Effects of Ultraprotective Ventilation, Extracorporeal Carbon Dioxide Removal, and Spontaneous Breathing on Lung Morphofunction and Inflammation in Experimental Severe Acute Respiratory Distress Syndrome

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ABSTRACT

Background: To investigate the role of ultraprotective mechanical ventilation (UP-MV) and extracorporeal carbon dioxide removal with and without spontaneous breathing (SB) to improve respiratory function and lung protection in experimental severe acute respiratory distress syndrome.

Methods: Severe acute respiratory distress syndrome was induced by saline lung lavage and mechanical ventilation (MV) with higher tidal volume (V_T) in 28 anesthetized pigs (32.8 to 52.5 kg). Animals (n = 7 per group) were randomly assigned to 6 h of MV (airway pressure release ventilation) with: (1) conventional P-MV with V_T ≈6 ml/kg (P-MV_{contr}); (2) UP-MV with V_T ≈3 ml/kg (UP-MV_{contr}); (3) UP-MV with V_T ≈3 ml/kg and SB (UP-MV_{spont}); and (4) UP-MV with V_T ≈3 ml/kg and pressure supported SB (UP-MV_{PS}). In UP-MV groups, extracorporeal carbon dioxide removal was used.

Results: The authors found that: (1) UP-MV_{contr} reduced diffuse alveolar damage score in dorsal lung zones (median[interquartile]) (12.0 [7.0 to 16.8] *vs.* 22.5 [13.8 to 40.8]), but worsened oxygenation and intrapulmonary shunt, compared to P-MV_{contr}; (2) UP-MV_{spont} and UP-MV_{PS} improved oxygenation and intrapulmonary shunt, and redistributed ventilation towards dorsal areas, as compared to UP-MV_{contr}; (3) compared to P-MV_{contr}, UP-MV_{contr} and UP-MV_{spont}, UP-MV_{PS} yielded higher levels of tumor necrosis factor-α (6.9 [6.5 to 10.1] *vs.* 2.8 [2.2 to 3.0], 3.6 [3.0 to 4.7] and 4.0 [2.8 to 4.4] pg/mg, respectively) and interleukin-8 (216.8 [113.5 to 343.5] *vs.* 59.8 [45.3 to 66.7], 37.6 [18.8 to 52.0], and 59.5 [36.1 to 79.7] pg/mg, respectively) in dorsal lung zones.

Conclusions: In this model of severe acute respiratory distress syndrome, MV with V_T ≈3 ml/kg and extracorporeal carbon dioxide removal without SB slightly reduced lung histologic damage, but not inflammation, as compared to MV with V_T = 4 to 6 ml/kg. During UP-MV, pressure supported SB increased lung inflammation. **(ANESTHESIOLOGY 2015; 122:631–46)**

PROTECTIVE mechanical ventilation (P-MV) with low tidal volume (V_T, 4 to 8 ml/kg of predicted body weight) and distending pressures (inspiratory plateau pressure) lesser than or equal to 30 cm H_2O reduces mechanical stress to lung tissue, decreasing lung inflammation, and improving survival in patients with the acute respiratory distress syndrome (ARDS).[1,2] However, even low V_T cannot avoid increased lung stress/strain, leading to ventilator-induced lung injury (VILI).[3] In fact, in a group of ARDS patients mechanically ventilated with V_T of 6 ml/kg, tidal hyperinflation could still be detected,[4] suggesting that P-MV in those patients would require even decreased V_T. However, carbon dioxide retention and respiratory acidosis may pose a limit to further reduction of V_T.

What We Already Know about This Topic

- Ultraprotective tidal volumes with extracorporeal carbon dioxide removal have been proposed to minimize ventilator-associated lung injury, as compared to conventional protective ventilation alone, but the impact of spontaneous breathing is not well defined

What This Article Tells Us That Is New

- In a model of severe acute respiratory distress syndrome in pigs, mechanical ventilation with 3 ml/kg tidal volume and extracorporeal carbon dioxide removal without spontaneous breathing slightly reduced lung histologic damage
- Spontaneous breathing during ultraprotective ventilation improved gas exchange and distribution of ventilation, but pressure support increased lung inflammation

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Extracorporeal carbon dioxide removal ($ECCO_2$-R) allows reduction of V_T beyond the threshold of 4 ml/kg (ultralow V_T), while keeping $Paco_2$ in a clinically acceptable range.[5,6] Nevertheless, reduced alveolar ventilation with ultralow V_T may favor alveolar collapse and further deterioration of oxygenation. Furthermore, most commercially available devices for $ECCO_2$-R provide adequate carbon dioxide elimination, but not oxygenation.[7]

To our knowledge, the role of ultra-P-MV (UP-MV) combined with $ECCO_2$-R in severe ARDS has not been definitely demonstrated. Furthermore, the effects of spontaneous breathing activity to counteract lung collapse and improve oxygenation during ultralow V_T ventilation have not been investigated.

Spontaneous breathing activity during mechanical ventilation may reverse alveolar collapse, redistribute ventilation and perfusion, and decrease cyclic collapse and reopening of alveoli, possibly leading to less VILI.[8] On the other hand, spontaneous breathing activity may also result in unpredictable inspiratory effort, increasing stress/strain, and worsening VILI.[9]

In this study, we evaluated the effects of UP-MV with and without spontaneous breathing activity on gas exchange, lung mechanics, hemodynamics, regional distribution of ventilation and perfusion, as well as on proinflammatory response, and histological damage in lungs, in a double-hit model of severe early ARDS in pigs. We hypothesized that during UP-MV combined with $ECCO_2$-R: (1) lung inflammation and damage are reduced compared to P-MV; (2) spontaneous breathing activity, whether supported by pressure or not, enhances oxygenation and further improves lung protection.

Materials and Methods

After approval by the governmental animal care committee (Landesdirektion Dresden, Dresden, Saxony, Germany), 28 pigs with mean body weight of 41.8 kg (32.8 to 52.5 kg, German landrace) were used for this study.

Anesthesia and Mechanical Ventilation

Animals were premedicated intramuscularly with 10 mg/kg ketamine (Ketamin-ratiopharm; Ratiopharm, Ulm, Germany) and 1 mg/kg midazolam (Midazolam; Ratiopharm), intubated with a cuffed 8.0-mm internal diameter endotracheal tube and mechanically ventilated (EVITA XL; Dräger Medical, Lübeck, Germany). Anesthesia was maintained by means of continuous intravenous infusion of midazolam (1 to 2 mg kg^{-1} h^{-1}) and ketamine (10 to 20 mg kg^{-1} h^{-1}). Muscle paralysis was achieved by continuous administration of atracurium (1 to 2 mg kg^{-1} h^{-1}). Animals were kept in the supine position during the whole experiment. Volume status was maintained with a continuous infusion of Ringer's acetate (RA—Ringer-Acetat-Lösung Bernburg; Serumwerk Bernburg AG, Bernburg, Germany) at 10 ml kg^{-1} h^{-1}.

Until induction of ARDS, animals were ventilated in volume-controlled mode with the following settings: fraction of inspired oxygen (Fio_2) = 1.0; V_T = 10 ml/kg; positive end-expiratory pressure (PEEP) = 5 cm H_2O; inspiratory to expiratory time ratio (I:E) = 1:1; the respiratory rate (RR) was adjusted to achieve a $Paco_2$ in the range of 35 to 45 mmHg.

Instrumentation and Measurement Devices

External jugular vein and internal carotid artery were cannulated with 8.5 French sheaths. The arterial line was used for continuous blood pressure measurements and blood sampling. A pulmonary artery catheter (Opticath; Abbott, Abbott Park, Chicago, IL) was advanced through the venous sheath into the pulmonary artery for continuous measurement of pulmonary arterial blood pressure, mixed venous blood sampling, and cardiac output measurements. The airflow signal was acquired from the internal flow sensor of the ventilator through a serial interface. The airway pressure (P_{aw}) was measured at the proximal end of the endotracheal tube with a T-piece connected to a differential pressure transducer (163PC01D48-PCB; Sensortechnics GmbH, Puchheim, Germany). Esophageal pressure (P_{es}) was measured with a balloon catheter (Erich Jaeger, Höchberg, Germany) that was advanced into the mid chest and connected to another differential pressure transducer (163PC01D48-PCB, Sensortechnics GmbH). For acquisition of airway flow, as well as airway and esophageal pressures, a LabVIEW-based data acquisition system (National Instruments, Austin, TX) was used, as described elsewhere.[10]

Blood Gas and Hemodynamics

Arterial and mixed venous blood samples were analyzed using a standard blood gas analyzer (ABL 505; Radiometer, Copenhagen, Denmark). Oxygen saturation and hemoglobin concentration were measured using an OSM 3 Hemoximeter (Radiometer) calibrated for swine blood. Heart rate, mean arterial blood pressure, central venous pressure, and mean pulmonary arterial pressures were measured using a standard monitor (IntelliVue Patient Monitor MP 50 Philips, Böblingen, Germany). Cardiac output was measured *via* the pulmonary artery catheter as the average of three repeated injections of 10 ml iced saline into the proximal lumen.

