Comparison of different degrees of variability in tidal volume to prevent deterioration of respiratory system elastance in experimental acute lung inflammation

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Abstract

Background: Variable ventilation improves respiratory function, but it is not known whether the amount of variability in tidal volume (VT) can be reduced in recruited lungs without a deterioration of respiratory system elastance.
Methods: Acute lung inflammation was induced by intratracheal instillation of lipopolysaccharide in 35 Wistar rats. Twenty-eight animals were anaesthetized and ventilated in volume-controlled mode. Lungs were recruited by random variation of VT (mean 6 ml kg\(^{-1}\), coefficient of variation 30%, normal distribution) for 30 min. Animals were randomly assigned to different amounts of VT variability (n=7 for 90 min per group): 30, 15, 7.5, or 0%. Lung function, diffuse alveolar damage, and gene expression of biological markers associated with cell mechanical stress, inflammation, and fibrogenesis were assessed. Seven animals were not ventilated and served as controls for post-mortem analyses.
Results: A VT variability of 30%, but not 15, 7.5, or 0%, prevented deterioration of respiratory system elastance [Mean (SD) –7.5 (8.7%), P<0.05; 21.1 (9.6%), P<0.05; 43.3 (25.9), P<0.05; and 41.2 (16.4), P<0.05, respectively]. Diffuse alveolar damage was lower with a VT variability of 30% than with 0% and without ventilation, because of reduced oedema and haemorrhage. A VT variability of 30, 15, or 7.5% reduced the gene expression of amphiregulin, cytokine-induced neutrophil chemoattractant-1, and tumour necrosis factor α compared with a VT variability of 0%.
Conclusions: In this model of acute lung inflammation, a VT variability of 30%, compared with 15 and 7.5%, was necessary to avoid deterioration of respiratory system elastance and was not associated with lung histological damage.
Key words: Escherichia coli; inflammation; respiratory mechanics; inspiratory positive-pressure ventilation; mechanical ventilation

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Tidal volume variability prevents deterioration of elastance

Editor’s key points

- Lung damage can be mitigated by varying tidal volumes during mechanical ventilation.
- The limits of such variation in tidal volume are unclear.
- In this study, using a rat model, tidal volumes were varied by up to 30%.
- Only 30% variability in tidal volume was able to prevent decreased respiratory function.
- This may have implications for clinical practice.

Controlled mechanical ventilation with variable tidal volumes (VT) has been shown to improve respiratory mechanics and pulmonary gas exchange1 and to reduce lung damage2 in animal models of the acute respiratory distress syndrome (ARDS). Increased lung surface area for ventilation appears to be a major mechanism explaining these effects.3 4

Lung recruitment resulting from variable ventilation is superior to, and lasts longer than, conventional recruitment manoeuvres.5 A previous investigation by our group showed that a coefficient of variation (amount of variability) in VT of 30% is able to stabilize respiratory function after conventional lung recruitment in experimental ARDS. In another study, a similar amount of variability optimized gas exchange and respiratory system mechanics during assisted mechanical ventilation, probably by promoting recruitment of the lungs.5 The ARDS Network has shown that a VT of 12 ml kg−1 is associated with lung inflammation and increased mortality in patients with ARDS compared with a VT of 6 ml kg−1.6 During variable ventilation with a mean VT of 6 ml kg−1, 30% variability results in periodic VT values higher than 12 ml kg−1, which could lead to volutrauma. In contrast, periodic increases in VT may recruit the lungs, contributing to reduced regional stress and strain. Thus, one could postulate that, once lungs are recruited and provided that recruitment is stable, the variability of the respiratory pattern could be reduced to avoid potentially harmful VT values, thereby maximizing lung protection. To our knowledge, this issue has not been addressed previously.

