Effects of Acute Blood Volume Expansion on Respiratory Mechanics in the Rat

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Key Words
Blood volume expansion \cdot End-inflation occlusion method \cdot Respiratory mechanics \cdot Respiratory system hysteresis \cdot Work of breathing

Abstract
Backgrounds: The effects of acute blood volume expansion (BVE) on the respiratory mechanics of normal animals have been not extensively studied. The subject is of both theoretical and practical interest since BVE is a frequent medical intervention, and the associated increase in cardiac output may occur in different physiopathological situations. Objectives: To describe the changes in the parameters of respiratory mechanics occurring as an effect of acute BVE and the related increase in cardiac output. Methods: We applied the end-inflation occlusion method in normal, positive pressure-ventilated rats to measure the respiratory mechanics under control and BVE conditions. Results: Under BVE conditions, we found a statistically significant increase in static respiratory system elastance ($E_{st,rs}$), ohmic airway resistance plus resistance of respiratory system tissues to movement ($R_{min,rs}$), and overall resistance including pendelluft and stress relaxation effects ($R_{max,rs}$). Under BVE conditions, the resistive component due to sole stress relaxation and pendelluft ($R_{visc,rs}$) increased almost significantly while a significant increment in mean respiratory system hysteresis surface area ($H_{ry}$) was also found. Conclusions: Increasing pulmonary blood flow by BVE increases the mechanical work of breathing because of the effects on $E_{st,rs}$, $R_{min,rs}$ and $R_{max,rs}$ and because of the increase in $H_{ry}$.

Introduction

The mechanical characteristics of lung tissue may change as a consequence of variations in pulmonary blood volume. In particular, an increase in pulmonary blood volume is expected to increase lung stiffness, and airway resistance might increase as a consequence of blood engorgement. A modification of cardiac output is thus expected to modify the respiratory system mechanics because of the associated effects on pulmonary blood volume. The latter should increase with cardiac output because of the high distensibility of pulmonary blood vessels, and/or their possible recruitment.

This aspect has been studied mainly in infants with various congenital heart defects causing different ratios of pulmonary to systemic blood flow [1–5], and in infants after surgical correction of left to right shunts [6, 7], after induction of a left to right shunt [8], or correction of pulmonary hyper- or hypoperfusion [9]. The results of these studies suggest that a high pulmonary blood flow may be
associated with increased pulmonary elastance and airway resistance [10, 11].

However, previously reported investigations compared data obtained before and after highly invasive, open-chest surgical interventions. Moreover, due to the chronic pathological circulatory conditions of the subjects, unknown effects of possible chronic modifications of the pulmonary parenchyma and/or vasculature could not be excluded.

Since there are no data on the possible effects of changes in cardiac output on respiratory mechanics in controlled, acute, minimally invasive laboratory experiments in healthy animals, we carried out such a study in rats before (control) and after blood volume expansion (BVE).

Respiratory mechanics were studied with the technique of rapid end-inflation airway occlusion during constant-flow inflation which, differently from most other previously used techniques, models the respiratory system as composed by two compartments. It has been used in many studies of respiratory mechanics both in humans [12–15] and experimental animals [16–18], but has never been used previously to study this subject.

No evidence exists in the literature about the possible effect of a change in pulmonary blood volume and cardiac output on the hysteresis of the respiratory system. The work of breathing (WOB) has never been measured in rats during positive-pressure inflation. Thus, we measured the effects of BVE on both total WOB (WOBtot) and its elastic (WOBel) and resistive (WOBres) components, and on respiratory system hysteresis (Hyrs).

Materials and Methods

The experiments were carried out on 12 consecutive Wistar albino rats, 6 males and 6 females (weight 318 ± 18 g, mean ± SD). The experiments were performed according to the Declaration of Helsinki and the European laws on animal experimentation (86/609/EEC). The experimental protocol was approved by the local Ethical Committee.

The rats were anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg), the anesthesia level being monitored by observing the suppression of the corneal reflex and checking the presence of spontaneous breathing.

