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TRANSLATIONAL STUDIES

Comparison between neurally-assisted, controlled, and physiologically variable ventilation in healthy rabbits

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Abstract

Background: Various ventilation strategies have been proposed to reduce ventilation-induced lung injury that occurs even in individuals with healthy lungs. We compared new modalities based on an individualised physiological variable ventilation model to a conventional pressure-controlled mode.
Methods: Rabbits were anaesthetised and ventilated for up to 7 h using pressure-controlled ventilation with (Group PCS, n=10), and without (Group PC, n=10) regular sighs. Variable ventilation in the other two groups was achieved via a pre-recorded spontaneous breathing pattern [Group physiologically variable ventilation (PVV), n=10] or triggered by the electrical activity of the diaphragm [Group neurally adjusted ventilation assist (NAVA), n=9]. Respiratory elastance, haemodynamic profile, and gas exchange were assessed throughout the ventilation period. Cellular profile, cytokine content of bronchoalveolar lavage fluid, and wet-to-dry lung weight ratio (W/D) were determined after protocol completion. Lung injury scores were obtained from histological analysis.
Results: Marked deteriorations in elastance were observed (median and 95% confidence interval) in Group PC [48.6 (22)% increase from baseline], while no changes were detected in Groups PCS [3.6 (8.1)%], PVV [18.7 (13.2)%], and NAVA [−1.4 (12.2)%]. In comparison with Group PC, Group PVV had a lower lung injury score [0.29 (0.02) compared with 0.36 (0.05), P<0.05] and W/D ratio [5.6 (0.1) compared with 6.2 (0.3), P<0.05]. There was no difference in blood gas, haemodynamic, or inflammatory parameters between the groups.
Conclusions: Individualised PVV based on a pre-recorded spontaneous breathing pattern provides adequate gas exchange and promotes a level of lung protection. This ventilation modality could be of benefit during prolonged anaesthesia, in which assisted ventilation is not possible because of the absence of a respiratory drive.

Keywords: pulmonary gas exchange; respiratory mechanics; ventilator-induced lung injury

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Editor's key points

- Prolonged ventilation can damage even healthy lungs.
- Pressure-controlled ventilation was compared with variable ventilation based on pre-recorded spontaneous breathing patterns in rabbits.
- All ventilation modes provided adequate gas exchange.
- Individual variable ventilation promoted lung protection.
- This may be useful during prolonged ventilation when there is no respiratory drive.

Mechanical ventilation is routinely used in general anaesthesia or the intensive care setting. However, there is a considerable body of evidence that indicates that prolonged positive pressure ventilation can induce or worsen injury to the lung tissue through exaggerated mechanical stress, a condition termed ventilator-induced lung injury (VILI).[1–3] These adverse changes result from the monotonous, cyclic alveolar opening and closure that exerts shear stress forces and increases strain in lung tissue. This in turn promotes lung inflammation with a subsequent deterioration of gas exchange.[3,4]

Various modes of mechanical ventilation have been suggested to overcome the deleterious effects of mechanical ventilation. While there is increasing evidence for the beneficial effects of mechanical ventilation with low tidal volumes associated with positive end-expiratory pressure (PEEP) and regular alveolar recruitment manoeuvers,[5,6] these measures do not fully prevent VILI.[7,8] Therefore, new ventilation modalities have been introduced recently in an attempt to minimise lung injury.[9] One of these attempts is to mimic the physiological variability of spontaneous ventilation.[10,11] This so called 'noisy' or variable ventilation consists of lung insufflations with variable tidal volumes, respiration rates, or both. Experimental and clinical studies have shown that mechanical ventilation with some degree of variability (or 'noise') in the amplitude of individual breaths is beneficial, both for the gas exchange and for the mechanics of the respiratory system.[12–26] However, these variable ventilation patterns were generated on the basis of mathematical models,[12,13] rather than on the physiological spontaneous ventilation of a given individual.

Such variable ventilation can be applied by either assisting the patient's respiratory activity via the modality called neurally adjusted ventilation assist (NAVA),[27] or by using a novel approach based on the pre-recorded spontaneous ventilation pattern to be applied in a controlled ventilation mode. We therefore compared these two variable ventilation modalities with conventional pressure-controlled ventilation with and without regular alveolar recruitment through sighs in normal lungs ventilated for a prolonged period of time. We hypothesised that the application of a degree of physiological variability would provide lung protection, whilst guaranteeing the same gas exchange and preserving normal respiratory function.[28,29]

Methods

All experiments and procedures were conducted under approval from the Swiss animal welfare committee (Geneva Cantonal Veterinary Office, registration number GE61-14 and GE54-15) and concurred with EU directive 2010/63/EU, and results are reported in compliance with the ARRIVE guidelines. Fifty-one female and five male New-Zealand White rabbits, weighing 3.4 (0.1) kg, were involved in the study. All animals came from the University animal farm (Arare, Geneva, Switzerland), and were housed 2 days before the experiment at the laboratory facility of the University, in a pathogen-free environment. Six animals died before randomisation and completion of the experimental protocol, consequent to either severe hypoxaemia because of difficulties in tracheal intubation or to haemodynamic instability. Eleven rabbits served as pilot study animals to optimise the sedation and anaesthesia protocol, to verify the feasibility and consistency of the physiologically variable ventilation (PVV) and NAVA recordings, and to master the techniques for cytological and histological analysis. Hence, 39 animals were randomised based on a list generated by a random number generator in Excel.