Respiratory Variables

Respiratory signals were acquired at a sample frequency of 200 Hz, using an A/D-card (NI USB-6210; National Instruments) connected to a laptop. Extraction of respiratory variables was performed offline from 45 min recordings of airflow, P_{aw}, and P_{es} at each time point. Transpulmonary pressure (P_L) tracings were computed as P_{aw} minus P_{es}, whereby peak and mean values were calculated cycle-by-cycle ($P_{L,mean}$ and $P_{L,peak}$, respectively) in all cycles (spontaneous, mixed, and mandatory). During controlled mechanical ventilation, the resistance and elastance of the respiratory

system (E_{rs} and R_{rs}, respectively) were calculated using the equation of motion, as shown in equation E1:

$$P_{aw}(t) = R_{rs} \cdot \dot{V}(t) + E_{rs} \cdot V(t) + P_0 \quad \text{(E1)}$$

with airway pressure P_{aw}, airway flow $\dot{V}$, volume V, time t, and the total airway pressure at end-expiration P_0.

Distribution of Ventilation

The distribution of ventilation was assessed using electric impedance tomography (EIT—EIT Evaluation Kit 2; Dräger Medical) as described elsewhere.[11] Shortly, a flexible belt equipped with 16 electrodes was mounted at the xiphoid level around the thorax to perform EIT. The output images were recorded at 20 frames/s, during 5 min. Impedance distribution was reconstructed offline using dedicated EIT software (Dräger EIT Data Review; Dräger Medical AG, Germany). Each frame consisted of 32 × 32 image values I (x, y), which were analyzed with a custom-made software as described elsewhere.[12]

Distribution of Perfusion

Regional pulmonary blood flow was marked with intravenously administered fluorescent, color-labeled microspheres as described in detail elsewhere.[13] A different color was administered at *Baseline 2* and *Time 6* to mark regional perfusion. Postmortem processing of lungs was performed as previously described.[13,14] Briefly, the left lung was flushed, air dried, coated with one-component polyurethane foam (BTI Befestigungstechnik, Ingelfingen, Germany), suspended vertically in a square box, and embedded in rapidly setting urethane foam (polyol and isocyanate; Elastogran, Lemförde, Germany). The foam block was cut into cubes and each cube was weighed and assigned a three-dimensional coordinate. The fluorescent dye was retrieved and read in a luminescence spectrophotometer (LS-50B; Perkin-Elmer, Beaconsfield, United Kingdom). The measured intensity of fluorescence in each probe was normalized according its own weight using equation E2:

$$\dot{Q}_{rel,i} = \left[x_i \Big/ \sum_{i=1}^{n} x_i \right] \Big/ W_i \quad \text{(E2)}$$

Where $\dot{Q}_{rel,i}$ is the weight-normalized relative pulmonary blood flow of the probe i; x_i is the obtained fluorescence probe i, W_i is the weight of the probe i, and n is the total number of probes. The distribution of pulmonary blood flow along the dorsal–ventral and caudal–cranial axes at each experimental condition was assessed by means of linear regression. Changes in the angular coefficients were used to characterize redistribution of perfusion along the respective axis.

Extracorporeal Carbon Dioxide Removal

In groups with ultraprotective ventilation, a 15 French and a 17 French catheter (Novalung; Heilbronn, Germany) were placed in the femoral artery and vein, respectively, and connected to an interventional lung assist device (ILA® Novalung) for $ECCO_2$-R. A mixture of oxygen and air was used as sweep gas, whereby the gas flow was titrated to $Paco_2$ = 50 to 70 mmHg. The oxygen fraction of the sweep gas was set to keep the partial pressure of oxygen in the blood flowing across the ILA® approximately constant, minimizing the membrane oxygenation effect.

Double-hit Lung Injury

Experimental ARDS was induced with a double-hit consisting of saline lung lavage and mechanical ventilation with high V_T. Saline lung lavage (first hit) was performed until Pao_2/Fio_2 was less than 200 mmHg for greater than or equal to 30 min. Following that, VILI (second hit) was performed with the following settings: driving pressure of 60 cm H_2O, PEEP = 0, RR = 10 per min, for 5 min. Lung injury was considered stable, when Pao_2 did not increase within 15 min.

Protocol of Measurements

The study was a prospective, randomized multiple arms study, evaluating the effects of four different ventilatory approaches, namely: (1) protective controlled MV according to the ARDS network (P-MV_{contr}); (2) controlled UP-MV (UP-MV_{contr}); (3) UP-MV with mandatory cycles and superposed unassisted spontaneous breathing (UP-MV_{spont}); and (4) continuous positive airway pressure combined with pressure supported (PS) spontaneous breathing (UP-MV_{PS}).

Figure 1 shows the time course of interventions. After instrumentation, baseline measurements were obtained (baseline 1), and experimental ARDS was induced. Following that, the ventilator settings of baseline 1 were resumed, a stabilization period of 15 min was maintained and measurements were performed (injury). P-MV was initiated in the airway pressure release ventilation mode with the following settings: inspiratory airway plateau pressure ($P_{aw,plat}$) targeted at V_T = 6 ml/kg, PEEP = 16 cm H_2O, I:E = 1:1, and RR ≤35 per min to pHa >7.30. V_T was reduced up to 4 ml/kg targeting at $P_{aw,plat}$ ≤30 cm H_2O. If RR was 35 per min and severe respiratory acidosis with pHa between 7.15 and 7.20 developed, V_T and $P_{aw,plat}$ were not further reduced. A stabilization period of 30 min was allowed and measurements taken (baseline 2 [BL2]). After BL2, a continuous infusion of heparin at a rate of 25 IU kg^{-1} h^{-1} including a loading dose of 80 IU/kg was started. Animals were then randomly assigned to one of the four modes of mechanical ventilation using sealed envelopes. In UP-MV_{contr}, UP-MV_{spont}, and UP-MV_{PS} groups, animals were instrumented and connected to the ILA® device. In P-MV_{contr}, a period of sham ventilation of 60 min was maintained to match the time needed for instrumentation and placement of the ILA® device in the other groups.

Ventilator settings in UP-MV_{contr}, UP-MV_{spont}, and UP-MV_{PS} groups were as follows: airway pressure release ventilation mode with driving pressure titrated to V_T ≈3 ml/kg,

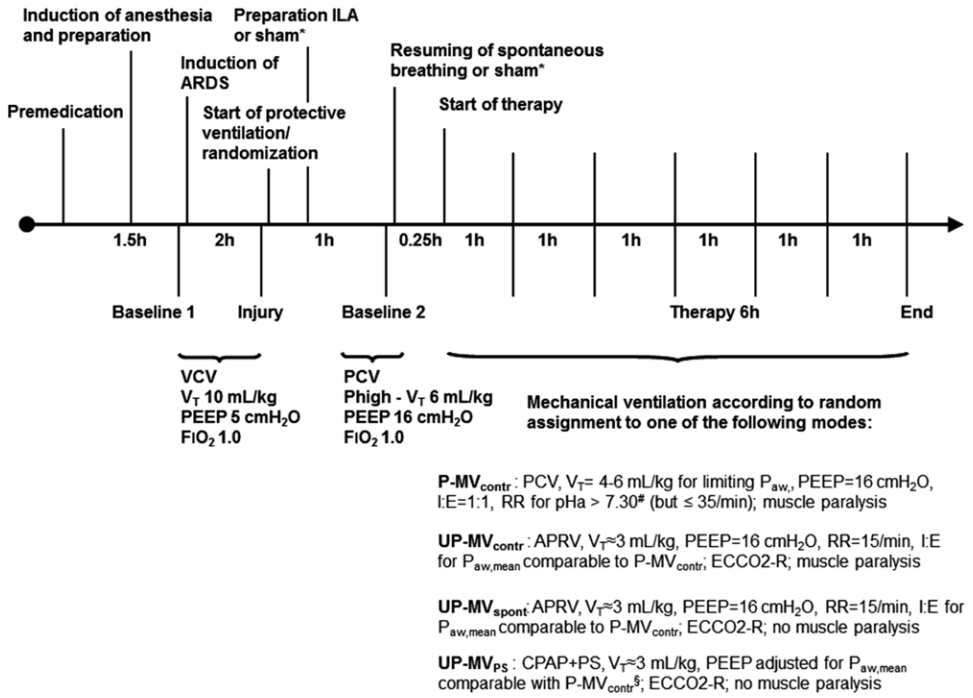

Fig. 1. Time course of interventions. *Period of sham ventilation in P-MV_{contr} as well as P-MV_{contr} and UP-MV_{contr} animals, respectively. #If RR was 35 per min and severe respiratory acidosis with pHa between 7.15 and 7.20 developed, P_{aw} was allowed to be increased above 30 cm H_2O to ensure sufficient alveolar ventilation. §In case inspiratory efforts disappeared, PEEP level was reduced until spontaneous breathing reoccurred. APRV = airway pressure release ventilation; CPAP+PS = continuous positive airway pressure + pressure support; $ECCO_2$-R = extracorporeal carbon dioxide removal; F_{IO_2} = inspiratory fraction of oxygen; I:E = ratio of inspiration to expiration; ILA = interventional lung assist; $P_{aw,mean}$ = mean airway pressure; PCV = pressure controlled ventilation; PEEP = positive end-expiratory pressure; pHa = arterial pH; P-MV_{contr} = controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; RR = respiratory rate; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing; VCV = volume controlled ventilation; V_T = tidal volume.

