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Effects of variable versus nonvariable controlled mechanical ventilation on pulmonary inflammation in experimental acute respiratory distress syndrome in pigs

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Abstract

Background: Mechanical ventilation with variable tidal volumes (V_T) may improve lung function and reduce ventilator-induced lung injury in experimental acute respiratory distress syndrome (ARDS). However, previous investigations were limited to less than 6 h, and control groups did not follow clinical standards. We hypothesised that 24 h of mechanical ventilation with variable V_T reduces pulmonary inflammation (as reflected by neutrophil infiltration), compared with standard protective, nonvariable ventilation.

Methods: Experimental ARDS was induced in 14 anaesthetised pigs with saline lung lavage followed by injurious mechanical ventilation. Pigs (n=7 per group) were randomly assigned to using variable V_T or nonvariable V_T modes of mechanical ventilation for 24 h. In both groups, ventilator settings including positive end-expiratory pressure and oxygen inspiratory fraction were adjusted according to the ARDS Network protocol. Pulmonary inflammation (primary endpoint) and perfusion were assessed by positron emission tomography using 2-deoxy-2-[18F]fluoro-D-glucose and 68Gallium (68Ga)-labelled microspheres, respectively. Gas exchange, respiratory mechanics, and haemodynamics were quantified. Lung aeration was determined using CT.

Results: The specific global uptake rate of 18F-FDG increased to a similar extent regardless of mode of mechanical ventilation (median uptake for variable V_T=0.016 min⁻¹ [inter-quartile range, 0.012–0.029] compared with median uptake for nonvariable V_T=0.037 min⁻¹ [0.008–0.053]; P=0.406). Gas exchange, respiratory mechanics, haemodynamics, and lung aeration and perfusion were similar in both variable and nonvariable V_T ventilatory modes.

Conclusion: In a porcine model of ARDS, 24 h of mechanical ventilation with variable V_T did not attenuate pulmonary inflammation compared with standard protective mechanical ventilation with nonvariable V_T.
Methods

The study protocol was approved by the Institutional Animal Care and Welfare Committee and the Government of the State of Saxony, Germany (AZ 24-9168.11-1/2013-53). 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 Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Animals were kept in an environment with controlled temperature (23°C) and light–dark cycles, with free access to water and food.

Animal preparation

Fourteen female pigs (German landrace, weighing 29.5–40 kg; Danish Specific Pathogen Free Certification; www.spf.dk) were pre-medicated with midazolam (1 mg kg⁻¹ i.m.) and ketamine (10 mg kg⁻¹ i.m.); intravenous anaesthesia was induced and maintained with midazolam (bolus 0.5–1 mg kg⁻¹, followed by 1–2 mg kg⁻¹ h⁻¹) and ketamine (bolus 3–4 mg kg⁻¹, followed by 10–18 mg kg⁻¹ h⁻¹). Neuromuscular block was achieved with atracurium (bolus 3–4 mg kg⁻¹, followed by 1–2 mg kg⁻¹ h⁻¹). Adequacy of anaesthesia was assessed continuously by lack of spontaneous movements, absence of reaction to painful stimulation between the front hooves, and absence of cardiovascular signs of sympathetic stimulation (increases in heart rate or arterial blood pressure). The trachea of the animals was intubated transorally with a cuffed single-lumen tracheal tube (8.0 mm internal diameter; Mallinckrodt, Athlone, Ireland), and lungs were ventilated with an EVITA XL (Dräger Medical AG, Lübeck, Germany) ventilator. All skin incisions were preceded by infiltration of 2–5 ml lidocaine 2%. After surgical preparation of the right internal carotid artery, an indwelling catheter was inserted and the mean arterial pressure continuously monitored. A 7.5 Fr pulmonary artery catheter (Opticath; Abbott, Abbott Park, IL, USA) was advanced through an 8.5 Fr sheath, placed in the right external jugular vein until typical pulmonary arterial pressure waveforms could be observed. Urine was collected with a bladder catheter inserted through a median mini-laparotomy. Parameters of gas exchange, respiratory signals, and haemodynamics were recorded before ARDS induction (time point BASELINE1).