Protocol groups

Rabbits were randomly assigned to one of the four protocol groups. Conventional pressure-controlled ventilation was applied to the animals in Group PC (n=10). Rabbits in Group PCS (n=10) received identical pressure-controlled ventilation with regular application of a lung inflation manoeuvre (sighs with peak airway pressure of 20 cm H_2O every 30 min). Ventilatory pressure level and cycle duration were identical to normal ventilation values during non-sigh periods). Animals in the other two groups underwent variable ventilation based either on the pre-recorded respiratory activity during spontaneous breathing (Group PVV, n=10), or the diaphragmatic activity detected by a NAVA catheter (Group NAVA, n=9).

Recording of spontaneous breathing

In animals assigned to the Group PVV, a lubricated nasogastric electrode was inserted to record the electrical activity of the diaphragm (Edi) (Maquet Critial Care, Solna Sweden), after premedication by i.m. injection of xylazine (5 mg kg^{-1}) and spraying the nostrils and the mouth with one push lidocaine 10% (Astra-Zenca®) to minimise sneezing. The Edi was recorded for 40 min (Supplementary Fig. S1). The PVV pattern was generated from these recordings using software to replay repeatedly this modality, taking into account ventilatory frequencies rates up to 55 bpm and an Edi higher than 2 μV. The characteristics of the PVV pattern are provided in Supplementary Table S1.

Anaesthesia and surgical preparation

Animals in all groups were sedated with an i.m. injection of xylazine (5 mg kg^{-1}) and a 22 G catheter was secured in a marginal ear vein. Anaesthesia was induced by i.v. injection of propofol (3 mg kg^{-1}) and maintained by a continuous infusion of propofol (15–20 mg kg^{-1} h^{-1}) and ketamine (5 mg kg^{-1} h^{-1}). All animals were intubated using a 3.0 mm cuffed tracheal tube. Mechanical ventilation was initiated in pressure-controlled mode using a paediatric respirator (Servo-i, Maquet Critical Care, Solna Sweden), with an FiO_2=30%, PEEP of 3 cm H_2O, and an inspiratory pressure of 6 cm H_2O above PEEP. Driving pressure (in group PC), ventilatory frequency, and NAVA level (in groups PVV and NAVA) were adjusted in order to maintain end-tidal CO_2 ($ETCO_2$) between 5.5% and 6%. The resulting tidal volume and ventilatory frequency values in these settings are detailed in Supplementary Table S1.

After ensuring proper depth of anaesthesia, muscle relaxation was induced by a continuous infusion of atracurium

(Tracrium, 0.3 mg kg^{-1} h^{-1}) in all animals except those assigned to the NAVA group. Fluid maintenance was provided by an i.v. infusion of lactated Ringer's solution (10 ml kg^{-1} h^{-1}) after an initial bolus of 10 ml at the time of anaesthesia induction. The central ear artery was cannulated using a 24 G polyethylene catheter for blood pressure monitoring. The electrocardiogram was monitored using s.c. needle electrodes (ADinstruments BioAmp, Dunedin, New Zealand). The animals were placed on a thermostatic heating pad and internal body temperature was continuously monitored and maintained at 38–39°C (Harvard Apparatus, South Natick, MA, USA). An alveolar recruitment manoeuvre was then performed by inflating the lungs to 25 cm H_2O for 5 s to standardise volume history. Fifteen minutes were allowed for the physiological parameters to stabilise before starting the experimental protocol.

Measurement of respiratory mechanics

The airway and respiratory tissue mechanical parameters were measured by using the forced oscillation technique, as detailed previously.[30] Briefly, a loudspeaker-in-box system was utilised to generate a small-amplitude (1 cm H_2O peak to peak) composite pressure forcing signal in a frequency range of 0.5–21 Hz through the tracheal cannula, during short (10 s) pauses interposed with mechanical ventilation. The pressure signal was led through a polyethylene wave tube (100 cm length, 0.375 cm ID) with lateral pressures measured at the loudspeaker end (P_1) and the tracheal end (P_2) using miniature pressure transducers (ICS 33NA00D, Silicon Microstructures Inc., Milpitas, CA, USA). The loudspeaker chamber was pressurised to the level of PEEP in order to maintain pressure constant during the recordings. These pressure signals were digitised at a sampling rate of 128 Hz after low-pass filtering at 25 Hz corner frequency. The pressure transfer function (P_1/P_2) was calculated by fast Fourier transformation from the 8 s recordings and was used to derive the input impedance of the respiratory system (Zrs). Four to five data epochs were collected under each experimental condition and Zrs spectra were ensemble-averaged for further processing.