PEEP = 16 cm H_2O, I:E ratio titrated to a mean airway pressure ($P_{aw,mean}$) equivalent to P-MV_{contr}, and RR = 15 per min. RR was reduced in order to restraint the mechanical stress inflicted by cycling of the ventilator, that is, the stress rate, which has been shown to influence VILI.[15] In UP-MV_{spont} and UP-MV_{PS}, muscle paralysis was stopped and spontaneous breathing resumed. In P-MV_{contr} and UP-MV_{contr}, another period of sham ventilation of 30 min was allowed to match the time of resuming spontaneous breathing in the UP-MV_{spont} and UP-MV_{PS} groups. In UP-MV_{spont}, animals were able to breathe spontaneously throughout the whole respiratory cycle. In UP-MV_{PS}, as soon as signs of spontaneous breathing efforts were observed in P_{es} tracings, the ventilator was switched to continuous positive airway pressure with PS with following settings: continuous positive airway pressure equivalent to $P_{aw,mean}$ during P-MV_{contr}, PS adjusted to V_T ≈3ml/kg. F_{IO_2} was maintained at 1.0 in all groups throughout the whole experiment. During a period of 6 h, measurements of gas exchange, hemodynamics, respiratory variables, and distribution of ventilation were performed once every hour (Times 1 to 6).

Postmortem Analyses

At the end of the observation period, heparin was administered (1000 IU/kg iv) (Ratiopharm) and animals were killed by iv injection of 2 g thiopental (Inresa, Arzneimittel GmbH, Freiburg, Germany) and 50 ml KCl 1 M (Serumwerk; Bernburg, Germany). Lungs were removed under continuous positive airway pressure equal to the PEEP level for further processing. Samples from gravitationally dependent (dorsal) and nondependent (ventral) areas of the right lower lung lobe were snap-frozen in liquid nitrogen and stored at −80°C until further analysis.

For analysis of wet/dry ratio, the right middle lobe was weighted (wet weight) and dried afterward in a microwave as described elsewhere (dry weight).[16] The wet-to-dry ratio was then calculated. Between weighing procedures, bronchoalveolar lavage fluid of the right middle lobe was obtained from three repeated instillations (in-and-out) using 50 ml 0.9% saline solution. The material was centrifuged for 15 min with 200 gauge at 4°C and aliquots of the supernatant were obtained and kept frozen at −80°C until processing.

For histology, the right upper lobe of the lung was perfused with 4% buffered formaldehyde solution although

a continuous positive pressure equivalent to the PEEP value during the observation period was maintained at the airway. Lung tissue samples of approximately 8 cm^3 were taken from ventral and dorsal zones of the right upper lobe. After perfusion fixation and immersion in 4% buffered formaldehyde solution for 7 days, tissue samples were embedded in paraffin, cut in slices of 5 μm thickness, and stained with hematoxylin–eosin for further analysis. Photomicrographs at magnifications of ×25, ×100, and ×400 were obtained from four nonoverlapping fields of view per section using a light microscope. Diffuse alveolar damage (DAD) was quantified by one of the authors (M.K.), who is an expert anatomist and was blinded to therapy groups, using a weighted scoring system, as described elsewhere.[17] Briefly, values from 0 to 5 were used to represent the severity of seven features of DAD, that is, alveolar edema, interstitial edema, hemorrhage, inflammatory infiltration, epithelial destruction, microatelectasis, and overdistension, with 0 standing for no effect and 5 for maximum severity. Additionally, the extent of each feature characteristic per field of view was determined with values of 0 to 5, with 0 standing for no appearance and 5 for complete involvement. The cumulated DAD Score was calculated as the sum of product of severity and extent of all features, being situated in the range, 0 to 175.

Total RNA from lung was isolated with TRI reagent (Sigma–Aldrich GmbH, Deisenhof, Germany) according to the manufacturer's protocol, followed by purification with NucleoSpin RNA II columns (Macherey&Nagel, Düren, Germany). The complementary DNA was synthesized with the Revert AidTM H Minus First Strand Synthesis Kit (MBI Fermentas, St. Leon Roth, Germany) from 1 μg total RNA according to instructions of the fabricant. The messenger RNA expression of the inflammatory mediators and markers tumor necrosis factor-α, interleukin 6 and 8 (IL-6 and IL-8), amphiregulin and tenascin-c was quantified using quantitative real-time polymerase chain reaction (Maxima SYBR Green qPCR MasterMix:, Fermentas, St. Leon Roth, Germany) with the iCycler MyiQ2 real-time polymerase chain reaction system (BioRad; Munich, Germany), with cyclophilin A and β2-microglobulin as housekeeping genes. The total protein content in broncho-alveolar lavage fluid and lung tissue was measured using the BioRad Protein Assay (BioRad). Protein levels of tumor necrosis factor-α, IL-6, and IL-8 were measured in lung tissue using commercial ELISA kits (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. Myeloperoxidase activity in broncho-alveolar lavage fluid was measured using a spectrophotometric assay using 50 mM potassium phosphate (pH 6.0) containing 0.167 mg/ml *o*-dianisidine dihydrocholrid and 0.0005% hydrogen peroxide.

Statistical Analyses

The sample size calculation for testing the primary hypothesis (UP-MV combined with $ECCO_2$-R reduces cumulative DAD score) was based on effect estimates obtained from pilot studies. Accordingly, we expected a sample size of seven animals per group to provide the appropriate power ($1\text{-}\beta = 0.8$) to identify significant ($\alpha = 0.05$) differences in DAD Score, taking a mean difference of 15 ± 8, two-tailed test and multiple comparisons (n = 6) into account ($\alpha^* = 0.0083$, α^* Bonferroni adjusted).

Data are presented as mean ± SD, unless stated otherwise. For functional variables, comparability of groups at injury and BL2 was tested with one-way ANOVA followed by Bonferroni *post hoc* test. *P* values were adjusted for multiple comparisons according to Bonferroni. Differences among and within groups (time effect T1 to T6) were tested with general linear model statistics using BL2 as covariate, and adjusted for repeated measurements according to the Sidak procedure. To test DAD Score, we used a linear mixed model for repeated measures (compound symmetry, repeated covariance type), including field of view and region (ventral *vs.* dorsal zones) as repeated, independent variables, treatment as fixed, independent variable, as well as their significant interactions, to analyze differences in the dependent variable DAD score. Adjustments for repeated measures were performed according to the Tukey Kramer procedure. Residual plots were used to examine model requirements. Other comparisons were explorative in nature. Inflammatory mediators and markers of cell stress were analyzed using Kruskal–Wallis test followed by pairwise Mann–Whitney U test with *post hoc* adjustment according to Bonferroni–Holm procedure. Statistical analysis was performed using SPSS (v. 17.0, Chicago, IL) and SAS (v. 9.2, procedure mixed, SAS Institute, Cary, NC). Statistical significance was accepted at *P* value less than 0.05.

Results

Due to technical problems with the EIT device, values were obtained from 24 animals in total ($P\text{-}MV_{contr}$ 7, $UP\text{-}MV_{contr}$ 6, $UP\text{-}MV_{spon}$ 5, and $UP\text{-}MV_{PS}$ 6 animals, respectively). Further measurements were performed in all 28 animals (n = 7 per group). As depicted in table 1, $P\text{-}MV_{contr}$ resulted in average V_T ≈5 ml/kg, and $P_{aw,peak}$ ≈33 cm H_2O. During UP-MV, V_T and $P_{aw,peak}$ were further reduced to less than 4 ml/kg and less than 30 cm H_2O, respectively. $UP\text{-}MV_{spont}$ was associated with decreased $P_{aw,peak}$ compared to $UP\text{-}MV_{contr}$ and $UP\text{-}MV_{PS}$. $P_{aw,mean}$ was comparable between $P\text{-}MV_{contr}$ and $UP\text{-}MV_{contr}$, but higher than $UP\text{-}MV_{spont}$ and $UP\text{-}MV_{PS}$. During $UP\text{-}MV_{PS}$, $P_{aw,peak}$ remained fairly constant, indicating that adjustments of PS were not necessary. $P_{L,mean}$ did not differ significantly among groups, but $P_{L,peak}$ was decreased during $UP\text{-}MV_{spont}$ as compared to $P\text{-}MV_{contr}$. During $P\text{-}MV_{contr}$, RR and minute ventilation were higher than in other groups. E_{rs} and R_{rs} were comparable during $P\text{-}MV_{contr}$ and $UP\text{-}MV_{contr}$, and pressure–time product did not differ significantly between $UP\text{-}MV_{spont}$ and $UP\text{-}MV_{PS}$.