In the present study, we investigated the effects of different amounts of VT variability on respiratory function, lung histologic damage, and markers of cell mechanical stress, in animal models of the acute respiratory distress syndrome (ARDS). Increased lung surface area for ventilation appears to be a major mechanism explaining these effects.3 4

Animal preparation and experimental protocol

Figure 1 depicts the time course of interventions. Thirty-five specific-pathogen-free adult male Wistar rats [weighing 347 (24 g)] were anesthetized by inhalation of sevoflurane 2% (Sevorane®; Cristália, Itapira, SP, Brazil), and acute lung inflammation was induced by intratracheal (i.t.) instillation of Escherichia coli lipopolysaccharide (LPS; O55:B5; Sigma Chemical Co., St Louis, MO, USA) 800 µg suspended in saline solution to a total volume of 200 µl. Animals were then allowed to recover from anaesthesia and observed for a period of 24 h. Thereafter, animals were premedicated intraperitoneally (i.p.) with diazepam 10 mg kg−1 (Compaz®, Cristália), ketamine 50 mg kg−1 (Ketamin-S®; Cristália), and midazolam 2 mg kg−1 (Dormicum; União Química, São Paulo, SP, Brazil). An i.v. catheter (Jelco 24G; Becton Dickinson, Franklin Lakes, NJ, USA) was inserted into the tail vein for continuous infusion of midazolam 2 mg kg−1 h−1, ketamine 50 mg kg−1 h−1, and Ringer’s lactate 7 ml kg−1 h−1 (B. Braun, Rio de Janeiro, Brazil). The adequacy of anaesthesia was assessed by the response to a nociceptive stimulus before surgery. Anaesthetized animals were kept in dorsal recumbency. Local anaesthetic (0.4 ml, lidocaine 2%) was infiltrated, and a tracheostomy was performed via a midline neck incision. Seven animals were not ventilated (NV). These were used as a control group for diffuse alveolar damage and molecular biology analysis. In the remaining 28 animals, a polyethylene catheter (PE-50) was introduced into the right internal carotid artery for blood sampling and mean arterial blood pressure (MAP) measurement. Heart rate (HR), MAP, and rectal temperature were continuously recorded (Networked Multiparameter Veterinary Monitor LifeWindow 6000 V; Digicare Animal Health, Boynton Beach, FL, USA). Body temperature was maintained at 37.5 (1°C) using a heating plate. To maintain MAP>60 mm Hg, Ringer’s lactate solution (B. Braun) was given i.v. as 1 ml boluses to a maximal volume of 5 ml. If further volume loading was necessary, Gelafulindin® (B. Braun) was administered in 0.5 ml increments.

Neuromuscular block was then induced with pancuronium bromide (Pancuron®; Cristália) given i.v. (0.4 mg), followed by 0.4 mg i.m. The lungs were mechanically ventilated (Inspira; Harvard Apparatus, Holliston, MA, USA) in volume-controlled mode (VCV), with VT=6 ml kg−1, respiratory rate (RR) adjusted to an arterial carbon dioxide partial pressure (PaCO2) target of 35–45 mm Hg, inspiratory to expiratory ratio (I:E) ratio=1:2, fraction of inspired oxygen (FiO2) = 0.4, and PEEP=4 cm H2O. After a 5 min stabilization period, arterial blood gases were measured with a Radiometer ABL80 FLEX (Copenhagen NV, Denmark) and lung mechanics recorded (BASELINE). To recruit the lungs, the VT variability was set at 30% and maintained for 30 min using an external controller for the mechanical ventilator, as described elsewhere. During variable ventilation, VT varied on a breath-to-breath basis using a self-looping sequence of randomly generated VT values (n=600, mean VT=6 ml kg−1, normal distribution). During the 30 min recruitment period, lung mechanics variables were continuously recorded. Values for the whole recruitment period were averaged and thus contained a few non-recruited and a majority of recruited lung breathing cycles. After the recruitment period, arterial blood gases and respiratory system mechanics were measured (START).

