

# Mild Hypothermia Attenuates Changes in Respiratory System Mechanics and Modifies Cytokine Concentration in Bronchoalveolar Lavage Fluid During Low Lung Volume Ventilation

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## Summary

Hypothermia was shown to attenuate ventilator-induced lung injury due to large tidal volumes. It is unclear if the protective effect of hypothermia is maintained under less injurious mechanical ventilation in animals without previous lung injury. Tracheostomized rats were randomly allocated to non-ventilated group (group C) or ventilated groups of normothermia (group N) and mild hypothermia (group H). After two hours of mechanical ventilation with inspiratory fraction of oxygen 1.0, respiratory rate 60 min<sup>-1</sup>, tidal volume 10 ml·kg<sup>-1</sup>, positive end-expiratory pressure (PEEP) 2 cm H<sub>2</sub>O or immediately after tracheostomy in non-ventilated animals inspiratory pressures were recorded, rats were sacrificed, pressure-volume (PV) curve of respiratory system constructed, bronchoalveolar lavage (BAL) fluid and aortic blood samples obtained. Group N animals exhibited a higher rise in peak inspiratory pressures in comparison to group H animals. Shift of the PV curve to right, higher total protein and interleukin-6 levels in BAL fluid were observed in normothermia animals in comparison with hypothermia animals and non-ventilated controls. Tumor necrosis factor- $\alpha$  was lower in the hypothermia group in comparison with normothermia and non-ventilated groups. Mild hypothermia attenuated changes in respiratory system mechanics and modified cytokine concentration in

bronchoalveolar lavage fluid during low lung volume ventilation in animals without previous lung injury.

## Key words

Rats • Mechanical ventilation • Inflammation • Cytokines • Hypothermia

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## Introduction

Hypothermia was shown to attenuate injury of the lung due to mechanical ventilation in several experimental studies that used large tidal volumes either alone or in combination with previous lung injury in both *in vivo* and *ex vivo* animal models of ventilator-induced lung injury (Lim *et al.* 2003, Suzuki *et al.* 2004, Hong *et al.* 2005). Although hypothermia could be considered as a

means for lung rest in the acutely injured lung, because it may ensure lower frequency ventilation (Lim *et al.* 2003), the results of published studies (Lim *et al.* 2003, Suzuki *et al.* 2004, Akinci *et al.* 2005, Hong *et al.* 2005) suggest that temperature modulates activation of pulmonary resident cells and this may, at least partially, explain protective effects of hypothermia. It remains unclear if the protective effect of hypothermia is maintained under less injurious mechanical ventilation in animals without previous lung injury.

This study was designed to investigate whether mild hypothermia could attenuate the changes in respiratory system mechanics and cytokine concentration in bronchoalveolar lavage fluid during low PEEP, high inspiratory fraction of oxygen (FiO<sub>2</sub>) and moderate tidal volume ventilation in animals without previous lung injury.

## Methods

### *Animal anesthesia and instrumentation*

The study protocol was approved by the Animal Care and Use Committee of the Faculty of Medicine in Hradec Králové, Charles University Prague.

Twenty-two male adult specific pathogen-free Sprague-Dawley rats (weighing 280 to 320 g) were anesthetized using ether chamber, placed supine and Silastic (0.012-inch I.D, 0.025 inch O.D) catheters were inserted in the left common carotid artery and in the right jugular vein. After awakening animals were returned to their cages and food and water was provided.

Twenty-four hours after instrumentation the rats were anesthetized by intraperitoneal administration of sodium pentobarbital (Nembutal, Abbott Laboratory, North Chicago, IL, USA) 50 mg/kg body weight. Additional pentobarbital was given freely as necessary. A tracheotomy was performed with a 14-gauge cannula (Vasocan Braunule, B.Braun, Germany) inserted to a depth of 1 cm and secured in place using three sutures. Mechanically ventilated animals were connected to a small animal ventilator (Inspiria ASV, Harvard Apparatus, MA) with an FiO<sub>2</sub> of 1.0, respiratory rate 60/min, tidal volume (TV) 10 ml/kg, inspiratory time 50 % of respiratory cycle and PEEP 2 cm H<sub>2</sub>O.