Heart rate was measured with ECG probes positioned on the limbs of the rats.

The rats were trachetomized, and a small polyethylene catheter (2 mm inner diameter, 3.5 cm long) was inserted through an incision performed on the second tracheal ring, and held tightly in place. The tracheal cannula was connected to a mechanical ventilator (Rodent Ventilator 7025, Basile, Italy) set to deliver a tidal volume (VT) of 3 ml at a breathing frequency of 100 breaths per minute. Mechanical ventilation was kept constant throughout the duration of the experiment, apart from the short time necessary for the constant-flow inflation tests needed for measuring respiratory mechanics and hysteresis (about 2 min each).

A small polyethylene cannula (24 G) was inserted into the right femoral vein, carefully advanced about 1 cm, and firmly held in place. The femoral cannula was connected to a waterfall saline-filled system to allow the measurement of systemic venous pressure (Pv). Care was taken to avoid air bubbles entering the venous system.

The rats were paralyzed by an intravenous injection of cis-atracurium (0.4 mg/kg), which is known not to substantially modify the heart rate [19], as opposed to other paralyzing drugs. Additional doses of cis-atracurium were occasionally administered when needed, as indicated by the onset of spontaneous breathing.

During the constant-flow inflation tests, the ventilator was disconnected, and the tracheal cannula connected to a constant-flow pump (SP 2000 Series Syringe Pump sp210iw; World Precision Instruments, USA) set to deliver a VT of 3 ml with a (nearly) square wave flow (F) of 4 ml/s. The time for the rise and fall of flow was about 30 ms. The precision of the pump settings was accurately checked before the experiments.

The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d; Honeywell, USA) and continuously recorded (1326 Econo Recorder, Biorad, Italy).

The end-inflation occlusion method [12, 13] was applied to measure respiratory mechanics: static elastic pressure of the respiratory system (Pels,rs), total resistive pressure drop (Pmax,rs) and the sudden Newtonian resistive pressure drop on flow interruption (Pmin,rs) were measured on adequately magnified tracings (fig. 1). Pmax,rs was measured as the difference between the maximum value of pressure at end inflation (Pdyn,max) and Pardc,rs. Pmin,rs was measured as the difference between Pdyn,max and Pvaso,rs, the pressure value measured immediately after flow interruption (fig. 1).

As previously stated [12, 13, 16, 17], Pmax,rs represents the resistive Newtonian pressure drop which would theoretically occur at infinite breathing frequency, i.e. without the pressure drop due to mechanical unevenness within the system and to stress relaxation, which are instead included in Pmax,rs.

Mean pressure data obtained from 3–5 inflations for each rat allowed the calculation of the respiratory system static elastance (Ers = Pdyn,max/VT) and respiratory system total resistance (Rrs = Pmax,rs/F) which, together with the Newtonian inspiratory airflow resistance provided by the airways and the respiratory system tissues (Rvaso,rs = Pmax,rs /F), includes the pressure drop due to uneven intrapulmonary airflow distribution and to the effect of stress relaxation. This last component of Rmax,rs was isolated and quantified as ‘viscous’ resistance (Rvisc,rs = Rmax,rs – Rmin,rs).

After these measurements of respiratory mechanics, which took about 2 min, mechanical ventilation was restored and maintained for 5 min. After this, in order to obtain a constant volume history for the measurement of respiratory system hysteresis, the lungs were consecutively inflated three times with a 10-ml syringe up to a static elastic pressure of 20–25 cm H2O. The respiratory system was then inflated in five 1-ml steps with a precision glass syringe starting from functional residual capacity and then deflated in a similar manner. The pertinent static elastic pressures were measured with a water-filled manometer, and the static inflation-deflation volume-pressure curves obtained. The hystere-
sis areas \( (H_{rs}) \) were quantified and expressed in cm H\(_2\)O/ml. The areas were determined by plotting them on a paper of known weight, and subsequently weighing the paper encompassing the hysteresis areas.