Animals were then randomly assigned to mechanical ventilation with one of four values of VT variability (n=7 per group), using closed sealed envelopes, as follows: (i) 30% (CV30); (ii) 15% (CV15); (iii) 7.5% (CV7.5); and (iv) 0% (CV0). Arterial blood gases and respiratory system mechanics were assessed every 30 min during a total

Methods

The study protocol was approved by the Animal Care Committee of the Health Sciences Centre, Federal University of Rio de Janeiro, Brazil. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the US National Academy of Sciences Guide for the Care and Use of Laboratory Animals and complied with relevant aspects of the ARRIVE guidelines. Animals were kept at a controlled temperature (23°C) and controlled light–dark cycle (12 h–12 h) with free access to water and food.

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time period of 90 min (TIME 30, TIME 60, and TIME 90). The mean \( V_t \) in all groups was 6 ml kg\(^{-1}\), and other ventilator settings were kept unchanged. Finally, animals were killed by i.v. injection of sodium thiopental 25 mg (Cristália), and potassium chloride (1 ml, 1 M). The left and right lungs were extracted at an airway pressure (\( P_{aw} \)) equivalent to PEEP for histological and molecular biology analysis, respectively. The lungs of NV animals were extracted in atmospheric conditions.

**Data acquisition and processing**

Airflow, \( P_{aw} \), and oesophageal pressure were continuously recorded throughout the experiments in a computer running custom software written in LabVIEW\textsuperscript{®} (National Instruments, Austin, TX, USA). The \( V_t \) was calculated by digital integration of the flow signal. All signals were filtered (30 Hz), amplified in a four-channel signal conditioner (SC-24; SCIREQ, Montreal, Quebec, Canada), and sampled at 1000 Hz with a 12-bit analog-to-digital converter (National Instruments). Peak and mean \( P_{aw} \) (\( P_{aw,peak} \) and \( P_{aw,mean} \), respectively) were computed. The elasticity and resistance of the respiratory system (\( E_{RS} \) and \( P_{RS} \), respectively) were calculated by fitting the respiratory signals to the equation of motion. Parameters obtained during lung recruitment were obtained from continuous recordings (30 min) and arithmetically averaged. Other respiratory variables were computed from the last 10 min of recordings preceding measurement time points using routines written in MATLAB (version 7.14; The Mathworks Inc., Natick, MA, USA).

**Lung histology**

Heparin (1000 IU) was injected into the tail vein. A laparotomy was performed at the end of the experiments (TIME 90). The left lung was carefully removed, fixed in 3% buffered formalin, paraffin embedded, and stained with haematoxylin and eosin. The left lung was first cut transversely at the hilar level. A second cut was performed approximately 5–8 mm caudal to the hilum. Sections for lung histology analysis were taken from the caudal part.

**Diffuse alveolar damage**

Photomicrographs at magnifications of \( \times100, \times200, \) and \( \times400 \) were obtained from seven non-overlapping fields of view per animal using a light microscope (Olympus BX51; Olympus Latin America-Inc., Brazil). Diffuse alveolar damage (DAD) was quantified using a weighted scoring system. Values from 0 to 4 were used to represent the severity of oedema, haemorrhage, inflammatory cell infiltration, atelectasis, and hyperdistension, with 0 standing for no effect and 4 for maximal severity. Additionally, the extent of each score characteristic per field of view was determined on a scale of 0 to 4, with 0 standing for no visible extent and 4 for complete involvement. Scores were calculated as the product of severity and extent of each feature, in the range of 0–80, which represented the DAD. Scoring was assessed independently by two co-authors (V.L.C. and M.K.) who are experts in lung pathology. Both assessors were blinded to group assignment. The scores of each expert were combined to yield a final score by arithmetic averaging.