Experimental ARDS

Lung injury was induced with a double hit model consisting of isotonic saline lung lavage alternating prone and supine position (four times each), followed by injurious ventilation with VT of 20 ml kg⁻¹ and zero PEEP until the PaO₂/FIO₂ is <13.3 kPa for at least 30 minutes. Measurements were performed immediately thereafter (injury). PET/CT imaging data were acquired (Day 1) and measurements performed (Baseline2). Thereafter, animals were randomly assigned to mechanical ventilation with: (i) variable VT (VV) or (ii) nonvariable VT (NV). In both groups, mechanical ventilation settings followed the recommendations of the ARDS Network protocol using the low...
FIO2/PEEP table. Measurements were performed in intervals of 6 h (Time1 to Time4). An illustration of the experiment’s time course is available in the Supplementary material. Intravascular volume was maintained with a crystalloid solution (E153; Serumwerk Bernburg AG, Bernburg, Germany) at a rate of 4 ml kg⁻¹ h⁻¹. Norepinephrine was used to maintain a mean arterial pressure of at least 60 mm Hg throughout the experiments. After 24 h, PET/CT imaging was repeated (Day 2) and animals were killed with an intravenous injection of thiopental (2 g), followed by potassium chloride (1 M, 50 ml). Postmortem lung tissue samples were obtained for histological and molecular biology analyses.

**Ventilator settings**

Until randomisation, animals were ventilated in volume-controlled mode with VT=6 ml kg⁻¹, FIO2=1.0, inspiratory/expiratory (I/E) ratio=1:1, PEEP=10 cm H₂O, constant inspiratory flow of 35 L min⁻¹, and ventilatory frequency (VF) adjusted to achieve an arterial pH between 7.38 and 7.42. During initial PET/CT, PEEP was set to 16 cm H₂O to standardise imaging conditions before randomisation. After randomisation, VF was set to reach a pH of >7.3, the lowest tolerable pH being 7.15 with a maximum VF of 35 breaths min⁻¹; otherwise, an increase of VT was allowed. Combinations of FIO2 and PEEP were set according to the low-PEEP table of the ARDS Network.²

**Variable VT ventilation**

During variable ventilation, VT changed from breath to breath, based on a predefined sequence of 600 random VT values (mean VT=6 ml kg⁻¹, normal distribution) which looped infinitely. The sequence of 600 variable VT cycles achieved the same minute volume as nonvariable ventilation. The targeted variance of the VT values was 30%, which is roughly the variability in healthy spontaneously breathing subjects.⁵ The flow rate was 35 L min⁻¹, and active inspiratory time was adjusted at each cycle to achieve the target VT. Because the I/E ratio was fixed at 1:1, the inspiratory pause paused.

**Lung imaging**

A low-dose helical CT scan (Biograph16 Hirez PET/CT; Siemens, Knoxville, TN, USA) of the thorax was obtained at mean airway pressure holds, and used for attenuation correction of the subsequent PET scans (attenuation correction CT [ACCT]). ACCT scans were used for manual segmentation of the lungs, excluding major airways and vessels. Segmented ACCT scans were used to define ventral, midventral, central, mid-dorsal, and dorsal lung regions (region of interest [ROI]) of equal lung tissue mass,¹⁵ and to calculate gas fraction (F_GAS) from the linear relation between tissue attenuation expressed in Hounsfield units (HU) and lung density (F_GAS=HU [–1000]⁻¹).

**Primary outcome: pulmonary inflammation**

After the ACCT scan, a bolus of ¹⁸F-FDG (~200 MBq) was infused to assess the infiltration of activated neutrophils which have a higher glucose uptake.¹⁵ Sequential PET frames and a series of blood samples were acquired over a period of 75 min. Blood samples were spun down, and the plasma activity was measured in a gamma counter, cross-calibrated with the PET scanner. The field of view (cranio-caudal extension: 15 cm) of the dynamic PET scan was set above the diaphragmatic dome to reduce motion artifacts. The Patlak two-compartment model¹⁴ was used to calculate the ¹⁸F-FDG uptake rate (Ki). To account for differences in lung inflation and blood volume between ROI, animals and time points, Ki was normalised to lung tissue fraction (F_TISSUE), thus computing the specific Ki (KIS) as shown (equation (1)):

\[
K_{IS} = \frac{K_i}{F_{Tissue}} = \frac{K_i}{1 - F_{GAS} - F_{BLOOD}}
\]

where F_BLOOD is the fractional blood volume obtained from the three-compartment Sokoloff model.¹⁵ KIS was determined for each ROI of comparable lung mass (regional KIS) and for the whole lung (global KIS).