Pancuronium (1 mg/kg; Organon, Oss, The Netherlands) was administered in mechanically ventilated animals intravenously after the depth of anesthesia was confirmed by the absence of response to paw compression. Depth of anesthesia was assessed every

15 min by monitoring the hemodynamic response to paw clamp. Anesthesia was maintained with intravenously administered pentobarbital (500 µg/kg/h), in case of response sodium pentobarbital (2.5 mg) was given intravenously.

Muscle relaxation was maintained with pancuronium (0.2 mg/g/h) administered by continuous infusion *via* the right jugular vein. Normal saline was also administered intravenously so that intravenous fluids totaled 4 ml/h including medications. The total volume of intravenous fluid and dosages of medications administered were recorded.

Airway pressures were monitored continuously (Gabarith PMSET 1DT-XX, Becton Dickenson, Singapore) and data recorded every 30 min. A catheter in the left common carotid artery connected to a pressure transducer (Gabarith PMSET 1DT-XX, Becton Dickenson, Singapore) was used to continuously monitor blood pressure (Datex-Ohmeda S/5, Instrumentarium Corp., Helsinki, Finland). A central venous catheter placed in the right internal jugular vein was used to administer medications and fluids.

### *Experimental groups and injurious ventilation*

After tracheostomy rats were randomly allocated to non-ventilated group (C, n=6) or ventilated groups of normothermia (N, n=8) and hypothermia (H, n=8). In the normothermia group, body temperature was maintained using an electric blanket at 37±0.5 °C throughout the study, as measured 7 cm deep in the rectum. In the hypothermia group, rectal temperature was reduced to 33±0.5 °C placing the bags with cold water (7 °C) to the sides of the chest and proximal part of the abdomen. Cooling took up to 10 min depending on the size of the animal. The temperature of these animals was maintained in the target range until the end of the study. Fifteen minutes after the beginning of mechanical ventilation, baseline physiological values were recorded and mechanical ventilation using the previously described setting was maintained for two hours.

After two hours (immediately after tracheostomy in C group animals) heparin (400 IU/kg) and additional pentobarbital (2.5 mg) were administered intravenously, abdomen was opened with a midline incision, the aorta visualized and animals were then killed by exsanguination from the abdominal aorta, the blood was collected into syringe. Exsanguination was performed on mechanical ventilation using unchanged ventilatory setting and inspiratory fraction of oxygen in both ventilated groups.

### Physiological variables

Systemic arterial pressure and peak airway pressure were measured and recorded throughout the protocol and recorded every 30 min in both ventilated groups.

### Blood sampling

At the end of the protocol an arterial blood sample was taken from the abdominal aorta and plasma was separated by centrifugation (centrifuge MPW-360, Mechanika Precyzyjna, Warszawa, Poland) at 10 °C, 1500 g for 10 min and stored at -70 °C.

### Pressure-volume curve measurement

Immediately after exsanguination the static pressure-volume (PV) curve was constructed. Animals were disconnected from mechanical ventilator for approximately 30 s and lungs were manually inflated using 20 ml syringe with 2 ml aliquots of air until total lung volume of 14 ml or an airway pressure above 30 mm Hg were reached. Airway pressures were recorded 5 s after every volume change.

### Bronchoalveolar lavage

Immediately after pressure-volume curve measurements bronchoalveolar lavage (BAL) was performed. BAL was carried out by intratracheal instillation of four aliquots (5 ml each) of normal saline and returned fluid was collected by free drainage. Second aliquot of BAL fluid was used for laboratory analysis. Samples from the second of BAL fluid (0.5 ml) were separated for the white blood cell count determination. The rest of BAL fluid samples were then centrifuged at 10 °C, 1000 g for 10 min and the supernatant was aspirated and stored at -70 °C BAL fluid.

### Determination of lung injury variables in BAL fluid and serum

The total protein concentrations were assayed as a measure of alveolar capillary permeability using the total protein colorimetric assay (Roche Diagnostics GmbH, Mannheim, Germany).

The white blood cell count was determined using a hemocytometer (Baxter Healthcare, McGaw Park, IL, USA).

Assays of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in the BAL supernatant and serum were performed with rat TNF- $\alpha$  UltraSensitive BioSource Immunoassay Kit (BioSource Europe S.A., Nivelles, Belgium).

IL-6 levels in the BAL supernatant and serum were measured using rat IL-6 BioSource immunoassay Kit (BioSource Europe S.A., Nivelles, Belgium).