**Biological markers of cell mechanical stress, inflammation, and fibrogenesis**

Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to measure biological markers associated with cell mechanical stress (amphiregulin), inflammation (these included interleukin (IL)-6, cytokine-induced neutrophil chemoattractant (CINC)-1, and tumour necrosis factor (TNF)-\( \alpha \)), and type III pro-collagen (PCIII) was used as a marker of fibrogenesis. Central slices were cut from the right lung, collected in cryotubes, snap frozen in liquid nitrogen and stored at \(-80^\circ \text{C} \). Total RNA was extracted from frozen tissues using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s recommendations. The RNA concentration was measured by spectrophotometry in a Nanodrop ND-1000 system. First-strand cDNA was synthesized from total RNA using a Quantitate reverse transcription kit (Qiagen, Hilden, Germany). The primers used are described in the Supplementary material (Table S1). Relative mRNA levels were measured with a SYBR green detection system using real-time PCR (ABI 7500; Applied Biosystems, Foster City, CA, USA). For each sample measured in triplicate, the gene expression was normalized to that of a housekeeping gene (acidic ribosomal phosphoprotein P0, 36B4) and expressed as fold changes relative to NV animals, using the 2\(^{ΔΔCt}\) method, where \( ΔCt=Ct(\text{reference gene})−Ct(\text{target gene}) \).
Statistical analysis

The number of animals per group did not follow formal sample size calculation and was based on previous experience of our laboratory with the i.t. LPS instillation model of acute lung inflammation and on unpublished data showing that variability in this model.

The effects of recruitment on gas exchange and lung mechanics were assessed by Student’s paired t-tests between the BASELINE and START time points. Comparisons among groups after randomization were conducted by means of a general linear model using START as covariate, and adjusted for repeated measurements according to the Sidák procedure (% effect: † vs CV7.5; ‡ vs CV30)

Results

All animals survived the induction of acute lung inflammation and the 24 h period that followed.

Gas exchange and haemodynamic variables are shown in Table 1. Lung recruitment led to an increase in oxygenation and arterial pH and a decrease in PaCO2. After randomization, oxygenation and haemodynamic variables were stable and comparable among groups.

Respiratory mechanics are depicted in Table 2. During recruitment, the VT variability was 26.5 (1.5)% and resulted in a decrease in Paw,peak, Paw,mean, ERS and Rs (data not shown). After recruitment, VT was slightly higher in CV30 than in other groups, whereas RR was higher in CV15 compared with CV30 and CV0. The Paw,peak and Paw,mean did not differ among groups, but ERS was lower in CV30. As shown in Fig. 2, ERS was stable throughout the experiments in CV30, but increased progressively in CV15, CV7.5, and CV0.

Photomicrographs of lung parenchyma from representative animals are available as Supplementary material Figure S1. In CV30, the cumulative DAD score was lower than in CV0 and NV animals (Fig. 3). The main histological features that contributed to this difference were oedema and haemorrhage (see Supplementary material Table S2 for details). Furthermore, CV30 animals had less haemorrhage than CV7.5 animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>BASELINE</th>
<th>START</th>
<th>TIME 30</th>
<th>TIME 60</th>
<th>TIME 90</th>
<th>BASELINE vs START</th>
<th>Group effect</th>
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<td>48.8 (8.7)</td>
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<td>P=0.36</td>
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<td>*P=0.001</td>
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<td>P=0.36</td>
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<td>4.4 (0.5)</td>
<td>*P=0.001</td>
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<tr>
<td>CV15</td>
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<td>4.9 (0.5)</td>
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<td>4.5 (0.3)</td>
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<td>4.8 (0.3)</td>
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<tr>
<td>CV30</td>
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<td>88 (17)</td>
<td>98 (18)</td>
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<tr>
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<td>106 (32)</td>
<td>104 (16)</td>
<td>118 (14)</td>
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<td></td>
<td>P=0.006</td>
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<tr>
<td>CV0</td>
<td>122 (43)</td>
<td>119 (28)</td>
<td>105 (18)</td>
<td>102 (19)</td>
<td>99 (20)</td>
<td></td>
<td>P=0.006</td>
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</table>
The detailed results of gene expression in lung tissue are shown in Fig. 4 and in the Supplementary material Figure S2. Levels of amphiregulin, CINC-1, and TNF-α mRNA were lower in CV30, CV15, and CV7.5 compared with CV0. In addition, CV0 animals had higher IL-6 mRNA expression than did NV and CV15 animals. There was no difference in PCIII expression between groups.