A global optimisation procedure was applied to fit a model to the Zrs spectra under each experimental condition. The validated model contained frequency-independent airway resistance (Raw) and inertance (Iaw), in series with a constant-phase tissue model[31] including damping (G) and elastance (H). As previously established,[32] Raw reflects mainly the flow resistance of the airways, Iaw is related to the cyclic acceleration and deceleration of the intra-thoracic gas, G describes the energy loss within the respiratory tissues (resistance), whereas H characterises the energy storage capacity of the respiratory tissues (elastance). Raw and Iaw values were corrected for by removing the instrumental components of the tracheal cannula and the connecting tubing.

Wet-to-dry lung weight ratio

At the end of the experiment, rabbits were culled by an i.v. injection of potassium chloride solution (2 mmol kg^{-1}), preceded by i.v. ketamine (40 mg kg^{-1}). A midline sternotomy was performed and the right main bronchi clamped. The right lung was removed and weighed using an analytical balance and then dried at 60°C for 24 h in an oven. The dry samples were weighed again in order to calculate the wet-to-dry lung weight ratio (W/D).

Assessment of lung inflammation

While keeping the right main bronchus clamped, a small catheter was introduced into the left main bronchus via the tracheal tube. The left lung was washed by filling with pre-warmed (38°C) phosphate buffered saline (PBS) containing bovine

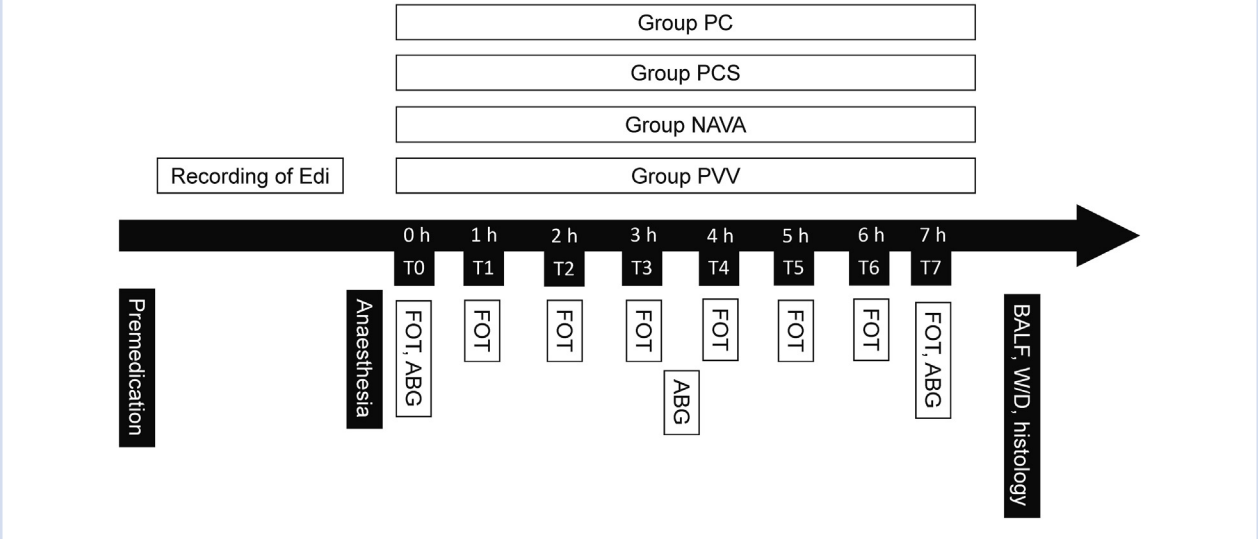

Fig 1. Scheme of the experimental protocol. Rabbits underwent mechanical ventilation with pressure controlled mode with (Group PCS, n=10), or without (Group PC, n=10) sighs, or variable ventilation based either on pre-recorded respiratory activity during spontaneous breathing (Group PVV, n=10), or diaphragmatic activity recorded by a neurally adjusted ventilation assist (NAVA) catheter (Group NAVA, n=9). Forced oscillatory measurements (FOT) were performed hourly, and arterial blood gas (ABG) and systemic haemodynamics were evaluated every 3.5 h. BALF, bronchoalveolar lavage fluid; Edi, electrical activity of the diaphragm; W/D, wet-to-dry lung weight ratio.

serum albumin (BSA) 1% at 20 cm H_2O hydrostatic pressure. Bronchoalveolar lavage fluid (BALF) was obtained very gently using gravity to avoid any tissue damage, which was then centrifuged at 412 *g* for 5 min at 5°C and the supernatant stored at −20°C until analysis. The cell pellet was re-suspended in PBS/BSA and cytospin preparations were obtained by centrifugation at 58 *g* for 7 min. The slides were then fixed and stained with May Grünwald Giemsa for differential cell counting and scanned using Mirax. Cells were counted using an image acquisition software (Panoramic viewer, 3DHISTECH Ltd, Budapest, Hungary). As the distribution of the cells was not homogeneous, the cells were counted within rectangles with an edge length equivalent to the radius of the circular cytospin. The number of cells was normalised to the surface area of the rectangles.

Cytokine assays

Undiluted BALF supernatant was for measurement of inflammatory cytokines using enzyme immunoassay: tumour necrosis factor-α (TNF-α) (MyBiosource MBS2021700, San Diego, CA, USA), interleukin (IL)-8, and IL-1β (RayBiotech, Norcross, GA, USA) following the manufacturer's instructions.