**Secondary outcomes**

**Lung perfusion**

After the acquisition of the residual ¹⁸F-FDG activity by a PET scan, ⁶⁸Ga-labelled (ITG Isotope Technologies Garching GmbH, Munich, Germany) microspheres (ROTOP Pharmaka AG, Dresden, Germany) were injected i.v. (~100 MBq) to quantify lung perfusion, as assessed by a static PET scan.¹⁶ Specific regional perfusion was determined for each ROI of comparable lung mass as count rate per ROI relative to that of the whole lung and multiplied by the cardiac output at the time point of the ⁶⁸Ga PET measurement.

**Lung aeration**

A static high-resolution helical CT scan of the thorax was acquired under a respiratory hold at mean airway pressure and muscle paralysis. In those high-resolution images, segmentation was performed in every fifth slice to define the lung contour. Segmented CT scans were used to differentiate hyper-aerated, normally aerated, poorly aerated, and nonaerated compartments, for which the respective mass relative to that of the whole lung was calculated.¹⁷,¹⁸

**Markers of lung injury and inflammation**

The wet/dry ratio of the right lung was measured. The diffuse alveolar damage score was evaluated by an expert blinded to group allocation. Gene expression and protein levels of markers associated with inflammation (interleukin [IL]-6 and IL-8), fibrosis (type III procollagen), and endothelial cell damage (vascular endothelial growth factor [VEGF] and intercellular adhesion molecule 1 [ICAM-1]) were analysed using quantitative real-time polymerase chain reaction (PCR) (iCycler IQ Real-Time PCR System; Bio-Rad Laboratories Inc., Hercules, CA, USA, and PerfeCta SYBR Green FastMix; Quanta Biosciences, Gaithersburg, MD, USA) and enzyme-linked immunosorbent assay (ELISA) (R&D Systems Europa, Abingdon, UK).

**Experimental protocol**

Gas exchange, respiratory signals (obtained from internal sensors of the ventilator), and haemodynamics were assessed at time points defined as Baseline1, Injury, Baseline2, and at 6
Table 1: Respiratory mechanics and gas exchange. Values are given as mean and standard deviation. Effects of Injury on variables were tested with paired t-test (Baseline vs Injury, \( P < 0.05 \)). Differences between and within groups (Group effect; Time \( \times \) Group effect) were tested with general linear model statistics with BASELINE2 as a covariate. Global statistical significance was accepted at \( P < 0.05 \).