**Discussion**

The main findings of the present study were that, after lung recruitment in a model of acute lung inflammation in rats: (i) CV30, but not CV15, CV7.5, or CV0, prevented deterioration of $E_R$; (ii) the DAD score was lower in CV30 than CV0, mainly because of reduced oedema and haemorrhage; and (ii) gene expressions of amphiregulin, CINC-1, and TNF-α were lower in CV30, CV15, and CV7.5 than in CV0.

We induced acute lung inflammation by i.t. instillation of LPS, because this model results in impaired lung mechanics, histological damage, and increased mRNA expression of markers of cell stress, inflammation, and fibrogenesis in pulmonary tissue, while still responding to different strategies for the treatment of acute lung injury. 2 In our experience, this model is associated with...
moderate to severe impairment of gas exchange and a high survival rate in the post-instillation period. Furthermore, data from our laboratory have shown that the acute lung inflammation induced by i.t. instillation of LPS responds to lung recruitment manoeuvres and to different mechanical ventilation settings.

Gene expression of amphiregulin is closely related to mechanical cell stress and is not influenced by inflammation, and IL-6, CINC-1, and TNF-α were chosen because they reflect inflammation during ventilator-induced lung injury. Type III pro-collagen mRNA expression was measured in control lung tissue from non-ventilated (NV) animals, and in lung tissue from animals mechanically ventilated with a coefficient of variation in tidal volume of 30% (CV30), 15% (CV15), 7.5% (CV7.5), or 0% (CV0). Values are means and standard deviations, and data were analysed by one-way ANOVA followed by Bonferroni’s multiple comparison test (significance accepted at P<0.05).

Fig 2 Respiratory system elastance $\Delta E_R$ relative to START of therapy. Measurements were performed during variable ventilation with coefficients of variation in tidal volume of 30% (CV30), 15% (CV15), 7.5% (CV7.5), or 0% (CV0). Statistical analysis revealed a significant group effect; $E_R$ was significantly lower in CV30 compared with all other groups at TIME 90; group effects analysis was by means of repeated-measures ANOVA and adjusted according to Sidak (elastance at START was used as covariate).

Fig 3 Cumulative diffuse alveolar damage (DAD) score, representing injury from alveolar oedema, haemorrhage, inflammatory cell infiltration, atelectasis, and hyperdistension in control, non-ventilated (NV) animals, and animals mechanically ventilated with a coefficient of variation of 30% (CV30), 15% (CV15), 7.5% (CV7.5), or 0% (CV0). Values are given as medians, interquartile range, and full range. Data were analysed by the Kruskal–Wallis test followed by Dunn’s multiple comparison test (significance accepted at P<0.05).

Fig 4 Gene expression of tumour necrosis factor-α (TNF-α). The TNF-α mRNA expression was measured in control lung tissue from non-ventilated (NV) animals, and in lung tissue from animals mechanically ventilated with a coefficient of variation in tidal volume of 30% (CV30), 15% (CV15), 7.5% (CV7.5), or 0% (CV0). Values are means and standard deviations, and data were analysed by one-way ANOVA followed by Bonferroni’s multiple comparison test (significance accepted at P<0.05).
mechanical properties of the respiratory system, especially $E_{RS}$, are closely related to the surface area available for gas exchange. However, such alterations can also result from changes in alveolar flooding and its redistribution. The findings of the present study show that improvement of $E_{RS}$ occurred during the variable ventilation recruitment period, as shown in a previous study. In CV30, $E_{RS}$ did not change significantly during the observation period. In contrast, the change from 2.98 (0.33) to 4.21 (0.78) cm H$_2$O litre$^{-1}$ in CV0 evinces a relevant deterioration of respiratory mechanics during non-variable ventilation of $\sim$40%. When the $E_{RS}$ worsens by that amount during volume-controlled ventilation, the driving pressure increases substantially, increasing the potential of mechanical ventilation to cause lung injury.