Lung histology

Lung histology was performed on the left lung after filling with formaldehyde 4% at a hydrostatic pressure of 20 cm H_2O. Three blocks (apical, middle, and basal lobe regions) were then excised and fixed before embedding in paraffin. Thick lung tissue sections (10 μm) were stained with hematoxylin and eosin, and histological assessment was performed by an expert technician who was blinded to the experimental group. In accordance with the American Thoracic Society guidelines,[33] a lung injury score was calculated taking into account neutrophil accumulation in the alveolar and interstitial spaces, the presence of hyaline membranes, proteinaceous debris filling the airspaces, and alveolar septal thickening in a weighted manner. The histology score was determined for each lung region separately (apical, middle, and basal), and a coefficient of variation [CV=standard deviation (SD)/mean] was derived as an indicator of the overall lung structural heterogeneity.

Study protocol

The study protocol is summarised in Fig. 1. After reaching a steady state respiratory and hemodynamic condition, a lung inflation manoeuvre (peak pressure of 25 cm H_2O) was performed to recruit the lungs and to standardise the volume history. The rabbits were then ventilated for the next 7 h, in accordance with their group allocations. In the PC group, inspiratory pressure over the PEEP (Ppi-PEEP) was adjusted in order to maintain $ETCO_2$ between 5.5 and 6 kPa. In the NAVA and PVV groups, NAVA level (cm H_2O/Edi) was adapted to obtain $ETCO_2$ between 5.5 and 6 kPa. Recordings of the Ppi-PEEP gradient and forced oscillatory measurements were performed hourly, while arterial blood gas, mean arterial pressure (MAP) and heart rate (HR) were evaluated every 3.5 h. At the end of the experimental protocol, BALF and histology samples were collected from the right lung, while the left lung was used to assess the W/D.

Statistical analyses

The parameters were expressed as mean (95% confidence intervals) values, except for non-normally distributed variables where inter-quartile ranges and 10th–90th percentiles are reported. A sample size estimation was performed with adjustments for multiple comparisons considering a 20% change within a group as clinically significant, and taking into account the mean values and the variabilities of the primary outcome (H), as established in our previous experiments in rabbits ventilated with pressure regulated volume controlled (PRVC) mode: 319 (32) cm H_2O $litre^{-1}$.[34] This sample size estimation revealed that nine rabbits per group were required at a *P* level of 0.05 with 85% power. The Shapiro–Wilk test was used to test data for normality. The absolute values of respiratory mechanics, blood gas analysis, and pulmonary hemodynamic parameters were normally distributed. These variables were evaluated by using two-way analysis of variance (ANOVA) repeated measures with a general linear model, with group allocation as independent factor and time as within-subject factor. The interaction between these factors was also incorporated in the analyses (i.e. group-by-time level). As the changes in the parameter values relative to the initial measurement condition (T0) were the centre of interest, the repeated comparisons were performed between T0 and those obtained at each following time point (T1–T7). Considering that the relative changes were not always normally distributed, one-way ANOVA on ranks were applied for these indices with pairwise Dunnett's multiple comparison procedures as a *post hoc* analysis. Changes in the parameters at T7 relative to T0, W/D data, BALF cellular profile, and histological outcomes were evaluated by using one-way non-parametric ANOVA tests with Dunnett's multiple comparison procedure. The statistical analyses were conducted using SigmaPlot (version 12.5, Systat Software, Inc. Chicago, IL, USA) and a *P* value of <0.05 was taken as significant.

Results

There was no statistically significant difference in body weight between the protocol groups (Supplementary Table S1).

Figure 2 depicts the changes in the pressure gradient (Ppi-PEEP) and airway and respiratory tissue mechanical parameters during the 7-h ventilation period. Statistically significant interactions were observed between group allocations and time for both respiratory tissue mechanical parameters G and H ($P<0.001$), indicating that the ventilation mode significantly affected their temporal changes. These temporal alterations were reflected in the relative changes of G and H, with reduced changes in G in Groups PCS ($P<0.001$) and NAVA ($P<0.02$), and in H in Groups PCS ($P<0.001$), PVV ($P<0.03$), and NAVA ($P<0.001$). The group-by-time interaction was not significant for Raw, demonstrating no effect of the ventilation mode on changes in airway mechanics.

The gas exchange parameters obtained in the protocol groups at three time points of the protocol are summarised in Supplementary Table S2. Blood gas indices remained in the normal range throughout the protocol in all groups, with only mild but statistically significant decreases in pH in PC, PCS, and NAVA groups ($P<0.05$ for all). $ETCO_2$ concentration and the difference in end-tidal and arterial CO_2 concentration remained in the normal range during the entire protocol with no difference within time or between groups. Haemoglobin concentration decreased in all groups ($P<0.05$), but there was no significant difference between groups.

Systemic hemodynamic indices during the 7-h ventilation period are shown in Fig. 3 and there were no differences in MAP or HR within or between groups.