| Variable | Group | Baseline1 | Injury | Baseline2 | Time1 | Time2 | Time3 | Time4 | Group effect | Time \( \times \) Group effect |
|----------|-------|-----------|--------|-----------|-------|-------|-------|-------|--------------|-----------------------------|
| \( V_T \) (ml kg\(^{-1}\)) | VV | 6.5 (0) | 6.5 (0.1) | 6.5 (0) | 6.2 (0.5) | 6.2 (0.5) | 6.2 (0.6) | 6.1 (0.5) | 0.931 | 0.20 |
| | NV | 6.6 (0.2) | 6.6 (0.2) | 6.9 (1.0) | 6.5 (0.3) | 6.4 (0.5) | 6.4 (0.5) | 6.5 (0.4) | 6.6 (0.2) | 0.20 |
| \( CV_V_T \) (%) | VV | 0.6 (0.1) | 0.6 (0.2) | 0.5 (0.2) | 28 (2.4) | 26.6 (6.8) | 27.6 (4.3) | 27.4 (4) | \( < 0.001 \) | 0.843 |
| | NV | 0.7 (0.3) | 0.6 (0.2) | 0.6 (0.4) | 0.5 (0.2) | 0.4 (0.1) | 0.5 (0.2) | 0.5 (0.1) | 0.522 | 0.162 |
| \( VF \) (min\(^{-1}\)) | VV | 33.6 (2.5) | 33.6 (2.5) | 35.1 (0.1) | 28.3 (7.2) | 27.5 (8.1) | 25.3 (8.6) | 26 (9.3) | 0.522 | 0.162 |
| | NV | 33.6 (2.5) | 33.6 (2.5) | 35.1 (0.0) | 32.9 (2.7) | 29.3 (6.1) | 27.9 (5.7) | 26.4 (5.6) | 0.333 | 0.349 |
| \( MV \) (L min\(^{-1}\)) | VV | 7.9 (0.6) | 7.9 (0.6) | 8.3 (0.7) | 6.2 (1.4) | 6.0 (1.6) | 5.4 (1.5) | 5.5 (1.5) | 0.333 | 0.349 |
| | NV | 7.6 (0.7) | 7.6 (0.6) | 8.2 (0.9) | 7.3 (0.6) | 6.4 (0.8) | 6.1 (1) | 6.1 (1) | 0.746 | 0.647 |
| \( ERS \) (cm H\(_2\)O L\(^{-1}\)) | VV | 24.1 (2.7) | 81.2 (7) | 69.2 (12) | 74.6 (22) | 74.1 (24) | 71.3 (23) | 70.1 (23) | 0.746 | 0.647 |
| | NV | 23.6 (4.3) | 67.7 (9.8) | 69.1 (8.8) | 79.3 (14) | 78 (14) | 74.6 (11) | 71 (10) | 0.479 | 0.101 |
| \( RRS \) (cm H\(_2\)O L\(^{-1}\) s) | VV | 7.3 (0.6) | 10.7 (2) | 7.4 (0.5) | 8.2 (0.6) | 9.1 (1.1) | 9.8 (2.1) | 10.7 (4.2) | 0.101 | 0.005 |
| | NV | 7.6 (1.1) | 10.1 (1.6) | 8.5 (1.9) | 7.9 (0.6) | 8.4 (2.1) | 9.4 (2.2) | 9.6 (2.4) | 0.479 | 0.101 |
| \( Ppeak \) (cm H\(_2\)O) | VV | 21 (0.7) | 34.4 (2.4) | 27.6 (4.2) | 27.1 (5.2) | 27.6 (6) | 26.8 (5) | 27.2 (4.7) | 0.713 | 0.152 |
| | NV | 20.9 (0.7) | 31.1 (2.6) | 30.5 (3.6) | 29.1 (3.9) | 28.1 (2.3) | 28 (2.5) | 26.6 (2.4) | 0.608 | 0.188 |
| | VV | 17.4 (0.7) | 30.8 (2.3) | 25.6 (4.3) | 24.6 (5.2) | 24.7 (7) | 23.5 (6.1) | 23.4 (6.2) | 0.479 | 0.101 |
| | NV | 17.3 (0.6) | 27 (2.3) | 27.8 (4.8) | 27.1 (4.2) | 25.7 (3.4) | 25.2 (3.1) | 23.6 (3) | 0.479 | 0.101 |
| | VV | 14 (0.2) | 19.2 (0.8) | 15.5 (3.2) | 14.2 (2.7) | 14.4 (3.5) | 13.8 (3.1) | 14 (3.2) | 0.479 | 0.101 |
| | NV | 14 (0.3) | 17.9 (0.9) | 17.3 (1.1) | 15.9 (2.8) | 15.3 (2.2) | 15.2 (2.1) | 13.9 (1.7) | 0.479 | 0.101 |
| | VV | 10 (0.0) | 9.8 (0.2) | 7.7 (2.9) | 6.2 (1.5) | 6.3 (2) | 5.8 (1.9) | 6.2 (2) | 0.519 | 0.088 |
| | NV | 10 (0.0) | 9.8 (0.2) | 9.2 (2.8) | 7.6 (2) | 6.9 (1.9) | 6.7 (1.9) | 5.6 (1.5) | 0.519 | 0.088 |
| \( PaO_2/FIO_2 \) (kPa) | VV | 80.1 (80.3) | 9.2 (2.1) | 27.1 (10.9) | 28.3 (10.5) | 29.3 (10.3) | 29.7 (9.9) | 30.4 (11.5) | 0.990 | 0.530 |
| | NV | 80 (8) | 8.5 (2) | 21.1 (5.6) | 22.4 (4.3) | 25.2 (5.9) | 26.4 (6.4) | 25.3 (5.6) | 0.349 | 0.483 |
| \( PaO_2 \) (kPa) | VV | 80.1 (80.3) | 9.2 (2.1) | 11.6 (1.7) | 11.1 (2.4) | 11 (2.1) | 11.1 (1.9) | 11.7 (2.4) | 0.349 | 0.483 |
| | NV | 80 (8) | 8.5 (2) | 10.8 (0.8) | 10.1 (1.5) | 9.9 (1.3) | 10.3 (1.4) | 9.9 (1.2) | 0.349 | 0.483 |
| \( PaCO_2 \) (kPa) | VV | 6.4 (0.8) | 11.9 (4.4) | 11.7 (2.4) | 11.2 (1.5) | 11 (1.5) | 10.9 (1.5) | 11.2 (1.4) | 0.237 | 0.587 |
| | NV | 6.8 (0.8) | 11.8 (3.7) | 11.9 (2.7) | 10.7 (2.0) | 10.7 (2.1) | 11.6 (2.6) | 11.2 (1.4) | 0.237 | 0.587 |
| pH | VV | 7.38 (0.04) | 7.23 (0.06) | 7.26 (0.08) | 7.30 (0.06) | 7.33 (0.06) | 7.32 (0.06) | 7.30 (0.07) | 0.188 | 0.401 |
| | NV | 7.36 (0.04) | 7.25 (0.11) | 7.22 (0.08) | 7.30 (0.05) | 7.32 (0.04) | 7.34 (0.05) | 7.35 (0.05) | 0.188 | 0.401 |
h intervals (Time1 to Time4). At the end of the experiments, the left and right lungs were removed for tissue analysis.