Table 1. Respiratory Variables

Parameter	Group	BL1	Injury	BL2	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Group Effect, *P* Value	*Post Hoc* Test, *P* Value
MV (l/min)	P-MV_{contr}	6.1±0.9	6.6±1.0	8.0±1.0	7.9±1.0	7.6±0.9	7.5±1.0	7.5±1.2	7.4±0.8	7.6±1.0	<0.001	
	UP-MV_{contr}	5.5±1.2	5.9±1.2	7.6±0.8	1.9±0.3	1.9±0.3	1.8±0.3	1.8±0.3	1.9±0.4	1.9±0.4		<0.001*
	UP-MV_{spon}	5.3±0.7	5.8±1.1	7.5±0.8	2.3±0.3	3.0±2.1	3.0±1.9	2.7±1.4	2.4±0.5	2.2±0.3		<0.001*, 0.363†
	UP-MV_{PS}	5.8±1.1	6.7±0.8	8.3±1.1	2.8±1.3	3.2±1.7	2.5±0.9	2.3±0.7	2.3±0.5	1.9±0.3		<0.001*, 0.570†, 1.000‡
			P = 0.260	*P = 0.293*								
V_T/kg (ml/kg)	P-MV_{contr}	9.8±0.1	10.7±0.2	5.4±0.4	5.3±0.4	5.2±0.4	5.1±0.5	5.1±0.6	5.1±0.5	5.2±0.5	<0.001	
	UP-MV_{contr}	10.0±0.1	10.7±0.6	5.5±0.4	3.1±0.2	3.1±0.3	3.0±0.3	3.0±0.4	3.1±0.4	3.2±0.3		<0.001*
	UP-MV_{spon}	9.9±0.2	10.9±0.2	5.6±0.4	3.5±0.5	3.8±0.7	3.7±0.7	3.4±0.6	3.5±0.6	3.6±0.5		<0.001*, 0.226†
	UP-MV_{PS}	10.1±0.3	10.8±0.5	5.8±0.3	3.1±0.4	2.9±0.4	3.0±0.5	2.9±0.4	3.1±0.5	2.6±0.3		<0.001*, 0.995†, 0.072‡
			P = 0.866	*P = 0.373*								
RR (breaths/min)	P-MV_{contr}	15±1	15±1	35±1	35±1	34±1	35±1	34±1	34±1	35±1	<0.001	
	UP-MV_{contr}	13±3	13±2	33±2	14±1	15±1	15±1	14±1	15±1	14±1		<0.001*
	UP-MV_{spon}	13±2	13±2	33±3	17±3	19±9	20±10	20±9	18±4	15±2		<0.001*, 0.315†
	UP-MV_{PS}	14±2	15±1	33±2	21±9	25±14	20±7	18±3	18±2	17±1		<0.001*, 0.073†, 0.977‡
			P = 0.560	*P = 0.474*								
T_i/T_{tot}	P-MV_{contr}	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	<0.001	
	UP-MV_{contr}	0.5±0.0	0.5±0.0	0.5±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0		<0.001*
	UP-MV_{spon}	0.5±0.0	0.5±0.0	0.5±0.0	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1		<0.001*, 0.015†
	UP-MV_{PS}	0.5±0.0	0.5±0.0	0.5±0.0	0.2±0.1	0.2±0.1	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0		<0.001*†‡
			P = 0.112	*P = 0.482*								
$P_{L,peak}$ (cm H_2O)	P-MV_{contr}	10.4±2.1	29.6±3.4	18.2±2.9	17.3±2.7	16.9±2.9	17.1±2.8	16.6±3.2	16.8±2.5	17.0±1.9	0.001	
	UP-MV_{contr}	9.4±3.3	25.1±4.3	14.6±1.3	9.4±1.7	11.6±1.9	12.2±1.8	11.3±2.7	11.9±3.3	11.5±2.3		0.272*
	UP-MV_{spon}	8.1±1.6	27.7±5.9	14.8±3.5	8.0±4.3	11.2±5.5	11.2±4.8	10.2±4.5	9.4±4.1	9.1±4.4		0.016*, 0.688†
	UP-MV_{PS}	7.3±2.9	25.9±3.2	13.4±2.0	10.4±2.5	10.5±2.6	12.1±3.2	10.3±3.6	9.9±3.3	11.3±1.6		0.504*, 1.000†, 0.472‡
			P = 0.299	*P = 0.023*								
$P_{L,mean}$ (cm H_2O)	P-MV_{contr}	3.8±2.2	11.5±1.5	8.3±2.3	8.0±1.9	7.6±1.8	7.9±1.9	7.3±1.8	7.6±1.0	8.1±1.0	0.048	
	UP-MV_{contr}	2.8±1.4	9.0±1.8	6.6±0.9	6.3±1.6	8.1±1.5	8.4±1.6	7.5±2.4	7.7±2.9	7.7±2.3		1.0*
	UP-MV_{spon}	2.2±0.7	10.0±2.3	6.5±2.2	5.1±2.6	6.6±2.5	7.0±2.9	6.7±3.1	5.9±2.9	5.9±3.4		0.708*, 0.786†
	UP-MV_{PS}	2.6±1.3	9.2±1.3	5.6±1.4	5.1±1.0	4.6±1.6	5.8±2.0	4.8±2.2	3.7±2.6	4.6±1.9		0.084*, 0.110†, 0.769‡
			P = 0.089	*P = 0.085*								
$P_{aw,peak}$ (cm H_2O)	P-MV_{contr}	21.1±2.0	40.8±3.5	35.8±2.1	35.1±2.2	35.1±2.7	34.8±2.5	34.5±2.9	34.3±2.7	34.1±2.7	<0.001	
	UP-MV_{contr}	18.8±2.9	36.6±4.1	32.4±1.7	26.1±1.6	26.9±2.1	27.2±2.2	27.1±2.6	27.6±2.2	27.0±2.2		<0.001*
	UP-MV_{spon}	19.0±2.2	38.0±6.3	32.3±3.2	24.1±3.5	24.0±3.2	23.6±3.1	23.4±3.1	23.1±3.2	23.4±3.2		<0.001*, 0.142†
	UP-MV_{PS}	17.6±4.3	37.1±3.3	32.9±2.4	28.4±2.3	28.5±2.4	28.5±2.6	27.3±3.2	28.0±2.6	28.0±1.8		<0.001*, 0.966†, 0.023‡

(*continued*)

Table 1. (*Continued*)