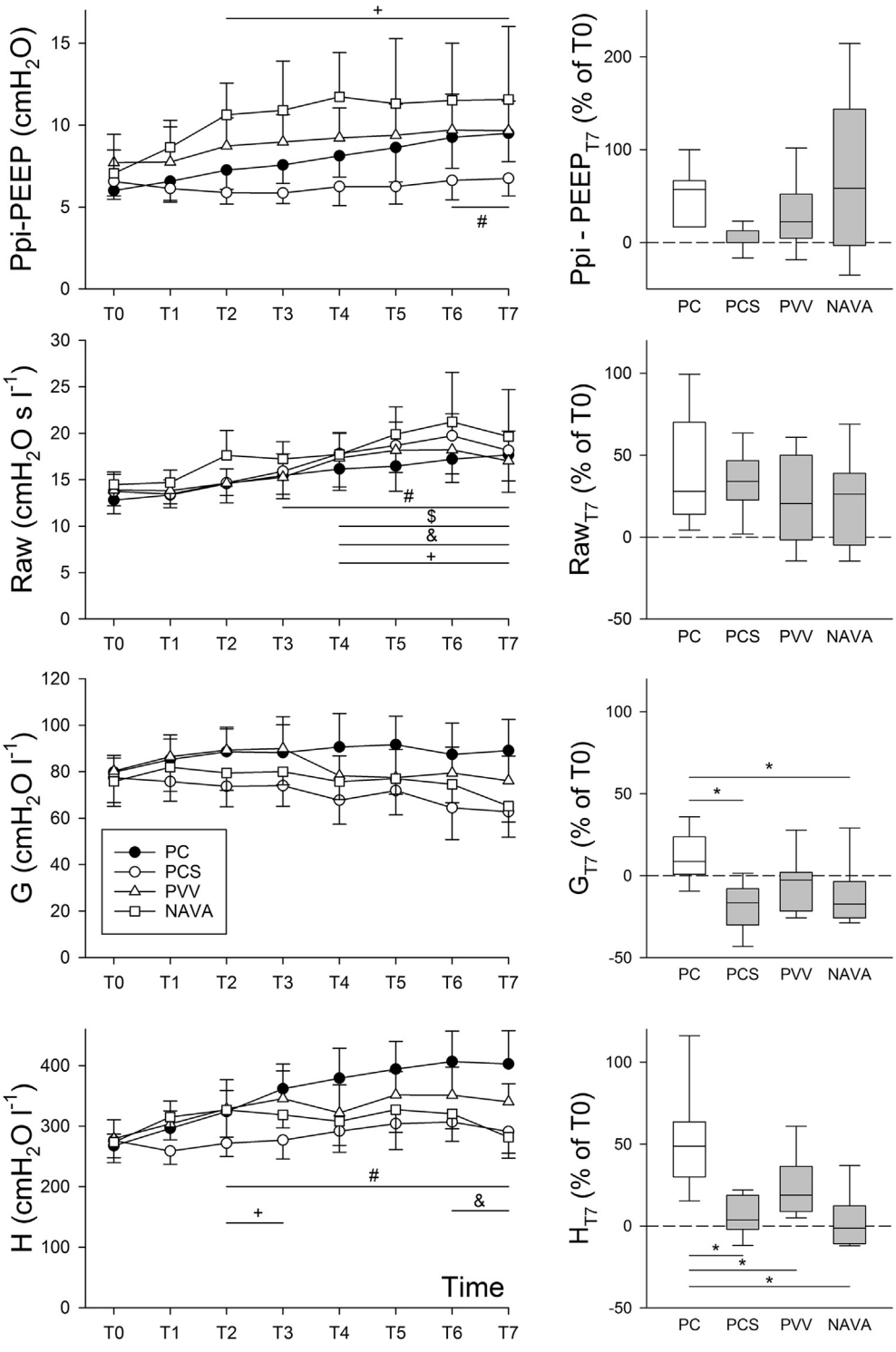

Fig 2. Absolute values of the pressure gradient (Ppi-PEEP), airway, and respiratory tissue mechanical parameters during the 7-h ventilation period (left) as mean and 95% confidence interval (CI), and their relative changes after 7 h of mechanical ventilation (right) as median, inter-quartile range, and 10th and 90th percentiles. Rabbits were ventilated with a pressure-controlled mode with (Groups PCS), or without (Groups PC) regular sighs, or received variable ventilation based either on the pre-recorded respiratory activity during spontaneous breathing (Group PVV), or the diaphragmatic activity recorded by a neurally adjusted ventilation assist (NAVA) catheter (Group NAVA). #$P<0.05$ in Group PC, \$$P<0.05$ in Group PCS, &$P<0.05$ in Group PVV and +$P<0.05$ in Group NAVA. *$P<0.05$ in the relative changes. G, damping; H, elastance; PVV, physiologically variable ventilation; Raw, airway resistance.