Statistical analysis
At the time of study planning, there was no literature available with suitable data for sample size estimation. Therefore, the number of animals per group was derived from the expertise of the group; thus, the analysis is exploratory in nature. Data are presented as median and 25% and 75% quartiles unless indicated otherwise. Student’s t-test, generalised linear model, Mann–Whitney U-tests, and Wilcoxon’s test were used as appropriate. Calculations were performed using the SPSS software package (SPSS version 22.0; IBM, Armonk, NY, USA); multiple comparisons were adjusted according to the Bonferroni–Holm procedure. Global statistical significance was accepted at \( P < 0.05 \).

Results
ARDS model
Respiratory variables were similar between each group, apart from the coefficient of variation of \( V_T \), which was close to 30% in the group with variable \( V_T \) ventilation (Table 1). Arterial pH was maintained at \( > 7.15 \) in both groups. All other parameters, including fluid therapy, anaesthetic requirements, and number of lavages were similar between groups (Supplementary material).

Primary outcome: pulmonary inflammation
Figure 1 shows the maps of the distribution of lung inflammation, aeration compartments, and perfusion of one representative animal of each group before randomisation at 24 h. At 24 h, global \( K_iS \) increased significantly in both groups, but values did not differ significantly between groups (VV: 0.016, 0.012–0.029 min\(^{-1}\); NV: 0.037, 0.008–0.053 min\(^{-1}\); \( P = 0.406 \)). Similarly, the increase in regional \( K_iS \) from before randomisation (Day 1) to 24 h after randomisation (Day 2) did not differ significantly between VV and NV in the investigated sub-regions. The same global and regional behaviour was observed when inflammation was assessed by non-normalised \( K_i \) measurements. Within each group, regional \( K_iS \) values were higher on Day 2 compared with Day 1 in all regions for group VV, and in ventral, mid-ventral, and mid-dorsal regions for group NV (Fig. 2).

Secondary outcomes
Lung aeration
The amount of normally aerated and hyper-aerated lung tissue decreased during the observation period in both groups.