### Statistical analysis

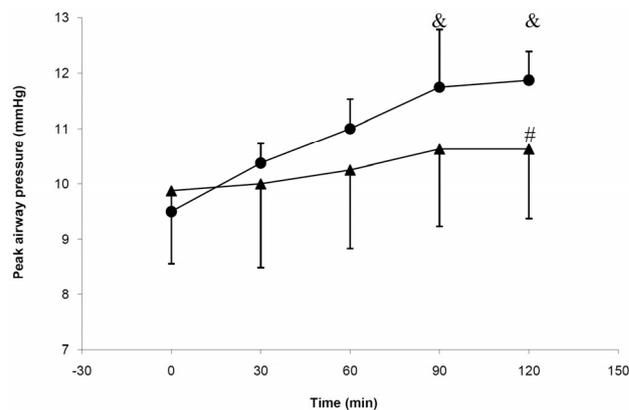
Statistical analysis was performed with SigmaStat 3.1 software (Systat Software Inc., San Jose, CA, USA). For continuous variables the results are expressed as mean  $\pm$  S.D. for normally distributed data and as median /interquartile range/ where non-normally distributed. Data was analyzed by one-way ANOVA followed by Student-Newman-Keuls test or by one-way ANOVA on ranks followed by Dunn's test, by t-test or Mann-Whitney U test as appropriate.  $P < 0.05$  value was accepted as statistically significant.

## Results

### Physiological variables

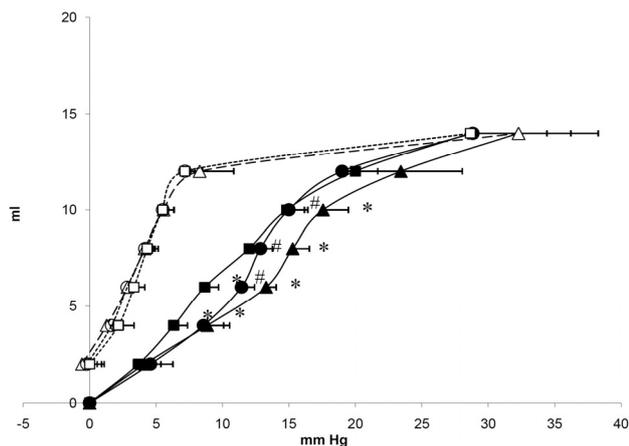
Arterial blood pressure values were stable during the study period and there were no significant differences between both ventilated groups.

Baseline peak airway pressures (mm Hg) did not differ between groups ( $9.5 \pm 1.3$  in normothermia group vs  $9.8 \pm 1.7$  in hypothermia group,  $p = 0.798$ ). Peak airway pressures increased significantly during the study period in the normothermia group. The difference between groups reached significance after 2 h of mechanical ventilation ( $11.9 \pm 1.2$  vs.  $10.6 \pm 1.1$ ,  $p = 0.049$ ) (Fig. 1).

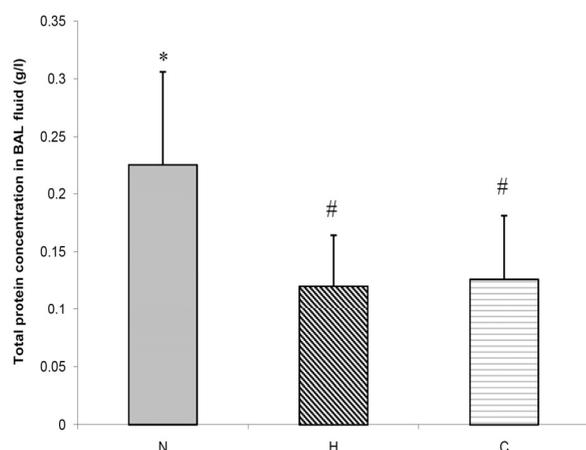


**Fig. 1.** Peak inspiratory pressures of the hypothermia (triangles) and normothermia (circles) groups. # $p < 0.05$  compared with normothermia group, & $p < 0.05$  compared with baseline value.

The PV curve of the normothermic group was significantly shifted to the right in comparison to the PV curve of the hypothermia group and the control group



**Fig. 2.** Pressure-volume curve of the normothermia (triangles), hypothermia (circles) and non-ventilated (squares) groups. \* $p < 0.05$  compared with non-ventilated group,  $p < 0.05$  compared with normothermia group (empty symbols – deflation limb).