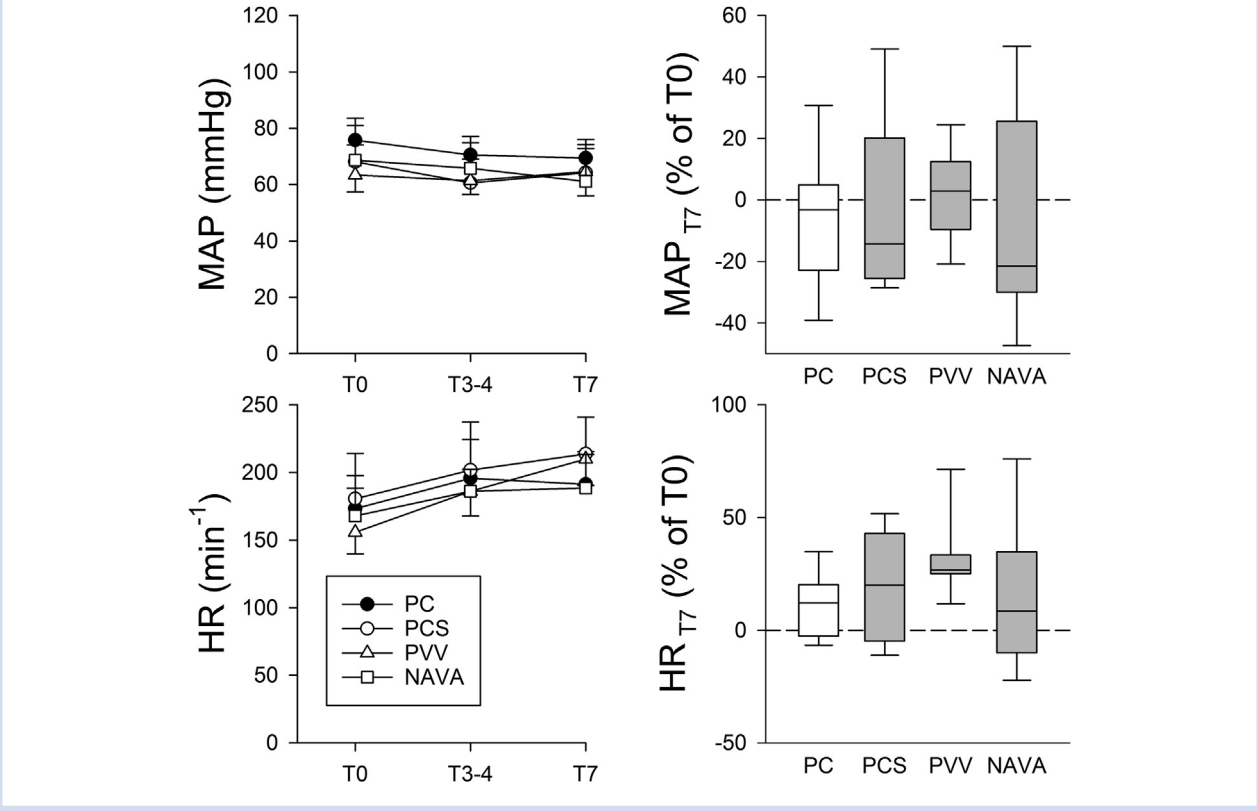

Fig 3. Absolute values of the systemic haemodynamic parameters (mean airway pressure: MAP, heart rate: HR) during the 7-h ventilation period (left) as mean and 95% confidence interval (CI), and their relative changes after 7 h of mechanical ventilation (right) as median, inter-quartile range, and 10th and 90th percentiles. Rabbits were ventilated with a pressure-controlled mode with (Groups PCS), or without (Groups PC) regular sighs or received variable ventilation based either on the pre-recorded respiratory activity during spontaneous breathing (Group PVV), or the diaphragmatic activity recorded by a neurally adjusted ventilation assist (NAVA) catheter (Group NAVA).

Figure 4 summarises the histological findings. Histology scores were significantly lower in Groups PVV ($P<0.05$) and NAVA ($P<0.05$), mainly attributed to the presence of differences at the medium lung level ($P<0.02$ for both PVV and NAVA groups). The CV of the histology scores was significantly lower for Group PVV than that obtained for Group PC ($P<0.01$).

Analyses of BALF revealed difference in the total cell count between the groups with counts of 457 (80), 434 (79), 382 (69), and 420 (71) cells μl^{-1} for Groups PC, PCS, PVV, and NAVA, respectively. Additionally, there was no difference in % macrophage, neutrophil, eosinophil, or lymphocyte cell counts: 60 (5.1)%, 37 (5)%, and 2.7 (0.2)%, respectively. BALF cytokine levels are reported in Supplementary Table S3, and there was no difference in TNF-α, IL-8, or IL-1β concentrations between the different ventilation modes.

The W/D values were the highest in rabbits of the PC Group [6.2 (0.3)], while significantly lower values were obtained for the PCS and PVV Groups [5.5 (0.2) and (5.6 (0.1), respectively, both $P<0.05$]. In the NAVA group the W/D ratio was 5.8 (0.1), $P=0.13$.

Discussion

Controlling mechanical ventilation by applying individualised long-term variable ventilation to healthy lungs based on a pre-recorded pattern, revealed similar beneficial effects on lung structure and function as compared with the NAVA-assisted ventilatory mode. Moreover, these PVV modes (PVV, NAVA), proved to be superior to conventional monotonous pressure-controlled ventilation in that there was less deterioration in respiratory tissue mechanics and a reduced level of lung injury from histological analysis.