As no major changes in oxygenation were observed among groups, the distribution of perfusion may have had no effect. The dynamic of instability, or opening, of lung units seems to reproduce the avalanche phenomenon. This can occur when a given airway (or mother airway) and its subordinated (or daughter) airways have different airway opening pressures. Daughter airways can be subjected to positive pressure from the mechanical ventilator only after the critical opening pressure of the mother airway has been exceeded (i.e. after the mother airway has been recruited). When daughter airways have lower opening pressures than the mother airway, they will subsequently open in a chain reaction that can be likened to an avalanche. In this context, variable airway pressures resulting from modulation of $V_T$ might provide a valuable means to exceed different opening pressures periodically. However, lung recruitment depends not only on opening pressures, but also on the time spent at such pressures.

Thus, it is conceivable that, with $V_T$ variability of 15% or lower, the time interval between instances of $V_T$ exceeding opening pressures is too great to avoid loss of aerated lung tissue.

It is worth noting that, even during a relatively short period of variable ventilation ($\sim$120 min), a $V_T$ variability of 30% led to a significant reduction in histological damage. Those effects were mainly determined by reduced oedema and haemorrhage, suggesting that alveolar flooding might have been the major determinant of alterations in the mechanical properties of the respiratory system and its structural response to a higher degree of variability. Given that variable ventilation permanently recruits lungs, these findings could be attributable to higher net alveolar fluid clearance. We also speculate that the reduced histological damage could be ascribed to increased release of surfactant, which represents a possible beneficial mechanism of variable ventilation. The fact that a reduction in the pro-inflammatory response to variable ventilation was observed at all values of $V_T$ variability compared with 0% variability suggests that mechanisms other than lung recruitment might be involved (e.g. because of a variable stretch pattern). In fact, recent data from our laboratory showed that, in type 2 alveolar epithelial cells challenged with LPS, variable stretch reduces the release of different cytokines.

The findings of the present study provide an important addition to previous investigations on variable ventilation. Our data suggest that, during apparently stable respiratory system mechanics, a reduction in the amount of $V_T$ variability may lead to progressive deterioration of $E_{RS}$. Therefore, limiting hyperdistension of sporadic cycles through a reduction of the $V_T$ variability cannot be recommended in acute lung inflammation.

This study has several limitations. First, acute lung inflammation was induced by i.t. instillation of LPS, and caution is advised when extrapolating our results either to other experimental models of ARDS or to patients. Second, we measured the mechanical properties of the respiratory system instead of those of the lungs. However, in small animals, chest wall elasticity is relatively low and not influenced by instillation of LPS into the lungs. Thus, its contribution to changes in $E_{RS}$ is almost negligible. Third, compared with humans, rats have fewer respiratory bronchiol and airway generation, with airways exhibiting a monopodial as opposed to a dichotomous branching pattern. Thus, both the response to the amount of $V_T$ variability and the dynamics of improvement and deterioration in $E_{RS}$ might be different in humans. Fourth, the observation period after randomization was relatively short (90 min), and we cannot rule out the possibility that $E_{RS}$ would also have deteriorated at a $V_T$ variability of 30% thereafter. To keep animals with endotoxin-induced acute lung inflammation alive for 4–6 h, greater amounts of fluid, inotropes, or both would have been required, which could interfere with gene expression. Finally, we did not measure protein levels of markers of cell stress, inflammation, or fibrogenesis in bronchoalveolar lavage fluid or lung tissue.

Future studies should determine which level of PEEP is necessary to avoid deterioration of respiratory system elastance mechanics when a 30% coefficient of variation in $V_T$ is used, and translation of our findings into large animal models and human populations would be an obvious next step.

In the rat model of acute lung inflammation used in the present study, a $V_T$ variability of 30% was necessary to avoid deterioration of $E_{RS}$ and was not associated with lung histological damage.

**Authors’ contributions**


**Supplementary material**

Supplementary material is available at British Journal of Anaesthesia online.

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**Declaration of interest**

None declared.

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