (64, 63, 168, and 95% for G, H, Rti, and Rrs, respectively; \( P < 0.0001 \) for all). It should be noted that, although Rti is derived from the model parameter G, it is also determined by RR (see METHODS above). Because RR was decreased at 60 h, the changes in Rti, and thus in Rrs, are greater than those in G.

**Relationships Between LFOT and WBP Parameters**

We also examined how the altered WBP indexes reflect the hyperoxia-induced changes in the airway and tissue mechanical parameters. The correlation between the WBP indexes and LFOT parameters are listed in Table 2. Raw exhibited a rather poor association with parameters determined by WBP, the closest relationship being that with PEF. PIF was found to correlate best with the LFOT parameters G and H. Concerning the independent parameters only, Rti and Rrs correlated most satisfactorily with the TI and flow indexes. Trivially, Rti and Rrs are not independent of RR (and thus \( V_E \)) because RR was used to calculate these parameters. Figure 3 illustrates the closest associations between the parameters determined via the LFOT and WBP.

The relationships between the forced oscillatory parameters and Penh are demonstrated in Fig. 4. The changes in Raw and Penh were fairly parallel but opposite, and the relatively small decreases in Raw (maximum 39%) were associated with massive (25-fold) increases in Penh. The associations between the changes in the tissue parameters (Rti, G, and H) and those in Penh were rather poor because Penh exhibited marked and statistically significant increases in the mice exposed to hyperoxia for 24 or 48 h, whereas no changes in tissue mechanics were apparent. Exposure to oxygen for 60 h caused marked increases in both tissue parameters and Penh, although the changes in the latter were far greater. Because Rti accounted for the majority of Rrs (71 ± 1.8%), the relationship between Rrs and Penh resembles that between the tissue parameters and Penh.

**Histology and Lung Weights**

Figure 5 illustrates lung histology in representative mice exposed to room air and to hyperoxia for 48 and 60 h. No pathological alterations were observed in the airways at any time of oxygen exposure. In agreement with previous findings (3, 4), no histological changes in the tissues were present in the mice exposed to 48 h of hyperoxia, whereas exposure to oxygen for 60 h induced interstitial edema and thickening of alveolar septa. Lung weights were not significantly higher in the mice exposed to hyperoxia for 48 h (0.185 ± 0.017 g) than those in the control group (0.175 ± 0.016 g, \( P = 0.69 \)), whereas significant increases were observed after longer oxygen exposures (0.25 ± 0.015 g, \( P < 0.02 \)).

**DISCUSSION**

In the present study, the effects of hyperoxia on the mouse respiratory system were investigated by measuring passive respiratory mechanics and analyzing indexes of spontaneous breathing patterns. The partitioning of Rrs into airway and tissue components revealed a decrease in Raw by 24 h of hyperoxia, whereas exposure to oxygen for 60 h induced interstitial edema and thickening of alveolar septa. Lung weights were not significantly higher in the mice exposed to hyperoxia for 48 h (0.185 ± 0.017 g) than those in the control group (0.175 ± 0.016 g, \( P = 0.69 \)), whereas significant increases were observed after longer oxygen exposures (0.25 ± 0.015 g, \( P < 0.02 \)).
the small size of the animal. We therefore adapted the wave-tube technique of forced oscillations, the low-frequency version of which has been validated in rats (23, 29) and has been shown to provide a separate estimation of airway and tissue parameters (1, 16, 19, 23, 29, 30). These previous investigations demonstrated that lung mechanical parameters identified from low-frequency input impedance data characterize the mechanical conditions of the airways and the respiratory tissues accurately in a healthy and moderately or homogeneously constricted lung (23, 29). In the severely constricted lung, enhanced interregional ventilation resulting from heterogeneously constricted peripheral airways may lead to a virtual increase in tissue damping, whereas the elastance remained relatively unaffected by this phenomenon (23, 29). Because the increases in G and H were proportional during hyperoxia in this study, the development of severe inhomogeneities was unlikely to occur.