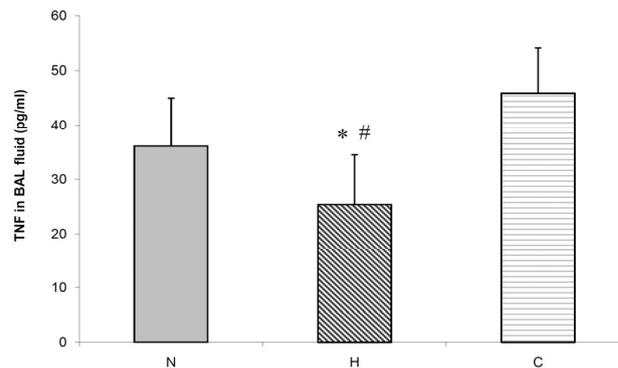


**Fig. 3.** The protein concentration in bronchoalveolar lavage (BAL) fluid in experimental groups (N – normothermia group; H – hypothermia group; C – non-ventilated group). \* $p < 0.05$  compared with non-ventilated group,  $p < 0.05$  compared with normothermia group.

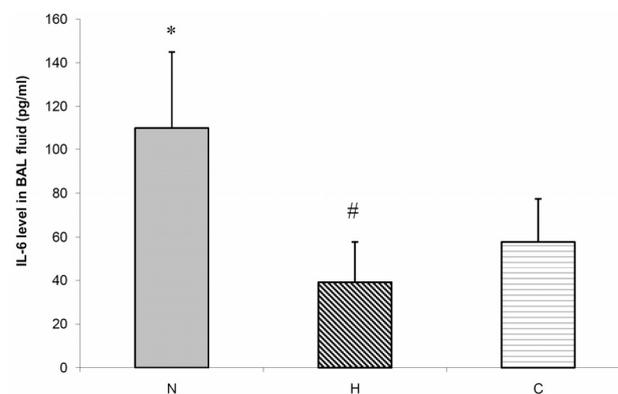
(Fig. 2). The static airway pressures of the hypothermia group were lower than those of the normothermia group at volumes 6, 8, and 10 ml above the end-expiratory volume (all  $p < 0.05$ ). Both ventilated groups showed higher static pressures in comparison with the control group at low lung volumes (4 and 6 ml above the end-expiratory volume, all  $p < 0.05$ ).

#### Lung injury variables in BAL fluid

The harvested amount of BAL fluid was not different between groups. The protein concentration (g/l) in the BAL fluid of the normothermia group was higher than that of the hypothermia group and control group ( $0.225 \pm 0.082$  vs.  $0.120 \pm 0.044$  vs.  $0.126 \pm 0.055$ , respectively,  $p = 0.009$ ) (Fig. 3).



**Fig. 4.** Tumor necrosis factor  $\alpha$  (TNF) level in bronchoalveolar lavage (BAL) fluid in experimental groups (N – normothermia group; H – hypothermia group; C – non-ventilated group). \* $p < 0.05$  compared with non-ventilated group,  $p < 0.05$  compared with normothermia group.



**Fig. 5.** Interleukin-6 (IL-6) level in bronchoalveolar lavage (BAL) fluid in experimental groups (N – normothermia group; H – hypothermia group; C – non-ventilated group). \* $p < 0.05$  compared with non-ventilated group,  $p < 0.05$  compared with normothermia group.

The WBC counts in the BAL fluid ( $10^4/\text{ml}$ ) were not significantly different among the normothermia, hypothermia and control groups ( $8.0 / 5.5; 15.5 / 6.0 / 4.0; 8.0 / 6.5 / 5.0; 12.0 / 6.0 / 4.0$ ,  $p = 0.433$ ).

The TNF level in the BAL fluid (pg/ml) was lower in the hypothermia group both in comparison with the normothermia group ( $25.4 \pm 9.1$  vs.  $36.1 \pm 8.9$ ,  $p = 0.036$ ) and the control group ( $25.4 \pm 9.1$  vs.  $45.9 \pm 8.2$ ,  $p < 0.001$ ). There was no significant difference between the control and normothermia groups ( $45.9 \pm 8.2$  vs.  $36.1 \pm 8.9$ ,  $p = 0.059$ ) (Fig. 4).