After ventilation was restored, BVE was induced by an intravenous infusion of 2.5 ml of a plasma expander solution \( (Voluven\textsuperscript{®}) \) containing 6% hydroxyethyl starch in saline (osmotic pressure 308 mosm/l) in about 3 min. It may be estimated that this infusion increased the circulating blood volume by about 15–18%, roughly corresponding to the infusion of about 1 liter of blood in humans. Venous pressure and heart rate were again measured (BVE values), and measurements of respiratory mechanics repeated as described above.

After this, the lungs were carefully dissected and weighed. The lungs were then subjected to a constant dry air flow for 24 h through the tracheal cannula, and weighed again. The weight of the cannula was subtracted from wet and dry weights, and the net wet to dry ratios of the lungs calculated. Prolonging the drying time for an additional 8 h did not modify the results.

The equipment resistance, including the tracheal cannula and a standard three-way stop-cock, was separately measured at a flow of 4 ml/s and amounted to 0.0575 cm H\(_2\)O/ml s\(^{-1}\). \( R_{eq} \) was subtracted from results, which hence represent intrinsic values.

WOB values were calculated according to the literature \([15, 20]\) as described in figure 2. We calculated WOB\(_{tot}\) by measuring the surface areas delimited by the total pressure \( (P_{dyn}) \) tracings from which the resistive pressure due to the tracheal cannula was subtracted. WOB\(_{cl} \) was obtained on the same diagram as the areas encompassing the static pressure/volume lines. WOB\(_{res} \) was obtained by subtraction: WOB\(_{res} = WOB_{tot} - WOB_{cl} \). WOB\(_{res} \) was also partitioned in the resistive work done to overcome ohmic airway resistance plus the viscous resistance to the movement opposed by the lungs and chest wall tissues. WOB\(_{res} \) = WOB\(_{ohm} \) + WOB\(_{visc} \), the mechanical work done to overcome the resistive effects of stress relaxation and pendelluft.

All measured variables were normally distributed (Smirnov-Kolmogorov test). On this basis, each rat being its own control, statistical analysis of the differences between control and BVE conditions was performed by Student’s t test for paired data. All values are expressed as means ± SE \( (n = 12)\).
Fig. 3. Heart rate and P_v. Mean values (± SE, n = 12) of heart rate (HR, a) and femoral vein pressure (P_v, b) under control and BVE conditions. Statistical significances of the differences are also indicated.

Fig. 4. P/V hysteresis loops. Mean (± SE, n = 12) V – P loops describing Hyrs under the control (a) and BVE (b) conditions. In order to demonstrate the increase in Hyrs under the BVE condition, the curves are superimposed in c.

Table 1. Mean respiratory mechanics under the control and BVE conditions (± SE, n = 12)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BVE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_res, cm H2O/ml</td>
<td>2.4 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>R_max, cm H2O/ml/s</td>
<td>0.66 ± 0.04</td>
<td>0.75 ± 0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>R_min, cm H2O/ml/s</td>
<td>0.06 ± 0.008</td>
<td>0.09 ± 0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R_visc, cm H2O/ml/s</td>
<td>0.59 ± 0.045</td>
<td>0.65 ± 0.035</td>
<td>0.07</td>
</tr>
<tr>
<td>P_dyn, max cm H2O</td>
<td>9.78 ± 0.5</td>
<td>11 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P1, cm H2O</td>
<td>9.55 ± 0.45</td>
<td>10.6 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P_el, cm H2O</td>
<td>7.2 ± 0.3</td>
<td>8 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2. Mean values of different components of inspiratory WOB (± SE, n = 12, cm H2O·ml) under the control and BVE conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BVE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOBtot</td>
<td>17.9 ± 0.8</td>
<td>21 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WOB_el</td>
<td>9.6 ± 0.4</td>
<td>11.6 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WOB_res</td>
<td>8.2 ± 0.5</td>
<td>9.3 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WOB_visc</td>
<td>0.74 ± 0.1</td>
<td>1.15 ± 0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WOB_visc</td>
<td>7.4 ± 0.4</td>
<td>8.2 ± 0.4</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Results

The results are reported in figures 3–5 and in tables 1–3.