Parameter	Group	BL1	Injury	BL2	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Group Effect, *P* Value	*Post Hoc* Test, *P* Value
			P = 0.368	*P = 0.064*								
$P_{aw,plat}$ (cm H_2O)	P-MV_{contr}	17.6±2.6	35.1±3.0	34.1±2.3	33.2±2.5	33.0±2.8	32.6±2.7	32.3±2.9	32.0±2.8	32.1±2.3	<0.001	
	UP-MV_{contr}	13.5±1.7	28.8±4.8*	29.7±3.1	24.1±1.3	24.7±1.6	25.0±1.6	24.6±1.9	25.1±1.9	24.8±2.0		<0.001*
	UP-MV_{spon}	15.8±1.8	30.2±4.2	30.7±3.4	22.4±3.1	22.6±3.2	22.3±3.1	21.9±3.3	21.7±3.1	21.9±3.1		<0.001*, 0.424†
	UP-MV_{PS}	15.0±3.3	30.9±3.0	31.8±2.2	28.5±2.1	26.5±3.3	26.7±3.7	26.2±3.4	25.8±4.2	27.7±1.9		0.003*, 0.678†, 0.024‡
			P = 0.043	*P = 0.072*								
$P_{aw,mean}$ (cm H_2O)	P-MV_{contr}	12.1±2.4	19.9±1.9	24.8±1.9	24.5±1.8	24.5±1.8	24.3±1.8	24.1±1.8	24.0±1.6	24.0±1.6	<0.001	
	UP-MV_{contr}	10.4±1.1	17.0±1.3	23.1±0.7	22.6±0.9	23.0±1.2	23.1±1.1	23.0±1.4	23.2±1.3	23.0±1.2		0.614*
	UP-MV_{spon}	10.5±0.8	17.5±2.4	22.7±1.6	20.1±2.0	20.4±2.1	20.3±2.0	20.3±1.9	20.2±2.1	20.3±2.0		0.001*, 0.025†
	UP-MV_{PS}	10.6±1.3	17.1±1.2	23.4±1.0	21.6±0.9	21.2±1.2	20.7±1.0	20.8±0.9	20.7±0.7	20.4±0.8		0.004*, 0.132†, 0.976‡
			P = 0.035	*P = 0.082*								
R_{rs} (cm H_2O /L/s)	P-MV_{contr}	7.1±0.8	13.2±2.8	7.1±1.9	6.5±1.1	6.2±1.0	5.9±0.8	5.7±0.9	5.6±0.8	5.7±0.9	0.118	
	UP-MV_{contr}	6.6±2.2	9.5±2.2	5.1±1.9	4.5±2.3	4.5±2.3	4.3±2.0	4.0±2.1	4.1±2.1	4.3±2.2		
	UP-MV_{spon}	7.2±1.1	12.1±3.8	5.4±0.9								
	UP-MV_{PS}	7.2±0.5	10.7±1.2	6.3±1.4								
			P = 0.083	*P = 0.110*								
E_{rs} (cm H_2O /L)	P-MV_{contr}	26.6±4.0	67.2±7.3	73.3±9.1	72.2±10.3	72.9±12.2	72.9±12.7	71.3±15.3	70.0±14.1	69.4±14.0	0.886	
	UP-MV_{contr}	23.2±8.9	57.3±4.0	61.0±5.3	66.0±6.9	71.1±7.0	75.0±7.5	73.7±10.7	74.9±7.1	73.3±7.5		
	UP-MV_{spon}	25.1±5.0	62.4±10.5	64.1±11.9								
	UP-MV_{PS}	22.1±3.9	58.0±9.1	58.5±12.5								
			P = 0.131	*P = 0.066*								
PEEP (cm H_2O)	P-MV_{contr}	4.9±0.2	4.9±0.1	15.9±0.1	15.9±0.1	15.8±0.2	16.0±0.1	15.9±0.1	15.9±0.02	16.0±0.1	<0.001	
	UP-MV_{contr}	4.9±0.1	5.0±0.1	16.0±0.2	16.0±0.4	16.0±0.3	15.9±0.2	16.1±0.4	15.9±0.4	15.9±0.2		1.000*
	UP-MV_{spon}	4.9±0.1	4.9±0.2	16.0±0.1	15.9±0.2	15.9±0.1	15.8±0.2	15.8±0.2	16.0±0.2	15.9±0.2		1.000*, 1.000†
	UP-MV_{PS}	5.0±0.1	4.9±0.1	16.0±0.1	19.8±1.0	19.4±0.7	19.4±0.7	19.5±0.8	19.3±0.8	19.2±1.0		<0.001*†‡
			P = 0.897	*P = 0.345*								
PTP (cm H_2O *s)	UP-MV_{spon}				20.1±17.	41.7±57.7	32.6±51.2	29.8±36.7	19.4±17.8	23.1±16.1	0.727	
	UP-MV_{PS}				8.0±7.8	53.0±56.8	35.8±39.7	17.5±17.5	7.6±6.6	11.4±10.8		

Values are shown as mean and SD, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Comparability of groups at injury and BL2 was tested using one-way ANOVA followed by Bonferroni *post hoc* tests, with *P* values given in italics below the respective columns. In case of significant differences at BL2, values at this time point were used as covariate during general linear model statistics. Differences among groups were tested with general linear model statistics (results are shown in column "Group effect.") and adjusted for repeated measurements according to the Sidak procedure (results of *post hoc* test are shown in column "*post hoc* test" and indicated as: *, *versus* P-MV_{contr}; † *versus* UP-MV_{contr}; ‡ *versus* UP-MV_{spon}).

BL1 = baseline 1; BL2 = baseline 2; E_{rs} = elastance of the respiratory system; IN = injury; MV = minute ventilation; $P_{aw,mean}$ = mean airway pressure; $P_{aw,peak}$ = peak airway pressure; $P_{aw,plat}$ = plateau airway pressure; PEEP = positive end-expiratory pressure; $P_{L,mean}$ = mean transpulmonary pressure; $P_{L,peak}$ = peak transpulmonary pressures; P-MV_{contr} = protective controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; PTP = pressure–time product; RR = respiratory rate; R_{rs} = resistance of the respiratory system; T_I/T_{tot} = inspiratory time to total cycle time; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing; V_T/kg = mean tidal volume per kg.

Table 2. Hemodynamics and Gas Exchange

Parameter	Group	BL1	Injury	BL2	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Group Effect, *P* Value	*Post Hoc* Test, *P* Value
Pa_{O_2}/FI_{O_2} (mmHg)	P-MV_{contr}	578.5 ± 26.1	46.0 ± 4.9	92.7 ± 19.1	131.2 ± 44.8	172.0 ± 70.5	222.1 ± 102.4	297.7 ± 100.4	344.4 ± 109.4	359.1 ± 93.8	<0.001	
	UP-MV_{contr}	577.6 ± 60.6	57.8 ± 15.1	162.1 ± 78.8	163.9 ± 84.3	137.3 ± 70.6	150.9 ± 81.8	152.4 ± 75.5	163.5 ± 80.2	176.6 ± 80.9		0.436*
	UP-MV_{spon}	580.4 ± 53.5	57.6 ± 17.4	163.7 ± 61.7	373.1 ± 142.3	399.6 ± 115.0	419.9 ± 113.5	440.1 ± 103.5	433.1 ± 100.2	440.8 ± 92.4		0.040*, <0.001†
	UP-MV_{PS}	563.9 ± 41.4	57.5 ± 14.1	135.6 ± 33.3	342.2 ± 107.6	357.6 ± 130.8	361.6 ± 147.8	3535.0 ± 140.9	389.9 ± 127.5	403.5 ± 122.0		0.266*, 0.005†, 0.940‡
			P = 0.377	*P = 0.104*								
$\dot{Q}_{VA}/\dot{Q}_r$ (%)	P-MV_{contr}	8.0 ± 3.6	59.9 ± 9.9	38.7 ± 11.2	29.3 ± 8.7	25.5 ± 7.8	21.8 ± 6.7	17.8 ± 4.6	15.1 ± 4.6	15.6 ± 4.8	<0.001	
	UP-MV_{contr}	7.3 ± 6.0	52.6 ± 15.0	28.3 ± 9.4	29.8 ± 6.8	33.2 ± 6.1	31.8 ± 4.7	34.6 ± 8.9	31.9 ± 5.7	32.0 ± 6.8		0.021*
	UP-MV_{spon}	7.3 ± 4.9	55.3 ± 16.6	26.3 ± 7.7	15.3 ± 8.6	13.1 ± 4.8	12.6 ± 6.0	12.3 ± 6.4	12.7 ± 5.9	14.2 ± 5.0		0.231*, <0.001†
	UP-MV_{PS}	9.8 ± 6.3	55.7 ± 13.7	28.1 ± 7.8	19.1 ± 8.3	18.2 ± 9.6	18.4 ± 11.0	19.4 ± 9.1	17.5 ± 8.3	15.7 ± 6.9		0.965*, 0.003†, 0.727‡
			P = 0.840	*P = 0.103*								
Pa_{CO_2} (mmHg)	P-MV_{contr}	41.5 ± 1.3	70.6 ± 18.2	83.2 ± 7.0	87.4 ± 7.7	90.2 ± 8.7	90.8 ± 11.7	92.7 ± 11.1	86.2 ± 10.6	84.3 ± 13.0	<0.001	
	UP-MV_{contr}	39.5 ± 3.7	67.4 ± 8.2	78.5 ± 10.3	68.0 ± 6.7	59.0 ± 2.8	57.0 ± 5.6	58.5 ± 4.4	58.4 ± 2.9	61.6 ± 2.5		<0.001*
	UP-MV_{spon}	40.7 ± 3.2	61.0 ± 14.7	80.4 ± 7.8	70.3 ± 5.2	67.5 ± 10.3	66.3 ± 6.9	63.7 ± 5.0	66.3 ± 6.7	64.6 ± 5.6		<0.001*, 0.478†
	UP-MV_{PS}	39.8 ± 3.2	68.6 ± 10.6	84.5 ± 3.2	65.6 ± 8.1	59.3 ± 9.7	58.4 ± 8.6	59.1 ± 7.5	59.9 ± 6.7	60.0 ± 8.1		<0.001*, 1.000†, 0.476‡
			P = 0.646	*P = 0.520*								
Arterial pH	P-MV_{contr}	7.48 ± 0.03	7.28 ± 0.05	7.22 ± 0.04	7.21 ± 0.04	7.22 ± 0.05	7.22 ± 0.05	7.23 ± 0.04	7.26 ± 0.04	7.27 ± 0.05	<0.001	
	UP-MV_{contr}	7.47 ± 0.04	7.27 ± 0.04	7.23 ± 0.04	7.30 ± 0.03	7.35 ± 0.02	7.37 ± 0.03	7.37 ± 0.03	7.37 ± 0.03	7.35 ± 0.02		<0.001*
	UP-MV_{spon}	7.45 ± 0.04	7.32 ± 0.07	7.22 ± 0.04	7.27 ± 0.04	7.30 ± 0.06	7.31 ± 0.04	7.34 ± 0.03	7.34 ± 0.04	7.35 ± 0.04		<0.001*, 0.934†
	UP-MV_{PS}	7.44 ± 0.04	7.26 ± 0.05	7.18 ± 0.01	7.28 ± 0.04	7.33 ± 0.05	7.34 ± 0.04	7.34 ± 0.04	7.33 ± 0.03	7.33 ± 0.05		<0.001*, 0.808†, 1.000‡
			P = 0.766	*P = 0.603*								
CO (l/min)	P-MV_{contr}	5.46 ± 1.16	5.55 ± 0.92	5.21 ± 1.45	4.79 ± 0.93	4.61 ± 0.67	4.53 ± 0.77	4.88 ± 1.29	4.41 ± 0.8	4.24 ± 0.84	0.647	
	UP-MV_{contr}	4.99 ± 0.52	5.74 ± 1.14	4.83 ± 1.25	5.32 ± 1.53	4.64 ± 0.63	4.60 ± 0.66	5.18 ± 1.08	5.37 ± 1.02	5.29 ± 1.43		
	UP-MV_{spon}	4.33 ± 0.99	6.00 ± 1.47	4.79 ± 1.27	4.65 ± 0.69	4.33 ± 0.79	4.45 ± 0.81	4.32 ± 0.86	4.74 ± 0.55	4.90 ± 0.71		
	UP-MV_{PS}	5.56 ± 1.22	5.32 ± 1.41	4.88 ± 1.12	5.00 ± 1.12	4.53 ± 0.96	4.68 ± 0.91	4.80 ± 0.98	4.98 ± 1.14	4.93 ± 0.98		
			P = 0.811	*P = 0.943*								
HR (l/min)	P-MV_{contr}	101 ± 7	105 ± 12	103 ± 9	98 ± 6	99 ± 8	98 ± 16	98 ± 18	92 ± 16	95 ± 18	0.131	
	UP-MV_{contr}	95 ± 6	105 ± 13	106 ± 13	108 ± 9	104 ± 8	99 ± 7	101 ± 10	104 ± 13	99 ± 9		
	UP-MV_{spon}	89 ± 11	101 ± 17	98 ± 16	95 ± 9	98 ± 7	96 ± 5	95 ± 5	96 ± 5	94 ± 6		
	UP-MV_{PS}	93 ± 10	88 ± 12	95 ± 15	96 ± 12	88 ± 14	90 ± 12	88 ± 14	88 ± 17	84 ± 13		
			P = 0.121	*P = 0.492*								
MAP (mmHg)	P-MV_{contr}	83 ± 9	90 ± 10	90 ± 14	93 ± 6	92 ± 6	88 ± 7	92 ± 7	91 ± 8	87 ± 10	0.352	
	UP-MV_{contr}	78 ± 15	98 ± 17	89 ± 9	97 ± 5	97 ± 5	92 ± 8	92 ± 9	90 ± 9	86 ± 9		
	UP-MV_{spon}	71 ± 7	92 ± 14	81 ± 3	96 ± 5	95 ± 6	91 ± 5	90 ± 5	89 ± 6	93 ± 5		
	UP-MV_{PS}	76 ± 5	91 ± 9	89 ± 9	92 ± 8	91 ± 7	91 ± 8	90 ± 7	84 ± 5	90 ± 5		
			P = 0.694	*P = 0.363*								