Prolonged mechanical ventilation of the lung has can induce VILI even in previously healthy lungs,[35] and the repeated monotonous closure and opening of terminal airspaces leading to shear stress is thought to be one of its triggers.[2,3] We applied a protective monotonous ventilation mode as a reference and compared two PVV modalities in an attempt to further characterise the value of such a strategy in a setting relevant to prolonged ventilation under general anaesthesia or deep sedation. Considering that NAVA requires triggering of assisted positive pressure ventilation, the development of a ventilatory mode at least as protective against VILI in subjects with suppressed respiratory drive, is of major importance. The results of the present study demonstrate that applying a controlled PVV based on the subject's own pre-recorded respiratory pattern in an untriggered mode, exhibits benefits on respiratory function over those obtained with a neurally-triggered assisted mode.

The novelty in this study was the application of a pre-recorded spontaneous breathing pattern to ventilate the lungs in a controlled fashion. The resultant applied ventilation in the PVV group seemed to afford similar protection in reducing deterioration as that observed in the NAVA group. These results

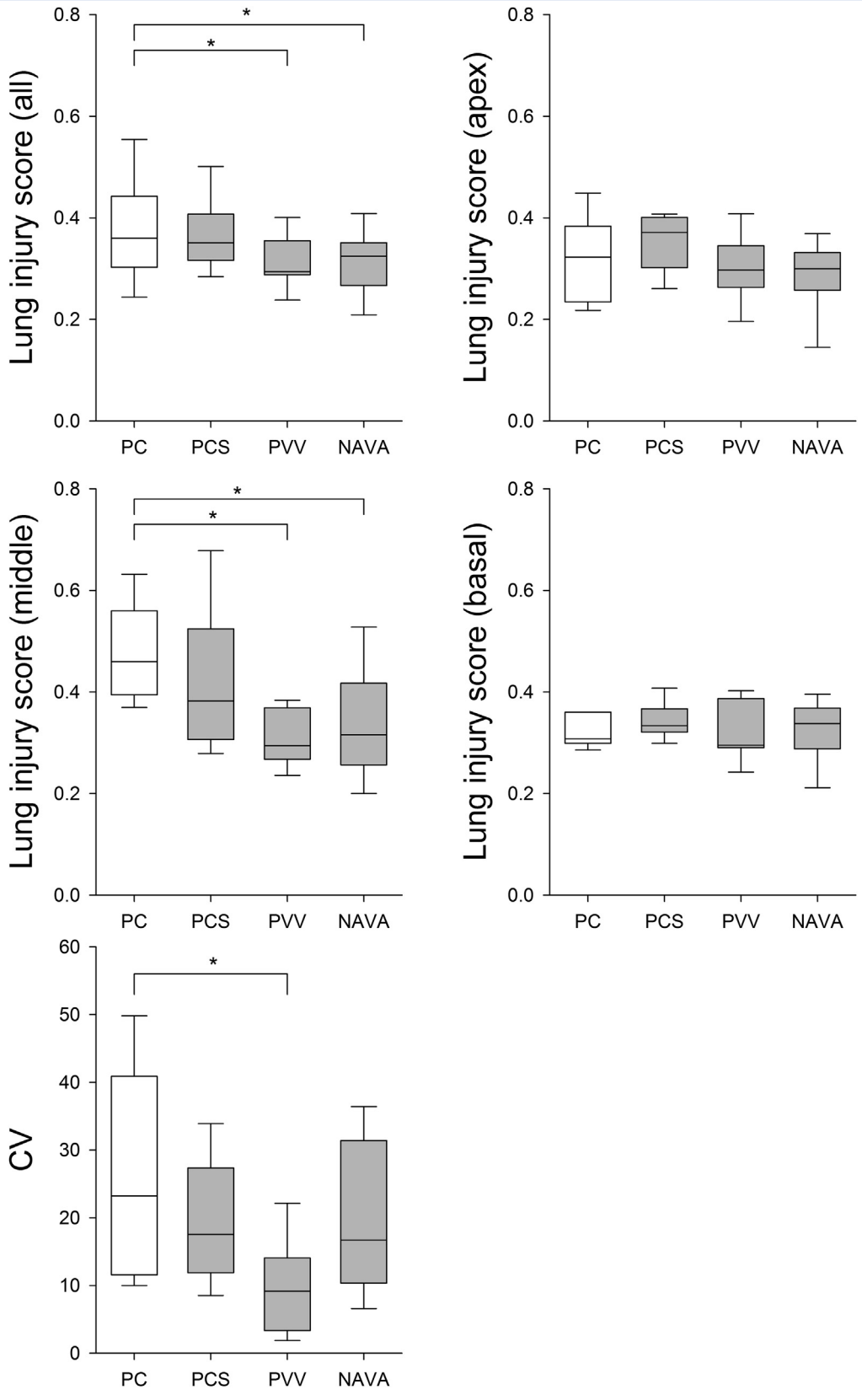

Fig 4. Lung injury score determined for the whole lung (top left) and for each of the lung regions separately (top right: apical level; middle right: middle level; middle left: basal level). The coefficient of variation (CV=SD/mean: bottom left) indicates overall lung structural heterogeneity. Data are presented as median, inter-quartile range, and 10th and 90th percentiles. *$P<0.05$ *vs* Group PC.