On BVE, heart rate decreased from 411 ± 14 to 389 ± 12 beats/min, and femoral vein pressure increased from 4.2 ± 0.55 to 8.3 ± 0.52 cm H₂O, both changes being significant (fig. 3).

We also observed an increase in E_{st,rs}, R_{min,rs} and R_{max,rs}, and the increase in R_{visc,rs} was almost significant (p = 0.07, table 1).

Table 1 also reports the mean values of P_{dyn,max}, P_{1} and P_{el,rs} measured under control and BVE conditions. Under the latter condition, all parameters increased significantly.

The values of WOB_{tot} and its components are reported in table 2. Under the BVE condition, we found a significant increase in WOB_{tot,rs}, WOB_{el,rs} and WOB_{ohm,rs}, and the increase in WOB_{visc} was almost significant (p = 0.07).

The mean values of P_{el,rs} after 1-ml stepwise inflations and deflations are reported in table 3, both under control conditions and after acute BVE. The mean values at any volume were statistically different during inflation compared to deflation, indicating the presence of hysteresis both under control and BVE conditions. As shown in figures 4 and 5, Hy_{rs} under the BVE condition was significantly higher than under the control condition (5.8 ± 0.37 vs. 4.5 ± 0.4 cm H₂O·ml, p < 0.001).

The E_{st,rs} values calculated from the stepwise-inflation pressure data in table 3 were similar to those obtained with the constant-flow occlusion technique reported in table 1.

The mean ratio of wet weight to dry weight was 4.05 ± 0.13 (n = 12).

Discussion

The discussion includes the following separate points: technique, venous pressure and heart rate, E_{st,rs}, R_{min,rs}, R_{max,rs} and R_{visc,rs}, WOB, and Hy_{rs}.

Technique

The present experiments were designed to investigate the effects of acute BVE (and the associated increase in
cardiac output, see below) on the mechanics of the respiratory system under minimally invasive and strictly controlled experimental conditions. In particular, surgical interventions were limited to the positioning of the tracheal and femoral vein cannulae, so that invasive maneuvers were kept to a minimum, leaving the cardiorespiratory system almost intact and without opening the chest wall.

This is different from previous investigations. The previous results were obtained mostly in children undergoing cardiac open-chest surgery, before and after highly invasive surgical maneuvers required to correct congenital heart defects [6–9]. Moreover, in our experiments, data were obtained from individual healthy rats under control conditions and immediately after acute BVE, so that each rat was its own control, and the possible confounding effects of chronic modifications of pulmonary parenchyma and/or vasculature were avoided. Other studies deal with different subjects presenting different ratios of pulmonary to systemic blood flow which had persisted for relatively long times [1–5].

Previous data were mostly obtained by means of different techniques based on single-compartment models, none by the constant-flow occlusion technique. Modeling the respiratory system as composed by two compartments allows to obtain both the ohmic airway resistance and the resistance of the respiratory system due to its viscoelastic properties and pendelluft [12–15].

For the constant-flow interruption technique to be ideally applied, the inflation flow should stop instantaneously, which in reality is not the case. A correction for this has been proposed, which renders the possible errors almost negligible [21]. We applied this correction to our data by manual extrapolation of the pressure tracings for the time necessary for a complete stop of the inspiratory flow, but the corrections were practically negligible, as previously reported in similar experiments in rats [18].

The wet to dry weight ratios we found are very similar to those reported in the literature for normal rats. A ratio of 4.3 was recently reported [22], which is even a little greater than the ratio we obtained. Thus, our results indicate that our rats did not develop substantial pulmonary fluid accumulation during the experiments. Visual inspection of the isolated lungs led to the same conclusion: absence of foam and no evidence of parenchymal fluid accumulation. Hence, our data compared control conditions against a higher cardiac output and pulmonary blood flow conditions in the absence of pulmonary edema, i.e., under physiological conditions.