![Fig 1. Transversal slices of inflammation, aeration, and perfusion of one representative animal per group before and 24 h after randomisation. Left column: transversal slice of the specific uptake rates of 2-deoxy-2-[\(^{18}\)F]fluoro-D-glucose (\( K_iS \)). \( K_iS \) was determined with positron emission tomography/CT and kinetic modelling according to the Patlak method. The resulting \( K_i \) values were normalised to the tissue fraction (\( K_iS = K_i / F_{TISSUE} = K_i / (1 – \text{gas fraction} – \text{blood fraction}) \); gas fraction determined from CT; blood fraction determined using the Sokoloff three-compartment model). Middle column: aeration compartments obtained from CT; hyper, hyper-aerated compartment; normally, normally aerated compartment; poorly, poorly aerated compartment; non, non-aerated compartment. Right column: distribution of perfusion obtained with \(^{68}\)Ga-labelled microspheres and positron emission tomography/CT. VV, variable ventilation; NV, nonvariable ventilation; Day 1, before randomisation; Day 2, 24 h after randomisation.](image-url)
The amount of nonaerated lung tissue increased in the NV group after 24 h, but not in the VV group. No significant differences were found between groups VV and NV (Fig. 3).

**Pulmonary perfusion**

The regional pulmonary perfusion increased in ventral and mid-ventral regions after 24 h in both groups. In the VV group, but not in the NV group, pulmonary perfusion decreased in the dorsal region after 24 h. No significant differences were found between groups VV and NV (Fig. 4).

**Histologic lung injury score, inflammatory markers, and wet/dry ratio**

The cumulative DAD score, the wet/dry ratio, and gene expression of IL-6 and IL-8, VEGF, ICAM-1, and type III procollagen did not differ significantly between groups (Table 2).

**Gas exchange and haemodynamics**

During the observation period, gas exchange and haemodynamics variables did not differ significantly between groups (Supplementary material).

**Discussion**

In this experimental model of ARDS in pigs, 24 h of mechanical ventilation according to the ARDS Network protocol with variable compared with nonvariable VT did not attenuate pulmonary inflammation. In addition, gas exchange, respiratory mechanics, lung aeration, pulmonary perfusion, histologic lung injury score, gene expression of inflammatory and endothelial damage markers and the wet/dry ratio did not differ between groups.

To our knowledge, this is the first study to address the effects of 24 h of variable ventilation on the distribution of lung inflammation. Of note, mechanical ventilation settings followed closely the clinical standard recommended by the ARDS Network. We used a double hit model consisting of saline lung lavage and injurious ventilation, as it reproduces several typical features of human ARDS, including alveolar haemorrhage, hyaline membrane formation, neutrophilic infiltration, decreased compliance, and gas-exchange deterioration, and results in a serious lung injury. A further strength of our study is that lung inflammation was determined with PET and the radiotracer 18F-FDG, which is an established method to assess lung inflammation in vivo. Our study also quantified the distribution of pulmonary perfusion in vivo using 68Ga-labelled microspheres and PET, which allows quantifying even minimal regional differences and assessed the distribution of aeration in lungs with a gold standard technique, namely CT.

Previous studies have reported conflicting results with respect to the effects of variable ventilation on lung damage and inflammation. In a porcine oleic acid model and a lavage-endotoxin-VILI model of ARDS in rabbits, variable ventilation with different PEEP levels did not reduce the level of pro-inflammatory cytokines (IL-8) and cell counts in bronchial alveolar lavage fluid and histological lung injury scores. In contrast, reduced lung damage has been observed in small animals ventilated with variable ventilation compared with conventional ventilation in hydrochloric acid injured and noninjured lungs. In pigs with saline lung lavaged lungs, the histologic damage, but not the level of cytokines, was reduced during variable ventilation compared with nonvariable ventilation when PEEP was ≥12 cm H₂O. In contrast, variable ventilation worsened epithelial cell damage when combined with higher PEEP levels, whereas we opted for the use of the ARDS Network low PEEP table, which represents current clinical practice.