The IL-6 level in the BAL fluid (pg/ml) was higher in the normothermia group both in comparison with the hypothermia group ( $110.1 \pm 34.9$  vs.  $39.2 \pm 18.7$ ,  $p < 0.001$ ) and the control group ( $110.1 \pm 34.9$  vs.  $57.9 \pm 19.6$ ,  $p = 0.001$ ). There was no significant difference between the control and hypothermia groups ( $57.9 \pm 19.6$  vs.  $39.2 \pm 18.7$ ,  $p = 0.168$ ) (Fig. 5).

### *Lung injury variables in serum*

Serum TNF (pg/ml) were similar in the normothermia, hypothermia and control groups ( $1.01 \pm 0.71$  vs.  $1.28 \pm 1.11$  vs.  $1.3 \pm 0.56$ ,  $p=0.720$ ). Serum IL-6 was below the detection limit in all groups.

## **Discussion**

Our main findings were that animals without previous lung injury ventilated at body temperature 33 °C had lower changes in mechanical properties of respiratory system and lower levels of lung injury markers (cytokines and total protein concentrations) in BAL fluid after two hours of mechanical ventilation with moderate tidal volumes, low PEEP and high inspiratory fraction of oxygen.

Prolonged mechanical ventilation at low lung volume and zero end-expiratory pressure using physiological tidal volumes has been shown to induce histological evidence of peripheral airway injury and parenchymal inflammation with a concomitant increase in airway resistance, which persists after restoration of physiological end-expiratory lung volume (D'Angelo *et al.* 2002, 2004). In these studies, morphological and mechanical alterations have been attributed to shear stresses caused by cyclic opening and closing of peripheral airways with tidal ventilation at low lung volumes, possibly combined with increased surface tension due to surfactant depletion or inactivation (Robertson 1984). Recent study (D'Angelo *et al.* 2005) also showed that mechanical ventilation at low volume with physiological tidal volumes causes an increase in airway resistance in rabbits. In closed-chest animals, this increase was more pronounced and associated with an increase of lung elastance, likely due to interstitial edema and surfactant depletion or inactivation.

We used low PEEP, high FiO<sub>2</sub> and moderate tidal volumes in this study to mimic the ventilatory setting sometimes utilized temporarily in patients with intracranial hypertension and low brain tissue oxygen tension. High concentration of oxygen is known to accelerate derecruitment in lung areas with low ventilation/perfusion ratio (Aboab *et al.* 2006). The shape of PV curves in both ventilated groups showed initial low compliance and lower inflection point that are considered markers of airway and/or alveolar collapse or closure (Martin-Lefevre *et al.* 2001). We therefore speculate that high inspiratory fraction of oxygen used in this study even in the presence of low PEEP increased the

magnitude of lung derecruitment observed to occur in both ventilated groups and that mechanical forces applied in this model were probably similar to previously published low lung volume injury models.

Increased total protein concentration in BAL fluid is considered to be a marker of increased alveolar capillary permeability (Behnia *et al.* 1996, Koay *et al.* 2002). The lower protein content in the bronchoalveolar fluid of the hypothermia group suggests a protective effect of hypothermia. Similar results have been observed in previous studies on hypothermia (Lim *et al.* 2003, Hong *et al.* 2005).

Mechanical ventilation itself may increase TNF- $\alpha$  in the lung (Imai *et al.* 1999) and cytokines levels in BAL fluid correlated with degree of lung injury due to mechanical ventilation (Tremblay *et al.* 1997). Hypothermia also decreases activation of nuclear factor- $\kappa$ B in the lung, (Koay *et al.* 2002) and this has been suggested to be one of the possible protective mechanisms of hypothermia (Hong *et al.* 2005). Lower TNF- $\alpha$  BAL fluid concentration observed in the hypothermia group probably reflects this mechanism.

Neutrophils are known as important inflammatory cells involved in the development of ventilator-induced lung injury (Kawano *et al.* 1987, Markos *et al.* 1993). In various settings, hypothermia has been shown to inhibit tissue accumulation of neutrophils (Toyoda *et al.* 1996, Whalen *et al.* 1997). In comparison with previous studies that used more injurious mechanical ventilation (Lim *et al.* 2003, Hong *et al.* 2005) we were not able to show a significant difference in number of WBCs in BAL fluid, although there was a non-significant trend towards higher values in the normothermia group.