We did not perform blood gas analyses, but pulmonary ventilation was kept constant throughout the experiments. Thus, it seems unlikely that changes in blood gases could have contributed to the observed differences between control and BVE conditions. It should be noted that an increase in cardiac output in the presence of constant pulmonary ventilation would eventually lead to a decrease in arterial pO2 and an increase in arterial pCO2. Although these possible changes may cause a decrease in airway resistance, an increment was in fact observed.

We have previously shown that circadian and estrous rhythms may significantly influence respiratory mechanics in the rat [23, 24]. The possible effects of these variables on the present results were avoided because the measurements under control and BVE conditions were separated by a short time only.

Venous Pressure and Heart Rate

In order to avoid the effects of thoracotomy on respiratory mechanics, we measured the peripheral (Pv) rather than the central venous pressure, before and after blood BVE. It is likely, however, that Pv was a good index of the filling pressure of the heart chambers. As expected, our results show that the femoral vein pressure increased significantly following BVE (fig. 3b), suggesting that cardiac output and lung perfusion were substantially increased as a result of Starling’s law of the heart.

A second indirect index of increased cardiac output is represented by the significant decrease in heart rate after BVE (fig. 3a). This is interpreted as a result of a baroreceptor-induced reflex elicited by increased systemic arterial pressure. Thus, since our rats exhibited bradycardia following BVE, they almost certainly had an increased mean arterial pressure secondary to increased cardiac output.

Static Respiratory System Elastance

The mean values of Est,rs obtained in the present study were similar to those previously reported in the literature in similar experiments. The reported mean values range between 1.75 and 5.5 cm H2O/ml [16, 18, 23–25].

We found a significant increase in Est,rs as after BVE and a related increase in lung perfusion. This is in line with a number of previous reports obtained under different experimental conditions [1, 2, 4–6, 8] and suggests that the amount of blood in the lungs has a substantial influence on tissue stiffness.

In contrast, Pellegrino et al. [26] found no change in respiratory system elastance after intravenous saline in-
fusion (30 ml/kg) in healthy humans. Taking into account the normal distribution of intra- and extravascular fluids, it may be estimated that in the experiments of Pellegrino et al. the resulting BVE was not higher than 4–5%, i.e. largely lower than that achieved in our experiments in the rat. Considering the normal rat blood volume and the fact that we used plasma as expander rather than saline, the achieved BVE in the present experiments may be estimated to be about 15–18%. This is fairly similar to BVE in clinical practice after infusion of about 1 liter of plasma in humans. This BVE was high enough to substantially increase cardiac output in our rats, while this did certainly not happen to a similar degree in the experiments by Pellegrino et al. as also suggested by the constancy of arterial pressure values in their subjects before and after saline infusion.

Moreover, the increment in the volumes of liquid in the extravascular spaces of the lung parenchyma of the subjects of Pellegrino et al. was probably not sufficient to cause a detectable change in respiratory system elastance. Although the saline infusion rate in their experiments may be considered similar to that commonly used in clinical practice, it is largely lower than that used in similar experiments in the rat by Dellacà et al. [22], who in fact did observe an increase in respiratory system elastance due to a substantial fluid accumulation in the lungs. Pellegrino et al. did not report a direct quantitative estimation of fluid accumulation in the lung interstitium, but only an indirect estimation of airway edema.

Thus, the finding of Pellegrino et al. of no change in respiratory system elastance is probably due to a rather small increase in BVE and cardiac output, and an associated pulmonary vascular engorgement, and to a minor degree to fluid accumulation in the lung interstitium of their subjects. This was effective in increasing airway resistance, but not in changing respiratory system elastance.

The $E_{st,rs}$ values measured with the constant-flow occlusion technique are very close to those calculated from the volume-pressure data reported in table 3, obtained for the measurement of $H_y_{rs}$, which range from 1.7 to 2.8 cm H$_2$O/ml, depending on the volume of expansion of the respiratory system.