(*continued*)

Table 2. (*Continued*)

Parameter	Group	BL1	Injury	BL2	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Group Effect, *P* Value	*Post Hoc* Test, *P* Value
MPAP (mmHg)	P-MV_{contr}	17 ± 3	32 ± 4	30 ± 3	30 ± 2	30 ± 3	29 ± 3	30 ± 3	29 ± 3	27 ± 2	<0.001	
	UP-MV_{contr}	18 ± 2	34 ± 5	31 ± 3	39 ± 5	42 ± 4	39 ± 3	38 ± 3	38 ± 2	38 ± 3		<0.001*
	UP-MV_{spon}	16 ± 3	34 ± 2	32 ± 2	34 ± 4	33 ± 6	31 ± 3	31 ± 5	32 ± 5	31 ± 4		0.591*, 0.006†
	UP-MV_{PS}	19 ± 3	34 ± 4	33 ± 3	33 ± 2	33 ± 4	34 ± 4	33 ± 4	31 ± 4	33 ± 5		0.321*, 0.017†, 0.999‡
			P = 0.801	*P = 0.246*								
CVP (mmHg)	P-MV_{contr}	6 ± 2	5 ± 1	7 ± 2	7 ± 2	6 ± 2	6 ± 2	6 ± 2	6 ± 2	6 ± 2	<0.001	
	UP-MV_{contr}	7 ± 2	8 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1		0.001*
	UP-MV_{spon}	6 ± 1	8 ± 2	9 ± 2	10 ± 1	9 ± 1	10 ± 1	9 ± 1	9 ± 1	10 ± 1		<0.001*, 0.941†
	UP-MV_{PS}	6 ± 2	7 ± 1	8 ± 1	10 ± 1	10 ± 1	10 ± 1	9 ± 1	9 ± 1	10 ± 1		<0.001*, 0.860†, 1.000‡
			P = 0.059	*P = 0.214*								
PO_2 gradient (mmHg)	P-MV_{contr}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.251	
	UP-MV_{contr}	n.a.	n.a.	n.a.	117.6 ± 86.0	109.4 ± 47.8	73.8 ± 51.3	63.6 ± 62.4	45.0 ± 54.8	23.4 ± 52.1		
	UP-MV_{spon}	n.a.	n.a.	n.a.	32.0 ± 78.1	−1.6 ± 90.5	−15.8 ± 86.4	−4.2 ± 37.3	28.2 ± 65.3	2.4 ± 31.2		
	UP-MV_{PS}	n.a.	n.a.	n.a.	56.1 ± 63.8	−17.4 ± 85.7	−7.2 ± 72.4	−14.3 ± 78.7	−3.1 ± 62.7	−3.3 ± 48.0		
PCO_2 gradient (mmHg)	P-MV_{contr}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.560	
	UP-MV_{contr}	n.a.	n.a.	n.a.	39.2 ± 5.8	33.4 ± 6.7	32.4 ± 4.8	29.9 ± 6.2	31.0 ± 5.1	33.0 ± 5.6		
	UP-MV_{spon}	n.a.	n.a.	n.a.	35.4 ± 16.6	36.8 ± 19.5	33.6 ± 15.3	31.2 ± 6.7	31.2 ± 6.3	30.0 ± 5.0		
	UP-MV_{PS}	n.a.	n.a.	n.a.	37.7 ± 7.5	34.6 ± 10.9	34.3 ± 8.8	30.8 ± 10.7	33.8 ± 7.3	32.9 ± 8.3		

Values are shown as mean and SD, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Comparability of groups at injury and BL2 was tested using one-way ANOVA followed by Bonferroni *post hoc* tests, with *P* values given in italics below the respective columns. Differences among groups were tested with general linear model statistics (results are shown in column "Group effect.") and adjusted for repeated measurements according to the Sidak procedure (results of *post hoc* test are shown in column "*post hoc* test" and indicated as: * *versus* P-MV_{contr}; † *versus* UP-MV_{contr}; ‡ *versus* UP-MV_{spon}).

BL1 = baseline 1; BL2 = baseline 2; CO = cardiac output; CVP = central venous pressure; HR = heart rate; IN = injury; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; $PaCO_2$ = partial arterial pressure of carbon dioxide; PCO_2 gradient = gradient of partial carbon dioxide pressure between inlet and outlet of the interventional lung assist; P/F Ratio = ratio of partial arterial oxygen pressure and fraction of inspired oxygen; P-MV_{contr} = protective controlled MV according to the Acute Respiratory Distress Syndrome network; PO_2 gradient = gradient of partial oxygen pressure between inlet and outlet of the interventional lung assist; $\dot{Q}_{VA}/\dot{Q}_t$ = venous admixture; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective MV with mandatory cycles and superposed unassisted spontaneous breathing.