Gradual rise of peak inspiratory pressure observed in normothermia group reflected deterioration of effective compliance of respiratory system. For technical reasons we did not measure inspiratory and expiratory pressures under static conditions and therefore we are not able to differentiate between increased resistance, decreased static compliance or increased intrinsic PEEP. PV curves of both mechanically ventilated groups showed the lower and upper inflection points. The inspiratory limb of the PV curve of the normothermia group was shifted to the right indicating more profound loss of aerated lung tissue and/or airway closure.

We observed similar course of PV curve during deflation in all groups. This could be explained by the

gentle nature of insult using moderate tidal volumes and by the reversibility of loss of aerated lung tissue and/or airway closure after full recruitment of the lung during the PV curve measurement. Taking into account lower total protein, IL-6 and TNF- $\alpha$  levels in BAL fluid of the hypothermia group we attribute observed changes in mechanical properties of respiratory system to less significant inflammatory response to applied insult.

Mechanical ventilation using the same setting of ventilator had to lead to lower arterial carbon dioxide tension in blood and tissues under hypothermia. The attenuated changes in mechanical properties and cytokine levels in BAL fluid observed in the hypothermia group appear to be in opposition to certain recent reports in which respiratory alkalosis was shown to be lung-deleterious while respiratory acidosis was lung-protective (Shibata *et al.* 1998, Laffey *et al.* 2000, 2004, Sinclair *et al.* 2002). It seems that the potential adverse effect of respiratory alkalosis is less important than the protective effect of hypothermia. Mild hypothermia may protect the lung by several mechanisms. Decreased metabolic rate may enable so called "lung rest" decreasing ventilation requirements (Hong *et al.* 2005). Since the same setting of ventilator was used in both groups, this mechanism does not explain the observed differences. Hypothermia may induce changes in cardiac output, lung perfusion and lung microcirculation; these parameters beside systemic blood pressure were not measured. Decreased lung perfusion was described to modify the extent of lung injury in *ex vivo* perfused lung models of ventilator-induced lung injury (Broccard *et al.* 1998). However, observations published by Suzuki *et al.* (2004) suggest that hypothermia protects the lung by mechanisms that are unrelated to lung perfusion. Our data are in concordance with previously published results (Sarcia *et al.* 2003, Lim *et al.* 2004, Suzuki *et al.* 2004) suggesting that temperature-dependent modulation of inflammatory response activation seems to be at least partially responsible for protective effect of hypothermia in setting of ventilator-induced lung injury.

Our study has several methodological limitations. Due to using high inspiratory fraction of oxygen we are not able to differentiate between the influence of hypothermia on the effects of hyperoxia from the influence of hypothermia on the effects of mechanical ventilation. However, a recent experimental

study showed that moderate hyperoxia does not increase lung injury in the absence of high lung volume injury (Sinclair *et al.* 2004). Even considering this information it remains unclear whether the inspiratory fraction of oxygen affected the results of our study.

For technical reasons we did not use classical markers of lung injury like histological score or lung wet/dry ratio for comparison between groups. Even considering absence of direct markers of lung injury in our study, we believe that used estimation of mechanical properties of respiratory system and used biochemical markers measured in BAL fluid are acceptable indirect indicators of adverse effect of mechanical ventilation on lung structure.

In theory, the differences in BAL fluid concentrations may be due to different dilution of bronchoalveolar fluid and different distribution of BAL fluid during bronchoalveolar lavage. The amount of harvested BAL fluid was not different between groups. BAL was performed after PV curve (e.g. recruitment maneuver) to sample most of the lung and the deflation limb of PV curve suggests similar lung volume at the end of procedure. Observed differences in levels of used markers between groups can not be simply explained by uneven dilution of bronchoalveolar fluid during BAL.

Last but not least the length of experiment was also limited and therefore we cannot exclude only temporary effect of hypothermia. This is a clear limitation, which could have been overcome by modifying the protocol only. Interspecies differences may also reduce translation of our results to the clinical setting.

In conclusions, mild hypothermia attenuated changes in respiratory system mechanics and modified cytokine concentration in bronchoalveolar lavage fluid during low lung volume ventilation.

### Conflict of Interest

The authors have no competing interests and financial relationships that influence the presentation of data in this article.

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