Our Zrs spectra are qualitatively similar to those obtained in previous studies in rats (29, 30), in guinea pigs (1), and in mice (16). In agreement with previous findings (1, 16, 19, 23, 29, 30), the model involving an airway and a constant-phase tissue compartment was consistent with the frequency dependence of Zrs. Hessel et al. (20) measured Zrs data in mice at frequencies above the rate of spontaneous breathing. Their Rrs values at 16 Hz (590 ± 51 cmH₂O·s⁻¹) are slightly lower than those obtained in the present study at the same frequency (720 ± 29 cmH₂O·s⁻¹) in the control
mice). Besides the obvious difference in the animal preparation (face mask vs. tracheostomy), Hessel et al. determined Zrs during spontaneous breathing at higher mean airway pressures (20), at which Raw, and thus Rrs, is expected to be lower (16). More recently, Gomes et al. (16) identified airway and constant-phase tissue parameters in anesthetized mechanically ventilated mice. Although our Raw values in the control mice are similar to their estimates, their measurements of G and H are ~20% lower than our findings. This discrepancy is likely to be attributed to the higher oscillatory amplitude applied by Gomes et al. (35% of the tidal volume), which results in lower tissue impedance because of the highly nonlinear characteristics of the chest wall (18).

We observed a striking decrease in Raw as early as by 24 h of oxygen exposure. To our knowledge, only one earlier study has reported hyperoxia-induced changes in the airway properties in vivo (13). In that study, Raw measured with a body plethysmograph in adult volunteers showed no statistically significant change at 6–11 h of hyperoxia. Nevertheless, it is difficult to compare their results with our findings because, besides the difference in species, the oxygen exposure of the humans was terminated quickly after the development of symptoms related to oxygen toxicity. Other authors found that the smooth muscle area measured in isolated bronchi was not affected by exposure to hyperoxia for 1 or 3 days (33). The lack of structural changes in the airways is in accordance with our finding that Raw did not increase but rather decreased further, even after 60 h of hyperoxia. Many factors may

Table 2. Spearman correlation coefficients between tidal breathing indexes obtained by means of whole body plethysmography and passive respiratory mechanical parameters determined by forced oscillations

<table>
<thead>
<tr>
<th></th>
<th>Raw</th>
<th>G</th>
<th>H</th>
<th>Rti</th>
<th>Rrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tt</td>
<td>−0.37⁺</td>
<td>0.45†</td>
<td>0.46†</td>
<td>0.77‡</td>
<td>0.74‡</td>
</tr>
<tr>
<td>Tc</td>
<td>−0.11</td>
<td>0.27</td>
<td>0.34⁺</td>
<td>0.75‡</td>
<td>0.75‡</td>
</tr>
<tr>
<td>TR</td>
<td>0.68‡</td>
<td>−0.54‡</td>
<td>−0.48†</td>
<td>−0.26</td>
<td>−0.24</td>
</tr>
<tr>
<td>Pause</td>
<td>−0.64‡</td>
<td>0.44⁺</td>
<td>0.39⁺</td>
<td>0.40⁺</td>
<td>0.35⁺</td>
</tr>
<tr>
<td>PIF</td>
<td>0.10</td>
<td>−0.54‡</td>
<td>−0.49†</td>
<td>−0.76‡</td>
<td>−0.79‡</td>
</tr>
<tr>
<td>PEF</td>
<td>0.69‡</td>
<td>0.22</td>
<td>0.21</td>
<td>−0.00</td>
<td>−0.08</td>
</tr>
<tr>
<td>Penh</td>
<td>−0.67‡</td>
<td>0.49†</td>
<td>0.41⁺</td>
<td>0.42⁺</td>
<td>0.36⁺</td>
</tr>
<tr>
<td>Vr</td>
<td>0.02</td>
<td>−0.46†</td>
<td>−0.36⁺</td>
<td>−0.55⁺</td>
<td>−0.62‡</td>
</tr>
<tr>
<td>Ve</td>
<td>0.08</td>
<td>−0.50†</td>
<td>−0.51†</td>
<td>−0.85‡⁺</td>
<td>−0.89‡⁺</td>
</tr>
<tr>
<td>RR</td>
<td>0.15</td>
<td>−0.45†</td>
<td>−0.51†</td>
<td>−0.86‡⁺</td>
<td>−0.86‡⁺</td>
</tr>
</tbody>
</table>

Raw, airway resistance; Rti and Rrs, tissue and total respiratory system resistance, respectively; G and H, tissue damping and elasticity, respectively. *P < 0.05, †P < 0.005, ‡P < 0.001; §pairs of not independent variables.

Fig. 3. Closest associations between parameters determined by low-frequency forced oscillatory technique and whole body plethysmographic measurements, according to the Spearman rank-order correlation test. Symbols with bars represent mean ± SE values in Ctrl mice and in mice exposed to hyperoxia for 24, 48, or 60 h. PEF and PIF, peak expiratory and inspiratory flows, respectively.
have contributed to the Raw decrease observed in the present study. Hyperoxia may have induced bronchodilation via neural pathways that were missing in the isolated bronchial preparations. The increase in functional residual capacity during hyperoxia was also suggested to lead to a decrease in Raw, although the changes in functional residual capacity were modest (13, 14). Moreover, hyperoxia might increase the levels of endogenous catecholamines in consequence of pain and discomfort (13) and thereby relax the bronchial smooth muscles (24).