The double hit injury resulted in $Pa_{O_2}/F_{I_{O_2}}$ less than 85 mmHg in all animals. As shown in table 2, UP-MV_{contr} was associated with decreased oxygenation and higher intrapulmonary shunt levels compared to P-MV_{contr}. The time needed to resume spontaneous breathing was 34 ± 14 and 33 ± 11 min in UP-MV_{spon} and UP-MV_{PS}, respectively. Both UP-MV_{spont} and UP-MV_{PS} yielded higher $Pa_{O_2}/F_{I_{O_2}}$ and lower intrapulmonary shunt than UP-MV_{contr}. $ECCO_2$-R reduced Pa_{CO_2} and increased pHa, as compared to P-MV_{contr}. Heart rate, mean arterial blood pressure, and cardiac output did not differ significantly among groups, whereas mean pulmonary arterial pressures was decreased during P-MV_{contr}, UP-MV_{spont}, and UP-MV_{PS} than UP-MV_{contr}. Also, central venous pressure was higher during UP-MV strategies. The partial pressure of oxygen gradient across the ILA® membrane was significantly higher than zero during UP-MV_{contr}, but not during UP-MV_{spont} and UP-MV_{PS}, while the partial pressure of carbon dioxide gradient was always higher than zero in all ultraprotective strategies.

Figure 2 shows the distribution of ventilation. UP-MV_{spont} and UP-MV_{PS} were associated with a redistribution of ventilation from central to dorsal lung zones compared to P-MV_{contr} and UP-MV_{contr}. However, we could not detect a redistribution of perfusion (differences of angular coefficients of relative pulmonary blood flow between Time 6 and BL2, median [interquartile range]) neither along the ventral–dorsal axis (P-MV_{contr}: 0.0019 [0.0000, 0.0042]; UP-MV_{contr}: 0.0007 [−0.0007, 0.0020]; UP-MV_{spont}: −0.0008 [−0.0022, 0.0006]; UP-MV_{PS}: −0.0009 [−0.0016, 0.0032]), nor along the cranial–caudal axis (P-MV_{contr}: −0.0010 [−0.0016, 0.0001]; UP-MV_{contr}: 0.0006 [−0.0001, 0.0014]; UP-MV_{spont}: −0.0006 [−0.0015, 0.0019]; UP-MV_{PS}: −0.0002 [−0.0010, 0.0010]).

As depicted in figure 3, UP-MV_{contr} reduced the DAD score in dorsal areas, as compared to P-MV_{contr}, mainly due to decreased alveolar edema and inflammatory infiltrates (table 3). The wet-to-dry ratio did not differ significantly among groups (P-MV_{contr}: 8.5 [7.8 to 8.9]; UP-MV_{contr}: 7.8 [7.0 to 9.7]; UP-MV_{spont}: 7.5 [7.4 to 7.7]; UP-MV_{PS}: 7.7 [7.1 to 8.0]).

UP-MV_{PS} was associated with higher levels of tumor necrosis factor-α and IL-8 both in ventral and dorsal lung regions compared to other groups (fig. 4). No differences were found in markers of inflammation in lung tissue among P-MV_{contr}, UP-MV_{contr}, and UP-MV_{spont}. Gene expression of inflammatory mediators and markers of cell stress in lung tissue (table 4), as well as total protein, cytokine, and myeloperoxidase levels in broncho-alveolar lavage fluid (table 5), were comparable among different groups.

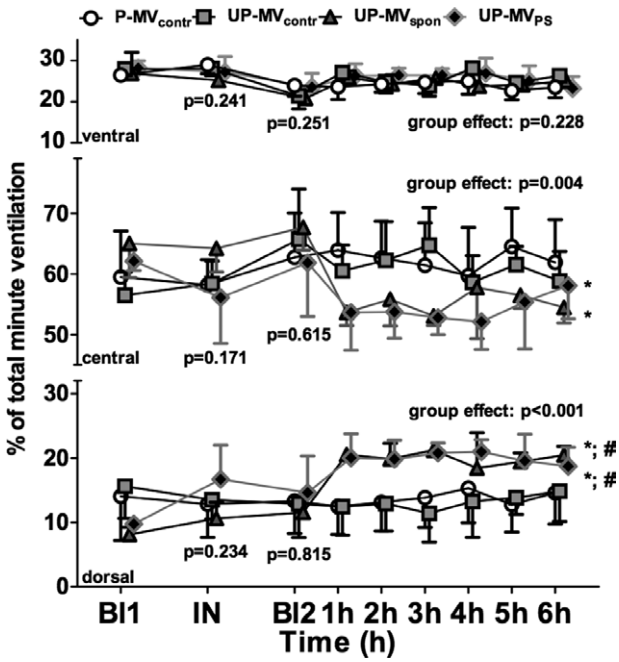

Fig. 2. Distribution of ventilation. Values represent percentage of total minute ventilation and are shown as mean and SD. Due to technical problems with the EIT device, values were obtained from 24 animals in total (P-MV_{contr} 7, UP-MV_{contr} 6, UP-MV_{spon} 5, and UP-MV_{PS} 6 animals, respectively). Statistical significance was accepted at *P* value less than 0.05. Comparability of groups at injury, BL2 was tested with one-way ANOVA followed by Bonferroni *post hoc* test. Differences among groups were tested with general linear model statistics and adjusted for repeated measurements according to the Sidak procedure; * *versus* P-MV_{contr}; # *versus* UP-MV_{contr}. *P* values in the figure represent group effect. *Post hoc* analysis, central: P-MV_{contr} = *versus* UP-MV_{contr} *P* = 0.945, *versus* UP-MV_{spon} *P* = 0.045, *versus* UP-MV_{PS} *P* = 0.013; UP-MV_{contr} = *versus* UP-MV_{spon} *P* = 0.161, *versus* UP-MV_{PS} *P* = 0.057 and UP-MV_{spon} = *versus* UP-MV_{PS} *P* = 0.999; dorsal: P-MV_{contr} = *versus* UP-MV_{contr} *P* = 1.000, *versus* UP-MV_{spon} *P* = 0.007, *versus* UP-MV_{PS} *P* = 0.004; UP-MV_{contr} = *versus* UP-MV_{spon} *P* = 0.003, *versus* UP-MV_{PS} *P* = 0.004 and UP-MV_{spon} = *versus* UP-MV_{PS} *P* = 1.000. BL1 = baseline 1; BL2 = baseline 2; central = central lung regions; dorsal = dorsal lung regions; EIT = electrical impedance tomography; IN = injury; P-MV_{contr} = controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing; ventral = ventral lung regions.

Discussion

In a model of severe ARDS in pigs, we found that: (1) UP-MV_{contr} reduced DAD score mainly in dorsal lung zones, but worsened oxygenation and intrapulmonary shunt, compared to P-MV_{contr}; (2) UP-MV_{spont} and UP-MV_{PS} improved oxygenation and intrapulmonary shunt, and redistributed ventilation towards dorsal areas, as compared to UP-MV_{contr}; (3) UP-MV_{PS} resulted in more inflammation in lung tissue than P-MV_{contr}, UP-MV_{contr}, and UP-MV_{spont}, mainly in dorsal zones.

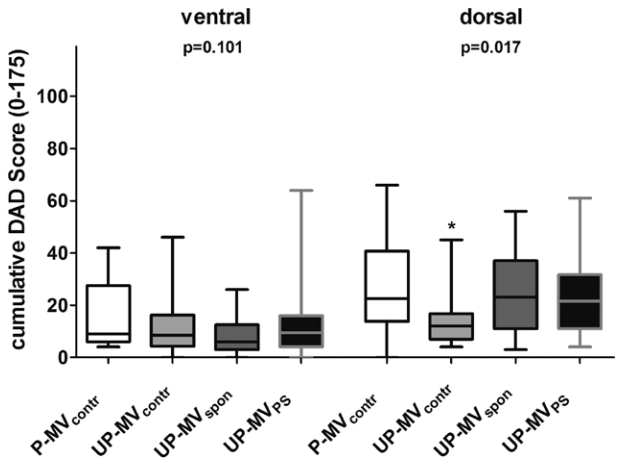

Fig. 3. Cumulative diffuse alveolar damage (DAD) score. Values are shown as median and interquartile range with *whiskers* indicating minimum and maximum and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Differences among groups were tested with mixed linear model statistics, and Tukey Kramer procedure used for *post hoc* test. Statistical significance of pairwise comparisons is indicated by $^{*}P < 0.05$ against P-MV_{contr}. *P* values in the figure represent group effect. *Post hoc* analysis: P-MV_{contr} = *versus* UP-MV_{contr} $P = 0.016$, *versus* UP-MV_{spon} $P = 0.964$, *versus* UP-MV_{PS} $P = 0.763$; UP-MV_{contr} = *versus* UP-MV_{spon} $P = 0.058$, *versus* UP-MV_{PS} $P = 0.177$ and UP-MV_{spon} = *versus* UP-MV_{PS} $P = 0.959$. Dorsal = dorsal lung regions; P-MV_{contr} = controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing; ventral = ventral lung regions.