Resistance of Respiratory System Tissues to Movement (Ohmic Resistance)

Previously reported mean values of $R_{min,rs}$ in similar experiments in the rat range from 0.037 to 0.4 cm H$_2$O/ml·s$^{-1}$ [18, 23–25], and our results are comprised in this range. They are near the lowest values previously report-
Changes in respiratory system mechanics following BVE should cause an increase in WOB during spontaneous breathing too.

**Mean Respiratory System Hysteresis Surface Area**

We found a statistically significant increase of the respiratory system hysteresis in BVE compared to the control condition (fig. 4, 5). This means that pulmonary blood flow influences the hysteretic properties of the respiratory system. These represent a complex phenomenon, mainly due to the plastic characteristics of the tissue elements and to true tissue hysteresis, surface hysteresis linked to alveolar surfactant activity, differences in the sequences of recruitment and derecruitment of lung units between inflation and deflation.

Previous studies measuring hysteresis in the isolated rat lung for the same lung volume expansions as those performed here report values comprised between 2.49 and 4.45 cm H₂O/ml [23, 24]. The present data are somewhat higher (fig. 4), thus suggesting that chest wall hysteresis is not negligible in the rat, as previously reported for the dog [29].

We previously demonstrated an increment in lung hysteresis in a rat model of (static) pulmonary vascular engorgement [30]. We cannot exclude that a change in pulmonary blood flow and/or volume may influence the sequences of lung unit recruitment and derecruitment during inflation and subsequent deflation, or the total extent of air-liquid interface in the lungs, but these results also suggest a possible effect of lung blood flow and/or volume on alveolar surfactant activity.

Confirming this hypothesis, Gutierrez et al. [31] found a decrease in surfactant protein expression in lambs with artificially induced increased pulmonary blood flow, suggesting this effect may be due to increased endothelial NO production.

The inspiratory limb of the volume-pressure loop under the BVE condition is shifted to the right with respect to the control condition, mostly at high lung volumes (fig. 4). This is in agreement with previously reported results in isolated cat lungs [32], suggesting that the effects of BVE on respiratory system elastance are more relevant at high lung volumes.

Although quantitatively limited, displacement to the right of the inspiratory pressure-volume curve during BVE contributes an additional increase in WOBel for a given VT.

Displacement to the left of the expiratory limb decreases the available elastic pressure during passive expiration (fig. 4).

Our data obtained in normal animals under controlled and minimally invasive conditions confirm that the increase in pulmonary blood flow significantly increases respiratory system elastance and resistance. In addition, an effect on respiratory system hysteresis is demonstrated for the first time.

These changes lead to a significant increase in both WOBel and WOBres, which were measured for the first time at different values of lung blood volume in the positive-pressure inflated respiratory system of the rat.

Thus, respiratory mechanics are significantly influenced as a result of increased cardiac output.

Our measurements of respiratory mechanics were done shortly after BVE.

We have no data describing how long the observed changes may last. This information is rather difficult to obtain because mechanical ventilation per se is known to alter respiratory mechanics with time [22]. We can speculate that the changes in respiratory mechanics we observed progressively decrease with time due to the urinary elimination of the excess of fluid. Svensen et al. [33] reported the rate of elimination of excess of fluid after intravenous hypertonic saline infusion to be about 20% of volume expansion per hour, and about the same result may be deduced from the data of Tollofsrud et al. [34] after plasma expander infusions. Thus, it may be expected that the mechanical parameters should return to baseline values in no more than 3–4 h. This estimated time seems to be long enough to cause possible deleterious effects in patients receiving large intravenous infusions. Even if the changes in respiratory mechanics we observed are rather small (10–20%), they are sufficient to significantly increase WOB, hence to cause a potentially harmful effect in severely ill patients, who might undergo respiratory failure.

Our findings suggest important clinical applications. Altered cardiac output is a frequent condition in many different diseases, and an increase in cardiac output may also result from frequently therapeutic manipulations of cardiac function such as acute BVE. The consequences of modifications of respiratory mechanics should be known and considered.

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References