Hyperoxia had no detectable effect on the resistive or elastic properties of the respiratory tissues at 48 h of hyperoxia, whereas oxygen exposure for 60 h was followed by marked increases in both G and H. According to our histological findings and in agreement with previous observations (2–5, 8, 14, 15, 34), these increases can be attributed to the development of interstitial or alveolar edema. In addition, lung inflammation (2–5, 10, 11) and/or the impaired surface activity of the surfactant (10, 11, 13, 14, 21) might have contributed to the impairment in parenchymal mechanics. The physiopathological changes in the surfactant function may affect the viscoelastic properties of the parenchyma or may lead to disperse atelectasis in the alveoli (10, 11, 13, 14, 21). Although our data do not allow to estimate the degree of a possible surfactant damage, our findings may provide an insight into the characteristics of the tissue processes. Heterogeneous lung injury leads to an additional rise in G, whereas it affects H to a far lesser degree (23, 29). Accordingly, the same magnitude of relative increases in G (64%) and H (63%) in the

---

**Fig. 4.** Relationship between parameters estimated from the forced oscillatory impedances of the respiratory system and the enhanced pause (Penh) determined by means of whole body plethysmography. Symbols with bars represent mean ± SE values in the Ctrl mice and in mice exposed to 24, 48, or 60 h of hyperoxia.
The present study indicates that the parenchymal damage was rather uniform throughout the lungs. Such pathological changes may be more characteristic of an edematous process, because atelectasis is expected to lead to a highly heterogeneous lung structure. The fairly uniform presence of edema evidenced from histology also confirms this conclusion. The restrictive changes in the lungs, causing increases in the parenchymal parameters but with no consequent increase in Raw, suggest that the pathological changes were limited to the alveolar compartment, without the development of obstructive changes in the conducting airways.

The previously reported time course of hyperoxia-induced lung tissue damage exhibits great variability and reveals a substantial species dependence. Significant decreases in lung compliance were reported by merely 11 h of oxygen exposure in humans (13), within 24 h in preterm rabbits (8), and at 48 h in rabbits (26) and piglets (10, 11). Adult rabbits, however, exhibited slight increases in Rrs and decreases in respiratory system compliance only after 69 h (35), and the increase in Rt became significant only after 96 h in sheep (14), whereas guinea pig pups displayed no change in Rrs after even 96 h of hyperoxia (31). Nevertheless, the results of the few studies in which mice were exposed to hyperoxia are in accordance with the current findings on lung morphology and oscillatory mechanics: no lung damage had occurred in this species by 48 h, whereas symptoms of oxygen toxicity were present at 72 h after exposure (2–5, 25). In addition, the results of the present study demonstrate that changes occur as soon as by 60 h of hyperoxia in mice, and the pathological changes in lung morphology are reflected in the compromised parenchymal mechanics. It should be noted that, besides the species dependence, the temporal changes during hyperoxia may also depend on many factors including age, gender, and strain of the particular species. We studied 6- to 8-wk-old female C57BL/6 mice inbred in a pathogen-free environment because the effects of oxygen toxicity are well characterized in these animals (3–5).

### WBP Indexes During Hyperoxia

We used WBP to assess hyperoxia-induced changes in the respiratory system. This approach has been demonstrated to detect changes in the spontaneous breathing pattern, which was proved (17) or assumed (9, 12, 22, 32) to reflect an altered lung resistance after a pulmonary constrictor challenge. In the present study, two different phases can be distinguished in the parameter changes during hyperoxia. In the first phase, up to 48 h of oxygen exposure, RR and PEF increased, whereas Te and TR decreased. We also observed a marked (fivefold) increase in Penh during this first phase. In agreement with the current findings, it has been demonstrated that a short exposure to hyperoxia stimulates breathing by increasing RR and V˙E (6, 7). These increases are reflected in the decreases in the expiratory time intervals (Te and TR). The increases in PEF either may be attributed to active expiration or may reflect changes in the dynamic mechanical properties of the respiratory system. However, the shape of the flow-time curves did not indicate qualitative changes between the control condition and that at 48 h of hyperoxia, which suggests that the expiration remained a passive process (Fig. 6). Therefore, it is more likely that the increases in PEF can be attributed to a bronchodilation effect, as also indicated by the decreases in Raw. Because Penh is proportional to PEF and Te – TR and inversely proportional to TR and PIF, we conclude that it was the superposition of the changes in the ventilatory pattern that resulted in the markedly in-
increased Penh during the first 48 h of hyperoxia and that no pathological changes in lung function or structure were reflected by the altered Penh.