obtained from using a pre-recorded physiologically variable breathing pattern are in agreement with those obtained by applying a mathematical algorithm in healthy[13,16,20] and injured lungs.[12,14,15,17,21,23–25] It is noteworthy that, in the present study, the benefit was apparent even in comparison with a conventional controlled protective ventilation mode set to a relatively small tidal volume and PEEP (Group PC). However, this positive effect on respiratory tissue mechanics could also be found in the group where regular recruitment manoeuvers were applied (Group PCS). Hence, regular sighs or controlled/assisted variable ventilation ensure a similar degree of alveolar recruitment during prolonged mechanical ventilation. Gas exchange parameters further confirmed sustained alveolar recruitment throughout the experiment. It is of note that these beneficial effects were achieved by performing a regular sigh every 30 min. There is a lack of consensus in the literature about the frequency of sighs in healthy anaesthetised individuals with rates ranging from two to six sighs per hour.[36–38] We chose a clinically meaningful rate that facilitates alveolar recruitment in a healthy lung without inducing lung injury subsequent to frequent lung over-distension and subsequent cytokine liberation.

One of the main findings of the present study is that efficient recruitment was associated with marked differences in lung oedema development and structural changes in the alveoli. These findings are in agreement with recent reports where variations in tidal volume were applied during volume-controlled ventilation,[25] or when the lungs were ventilated with a synchronised and assisted modality, the NAVA.[26] Regular sighs in the PCS group were able to ensure alveolar recruitment. However, regular application of sighs, at a relatively moderate level (20 cm H_2O) led to comparable degree of lung injury as observed with a conventional pressure-controlled mode (Fig. 4). Accordingly, our findings suggest a lack of protection against lung injury in the PCS group.

Histological characterisation revealed spatial heterogeneities in the development of lung damage. It is of note that the PVV group had the most homogenous alveolar structure by the end of the 7-h ventilation period, as expressed by the lowest CV (Fig. 4). This suggests that variable ventilation based on a pre-recorded breathing pattern is a promising ventilation modality that provides adequate gas exchange, whilst preventing deteriorations in alveolar structure and respiratory function with time.

To further characterise the severity of lung injury, we measured key inflammatory cytokines in the BALF.[39,40] Previous studies report conflicting results regarding the effect of variable ventilation on cytokines, with some studies demonstrating a benefit[12,15,24] and others finding no difference.[17,19,26] This may be attributed to variability in cytokine concentrations,[41] which precludes a clear conclusion. In line with earlier observations,[17,19,26] we did not find any effect on the most relevant inflammatory cytokines in BALF, which may reflect that the present study was not powered to detect any differences.

Out study has limitations. In order to compare the protocol groups, we aimed at maintaining physiological homeostasis. As lung compliance gradually decreased in Group PC, the pressure gradient was adjusted to fulfil this criterion (Fig. 2). It is unlikely that this adjustment may have resulted in over-distension of lung tissue and increased shear stress, as the tidal volume was maintained at 7 ml kg^{-1} and $ETCO_2$-$PaCO_2$ remained constant. Further, to maintain haemodynamic stability during prolonged i.v. anaesthesia, without the administration of vasoactive inotropic agents, animals received continuous i.v. crystalloid infusion. This resulted in haemodilution, as seen by the decreased haemoglobin concentrations in all groups. However, it is unlikely that the fluid infusion may have affected respiratory function, as the partial pressure of oxygen remained stable in all groups and the W/D ratio was elevated only in Group PC. A further factor that warrants consideration is the potential effect of premedication on the spontaneous breathing pattern. However, based on previous data obtained in spontaneously breathing rats,[42,43] even much higher doses of xylazine (80–100 mg kg^{-1}) than we used (5 mg kg^{-1}), had no effects on augmented breaths (equivalent to sighs). Thus, it can be implied that premedication was unlikely to affect breathing variability.

In summary, conventional pressure-controlled ventilation was compared with two modes of PVV to assess their value in protecting against VILI. A variable ventilation pattern within an individual animal was based on either a pre-recorded physiological breathing signal, or established from the electric activity of the diaphragm. These ventilation modalities exhibited comparable protective effects on respiratory function as applying regular sighs during pressure-controlled ventilation. However, the application of an individualised pre-recorded breathing pattern maintained a more uniform alveolar structure with less oedema development. Of note, a recent clinical trial found no improvement in postoperative respiratory function with controlled variable ventilation compared with non-variable ventilation during general anaesthesia for abdominal surgery.[44] These findings suggest that physiological variable ventilation extrapolated from the spontaneous breathing pattern provides optimal gas exchange with minimal deleterious effects on the lung. Hence, these results may stimulate further investigations towards a more personalised protective mechanical ventilation.

Authors' contributions

Study design, data collection and analysis, manuscript drafting: M.W.
Study design, data analysis and interpretation, manuscript drafting: F.P., S.B.
Study design, data collection, and data analysis: G.A.
Data collection, cytology and histology analysis, manuscript drafting: A.B.
Study design, data interpretation, manuscript drafting: W.H.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bja.2018.01.020.

Declaration of interest

Engineering support was provided by Getinge AB (Solna, Sweden) for developing the ventilation pattern based on the pre-recorded breathing signal.

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