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Fig 2. Regional specific uptake rates of 2-deoxy-2-[18F]fluoro-D-glucose ($K_i$) before and 24 h after randomisation. $K_i$ were determined by positron emission tomography/CT and kinetic modelling according to the Patlak method. Resulting $K_i$ values were normalised to tissue fraction ($K_i/F_{TISUE}=K_i/(1-\text{gas fraction}–\text{blood fraction})$; gas fraction was determined from CT; blood fraction determined using the Sokoloff three-compartment model). Symbols and horizontal lines represent the median and inter-quartile range. Global statistical significance was accepted at $P<0.05$, Bonferroni–Holm adjustment for multiple testing. Differences between Day 1 and Day 2 within the same region and group were tested with Wilcoxon test (depicted P-values). No differences were found between groups VV and NV (Mann–Whitney U-test). n=7 per group. VV, variable ventilation; NV, nonvariable ventilation. Day 1, before randomisation; Day 2, 24 h after randomisation.
The present observation that 24 h of variable ventilation did not improve gas exchange nor respiratory mechanics, differs from recent experimental and clinical reports. However, in line with the results of our study, variable ventilation compared with conventional ventilation did not improve gas exchange in oleic acid-induced ARDS in pigs and dogs. Furthermore, variable ventilation did not improve ERS in oleic acid injury in pigs. These results might be partly explained by differences in the severity of the lung damage. In fact, in saline lung lavage, variable ventilation resulted in lower mean and peak airway pressures, lower ERS, and higher arterial oxygenation values, whereas these beneficial effects could not be seen in the present double hit model of ARDS. Furthermore, PEEP values used in that study were higher (12 cm H₂O) than those used in clinical practice. These higher PEEP values might have favoured stabilisation of lungs after recruitment with variable ventilation. Also, the observation period was much shorter (6 h) than in the present study, and it cannot be ruled out that respiratory mechanics would have deteriorated after that period.

Our finding that variable ventilation did not increase lung aeration compared with nonvariable ventilation differs from similar studies in the field. In a previous study, variable ventilation resulted in a significant recruitment of nonaerated and poorly aerated lung volume and a significant increase of normally aerated lung volume in oleic acid-induced ARDS in pigs. Furthermore, redistribution of perfusion towards dependent lung zones was observed after saline lung lavage in pigs treated with variable ventilation, suggesting improved lung aeration in dorsal areas. In our study pulmonary perfusion was redistributed from dependent towards nondependent lungs, which is probably attributable to hypoxic pulmonary vasoconstriction. These differences in aeration and perfusion are likely explained by the use of higher PEEP values in other studies. Another possible explanation is that the time constant for derecruitment could have been lower in our study, leading to increased end-expiratory lung collapse. In fact, in a study with a computer model of lung recruitment, variable ventilation combined with low PEEP levels was not able to recruit lungs when the time constants of recruitment and derecruitment were the same.

Limitations

Our study has several limitations. First, the double hit model does not fully mimic all features of the human ARDS, even though this model has been considered clinically relevant. Second, the metabolic activity as indicated by 18F-FDG uptake was used as a surrogate of VILI, which involves not only metabolism, but also structural damage. Nevertheless, different studies showed that Kᵢs is a reliable marker of lung injury. Third, we set PEEP in both groups according to minimal adequate oxygenation; thus we did not evaluate the effects of variable ventilation in combination with alternative ways of PEEP titration aimed at stabilising lungs. However, in large clinical trials, the low PEEP table of the ARDS network has proved superior to other strategies that aim at an open lung with respect to clinical outcome. Fourth, we did not determine the pressure vs volume curve of the respiratory system. A previous study suggested that PEEP values according to the low PEEP table most likely centre the ventilation at the lower part of that curve. Thus, variable ventilation was likely conducted in a low compliance range, that is, was not optimised for respiratory mechanics. Fifth, we have targeted for a mathematically derived VT variance of 30% during VV. Although we cannot rule out that different levels or distributions of variance would have yielded different results, this value corresponds to the value of young healthy volunteers and led to most pronounced improvement in lung function in two experimental studies in rats and pigs. Sixth, the sample size was mainly derived from the expertise of the
authors. An increased sample size might have resulted in a statistically significant difference presumably not being clinically relevant.

**Conclusion**

In this model of ARDS in pigs, 24 h of ventilation according to the ARDS Network protocol with variable VT did not attenuate pulmonary neutrophilic inflammation compared with non-variable VT. These data reinforce the need to compare emerging ventilatory strategies with established gold-standard therapy.