In the second phase, at 60 h of hyperoxia, the changing trends in RR, Te, Ve, VT, and PIF were abruptly reversed, whereas TR continued its decreasing tendency and Ti, pause, and Penh were dramatically increased. All of these changes, i.e., the sharp decreases in PIF, RR, VT, and Ve and the increase in Te, suggest the occurrence of a profound change in respiration, induced by the prolonged hyperoxia, that was also obvious in morphology and in the marked increases in the tissue mechanical parameters measured by the LFOT.

Relationships Between LFOT and WBP Parameters

As a result of its demonstration of significant increases in Penh after administration of methacholine (17, 22, 32), histamine (9), or an allergen (12), WBP was proposed as a means of detecting altered lung resistance via the recording of the changes in the spontaneous breathing pattern in unrestrained, awake mice. The changes in spontaneous breathing, however, can also be associated with a number of other phenomena, such as modified neural control, pain, or discomfort (28), besides altered respiratory mechanics. Although it has been established clearly that a constrictor challenge increases Penh (9, 12, 17, 22, 32), the inverse question of whether an increased Penh is a consequence of changes in the mechanical properties of the respiratory system has not been investigated thoroughly. In particular, the validity of Penh in the estimation of the respiratory mechanical consequences during a generalized pulmonary disease affecting the airway and parenchymal compartments has not been tested. In the present study, we obtained an impressive (fourfold) and statistically significant increase in Penh at 48 h of hyperoxia, which was not accompanied by histological alterations or changes in tissue parameters, whereas Raw even slightly decreased. Although these opposite changes resulted in some correlation between Raw and Penh, it is very difficult to interpret this relationship because increases in Penh have been suggested to reflect a constrictor response (9, 12, 17, 22, 32). It should be stressed again that, in agreement with our lung weight, histology, and oscillatory data, there is a consensus in the literature that no lung injury develops in mice within the first 48 h of oxygen exposure (2–5, 24). Hence, the current study not only justifies the concerns regarding the interpretation of the quantitative changes in Penh (27) but also demonstrates that the changes in Penh do not even qualitatively follow the changes in the respiratory mechanical properties during oxygen exposure.

We additionally examined which indexes measured by means of WBP reflect the changes observed in the oscillatory mechanical parameters under the conditions of generalized lung disease. The correlation analysis revealed the strongest relationship between Raw and PEF; moreover, the tissue parameters extracted from the LFOT measurements exhibited the best correlations with the length (Ti) and the intensity (PIF) of the inspiration. Whereas an increased PEF associated with a decreased Raw may be explained on the basis of passive mechanical properties, the correlations between the inspiratory indexes and both G and H are not plausible, and the relationships between Penh and the Zrs parameters are rather puzzling.

In conclusion, partitioning of the airway and tissue mechanical properties of the respiratory system during normobaric hyperoxia in mice revealed distinct responses of the two compartments. Mild bronchodilatation was detected as early as by 24 h of oxygen exposure. In contrast, no adverse effect of hyperoxia was evident in the respiratory tissues during the first 48 h, whereas marked alveolar edema associated with increases in tissue damping and elastance had developed by 60 h. Comparison of the forced oscillatory and plethysmographic parameters revealed that the changes in the direct measures of the airway and tissue mechanical properties during hyperoxia are not reflected by those in Penh, and the statistical relationships with other plethysmographic indexes are not easily interpretable either. We conclude that, as a shape factor characterizing the breathing pattern via the plethys-

Fig. 6. Traces of box pressure signals in the whole body plethysmograph in representative mice from the Ctrl group and from the groups exposed to 48 or 60 h of hyperoxia. Inspirations are in negative directions.
mographic pressure fluctuations, Penh may become unrelated to changes in lung function and, in particular, airway resistance in the case of chronic lung disease.

We thank P. Rimensberger, J. Savoy, and P. F. Piguet for pertinent advice.

This study was supported by the Swiss National Science Foundation Grants 056717.99/1 and 056949.99/1 and Hungarian Scientific Research Grant OTKA T30670.

REFERENCES