**Authors’ contributions**

Acquisition of data: JW, MS, AB, RH, TB, MH, AG, LB, IR, MGA, TKi

Data processing: JW, MS, AB, RH, TB, MH, AG, LB, IR, MGA, TKi

Drafting of the manuscript: JW, MS, AB, RH, PRMR, PP, MGA, TKi

All authors contributed to the conception and/or design of this study, analysis and interpretation of data; critically revised the manuscript for important intellectual content; and approved the final version to be submitted.

**Acknowledgements**

We thank Susanne Henninger Abreu, and the research fellows of the Pulmonary Engineering Group, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany, for their assistance in conducting the experiments. We also thank Gabriele Kotzerke and Kathrin Rosenow, technical radiology assistants, and Liane Oehme, physicist, at the Department of Nuclear Medicine, University Hospital Dresden, Dresden, Germany, for their valuable support.

**Declarations of interest**

MGDeA and TK were granted a patent on the variable pressure support ventilation mode of assisted ventilation, which has been licensed to Dräger Medical AG (Lübeck, Germany). All other authors have disclosed that they do not have any conflicts of interest.

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**Table 2** DAD score, protein levels [pg mg⁻¹], and gene expression of pulmonary markers [relative mRNA expression, fold-change compared with the house-keeping-gen hypoxanthin-phosphoribosyl-transferase-1]. Values are given as median and quartiles. Statistical significance was accepted at P<0.05. Dad score diffuse alveolar damage score; IL-6, interleukin 6; ELISA, enzyme-linked immunosorbent assay, IL-8, interleukin 8; mRNA, messenger ribonucleic acid; VEGF, vascular endothelial growth factor; ICAM-1, intercellular adhesion molecule 1. VV, variable ventilation; NV, nonvariable ventilation; Day 1, before randomisation; Day 2, 24 h after randomisation.

| Group | DAD score | IL-6 ELISA | IL-8 ELISA | IL-6 mRNA | IL-8 mRNA | VEGF mRNA | ICAM-1 mRNA | Type III procollagen mRNA |
|-------|-----------|------------|------------|-----------|-----------|-----------|-------------|--------------------------|
| VV    | 15.1 (12.5) | 44.2 (38.6) | 109.3 (99.4) | 0.6 (0.5–1.1) | 0.8 (0.8–1.2) | 0.9 (0.9–1.2) | 1.0 (0.9–1.2) | 1.2 (1.0–1.3) |
|       | –17.1     | –49.3      | –136.3     |           |           |           |             |                          |
| NV    | 10.4 (8.0) | 47.0 (38.5) | 165.6 (111.4) | 0.7 (0.4–1.7) | 1.7 (1.1–4.0) | 1.1 (0.9–1.2) | 1.0 (0.9–1.4) | 1.1 (0.9–1.1) |
|       | –23.3     | –60.3      | –272.2     |           |           |           |             |                          |
| P-value | 0.565   | 0.482     | 0.085      | 0.655     | 0.110     | 0.749     | 0.565       | 0.142                    |

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**Fig 4.** Specific regional pulmonary perfusion in regions of equal lung mass from ventral to dorsal determined by positron emission tomography/CT and ⁶⁸Ga-labelled microspheres. Symbols and horizontal lines represent median and inter-quartile ranges. Global statistical significance was accepted at P<0.05, Bonferroni–Holm adjustment for multiple testing. Differences between Day 1 and Day 2 within the same group and same region were tested with Wilcoxon tests (depicted P-values). No differences were found between groups VV and NV (Mann–Whitney U-test). n=7 per group. VV, variable ventilation; NV, nonvariable ventilation; Day 1, before randomisation; Day 2, 24 h after randomisation.
Funding

German Research Foundation (Deutsche Forschungsgemeinschaft), Bonn, Germany (grant number GA 1256/6-2). The positron emission tomography/computed tomography device was a gift from the German Federal Ministry of Education and Research (BMBF contract 03ZIK42/OncoRay), Bonn, Germany. National Institutes of Health (NIH; Bethesda, MD, USA) (grant number R01 HL121228 to MFVM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2019.12.040.

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Handling editor: Gareth Ackland