To our knowledge, this is the first study investigating the impact of different ventilatory strategies, including spontaneous breathing, during UP-MV and $ECCO_2$-R on lung morphofunction and inflammatory markers in a model of severe ARDS. We used a double-hit consisting of saline lung lavage and VILI, which reproduces most histological features seen in human ARDS.[18,19] The levels of hypoxemia were compatible with severe ARDS according to the Berlin definition.[20] We chose pressure-controlled and pressure support ventilation because these modes share similar inspiratory flow patterns. Furthermore, in presence of spontaneous breathing, volume assist-control ventilation may yield breath stacking. In P-MV_{contr}, settings of V_T, RR, and I:E were based on recommendations of the ARDS network. However, in some animals, inspiratory plateau pressure could not be set lesser than or equal to 30 cm H_2O due to severe respiratory acidosis, but $P_{L,peak}$ was less than 20 cm H_2O during P-MV_{contr}, a level that appeared to be safe during the ventilation of pigs without lung injury in a study by Protti *et al.*,[21] when sufficient PEEP was used, and is far less than the safety limit of 27 cm H_2O proposed in humans elsewhere.[22] The PEEP level was chosen in agreement with the higher PEEP strategy.[2] In fact, a recent meta-analysis showed that higher PEEP levels improve survival in patients with severe ARDS.[23] The F_{IO_2} was kept at 1.0 to allow direct comparison with UP-MV strategies, where accumulation of nitrogen may impair oxygenation.[24] In order to minimize differences in $P_{aw,mean}$ among groups, we increased the I:E ratio in UP-MV_{contr} and UP-MV_{spont}, and the PEEP in UP-MV_{PS}. Indeed, $P_{aw,mean}$ may impact on gas exchange, hemodynamics and lung injury,[25] affecting the comparability among different MV strategies.

The deterioration of oxygenation and intrapulmonary shunt during UP-MV_{contr}, compared to P-MV_{contr}, may be ascribed to alveolar derecruitment due to decreased V_T and $P_{aw,peak}$, despite comparable $P_{aw,mean}$.[26] Spontaneous breathing activity, whether PS or not, improved oxygenation and intrapulmonary shunt, reducing also the mean pulmonary arterial pressure. Previous studies have shown that these effects could be explained by redistribution of perfusion towards better aerated, nondependent lung regions[27,28] or recruitment of collapsed, dependent zones.[29] In the current study, we found that UP-MV_{spont} and UP-MV_{PS} redistributed ventilation towards dorsal areas, although not affecting regional perfusion. These observations suggest that spontaneous breathing activity induced recruitment in those areas due to higher regional P_L, because $P_{L,peak}$ and $P_{L,mean}$ were comparable among UP-MV strategies. The decrease in mean pulmonary arterial pressures during P-MV_{contr}, as well as UP-MV_{spont} and UP-MV_{PS}, compared to UP-MV_{contr} may be explained by the improved oxygenation. The higher central venous pressure during UP-MV strategies could be attributed to the arterial-venous pressure gradient across the artificial membrane, which may have increased the pressure in the inferior cava vein.

Improved oxygenation in UP-MV_{spont} and UP-MV_{PS} compared to UP-MV_{contr} cannot be attributed to oxygen uptake in the extracorporeal gas exchange device. In fact, we found that the partial pressure of oxygen gradient across the artificial membrane was higher in UP-MV_{contr} than UP-MV_{spont} and UP-MV_{PS}, suggesting that the beneficial effects of spontaneous breathing activity during UP-MV on oxygenation were even underestimated.

The decrease of histological damage in dorsal areas during UP-MV_{contr}, as compared to P-MV_{contr}, can be explained by two mechanisms: reduced stress/strain, as indicated by decreased $P_{aw,peak}$ and $P_{L,peak}$, as well as V_T, and decreased stress rates, as suggested by decreased respiratory rate.[30] The beneficial effects of UP-MV on histological damage could not be detected when spontaneous breathing activity was resumed, despite comparable values of $P_{L,peak}$ and $P_{L,mean}$. This suggests that inspiratory effort might have partially counteracted lung protection. In fact, UP-MV_{PS}, but not UP-MV_{spont}, was associated with an

Table 3. Diffuse Alveolar Damage Score

Feature	Group	Ventral Region	Group Effect, *P* Value	*Post hoc* Test	Dorsal Region	Group Effect, *P* Value	*Post Hoc* Test, *P* Value
Alveolar edema	P-MV_{contr}	1 [0–2]	0.199		2 [0–11]	0.004	
	UP-MV_{contr}	0 [0–1]			0 [0–2]		0.003*
	UP-MV_{spon}	0 [0–1]			2 [1–6]		0.116*, 0.573†
	UP-MV_{PS}	0 [0–1]			1 [1–4]		0.020*, 0.938†, 0.894‡
Intersitial edema	P-MV_{contr}	1 [0–2]	0.011		1 [0–1]	0.695	
	UP-MV_{contr}	1 [0–2]		0.732*	1 [0–2]		
	UP-MV_{spon}	1 [0–2]		0.381*, 0.940†	1 [1–1]		
	UP-MV_{PS}	0 [0–1]		0.007*, 0.107†, 0.319‡	1 [0–2]		
Hemorrhage	P-MV_{contr}	0 [0–0]	0.110		3 [0–3]	0.445	
	UP-MV_{contr}	0 [0–2]			0 [0–3]		
	UP-MV_{spon}	0 [0–1]			2 [1–4]		
	UP-MV_{PS}	0 [0–2]			2 [0–6]		
Inflammatory infiltration	P-MV_{contr}	1 [0–11]	0.041		7 [3–15]	0.005	
	UP-MV_{contr}	1 [0–3]		0.216*	3 [3–6]		0.024*
	UP-MV_{spon}	1 [0–2]		0.038*, 0.864†	8 [3–15]		0.975*, 0.007†
	UP-MV_{PS}	2 [0–4]		0.809*, 0.719†, 0.265‡	8 [3–12]		0.998*, 0.040†, 0.930‡
Epithelial destruction	P-MV_{contr}	2 [1–4]	0.151		1 [1–4]	0.048	
	UP-MV_{contr}	1 [0–2]			1 [0–2]		0.131*
	UP-MV_{spon}	1 [0–4]			2 [1–4]		0.749*, 0.633†
	UP-MV_{PS}	1 [0–4]			4 [1–4]		0.973*, 0.048†, 0.483‡
Microatelectasis	P-MV_{contr}	2 [0–4]	0.003		4 [1–4]	0.083	
	UP-MV_{contr}	1 [1–1]		0.230*	1 [1–2]		
	UP-MV_{spon}	1 [0–1]		0.001*, 0.230†	2 [1–4]		
	UP-MV_{PS}	1 [0–2]		0.097*, 0.974†, 0.450‡	2 [1–4]		
Overdistension	P-MV_{contr}	3 [1–3]	0.010		3 [1–6]	0.686	
	UP-MV_{contr}	3 [2–9]		0.089*	3 [1–4]		
	UP-MV_{spon}	3 [0–3] †		0.888*, 0.013†	3 [1–6]		
	UP-MV_{PS}	4 [3–4]		0.414*, 0.843†, 0.113‡	3 [2–4]		

Diffuse alveolar damage in ventral lung regions (ventral) and dorsal lung regions (dorsal). Values are shown as median and interquartile range, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Differences among groups were tested with mixed linear model statistics (results are shown in column "Group effect."), and Tukey Kramer's procedure used for *post hoc* test. Results are shown in column "*post hoc* effect" and indicated as: *$P < 0.05$ against P-MV_{contr}, †$P < 0.05$ against UP-MV_{contr}, and ‡$P < 0.05$ against UP-MV_{spon}. P-MV_{contr} = protective controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing.

increase in markers of inflammation in lung tissue, mainly in dorsal areas. Since I:E was decreased during UP-MV_{PS} than UP-MV_{spont}, it is possible that UP-MV_{PS} favored the collapse/reopening of most lung units. This finding differs from previous studies from our group showing that pressure support reduces lung inflammation compared to controlled MV.[31,32] This difference could be explained by higher severity of lung injury in the current study as compared to previous ones. In fact, spontaneous breathing has been reported to increase lung injury in an experimental model of severe, but not mild ARDS.[9] However, we cannot exclude that higher PEEP during UP-MV_{PS} contributed to increase inflammation in our animals. Furthermore, differences in time-cycled *versus* flow-cycled assisted breaths may have led to different patterns of distribution of regional stress in lungs. Also, the impact of spontaneous breathing on lung injury may differ between protective and ultraprotective strategies. It is worth noting that spontaneous breathing during UP-MV_{spont} was not associated with injurious values of $P_{L,peak}$, which were decreased than during P-MV_{contr}. It must be kept in mind, however, that P_L derived from P_{aw} and P_{es} does not allow distinguishing the distribution of stress within the lungs on a regional level, where local phenomena, such as *pendelluft*, which can be caused by spontaneous efforts, may contribute to lung injury.[33] During UP-MV_{PS}, the relatively low RR and pressure–time product suggests animals were not uncomfortable.

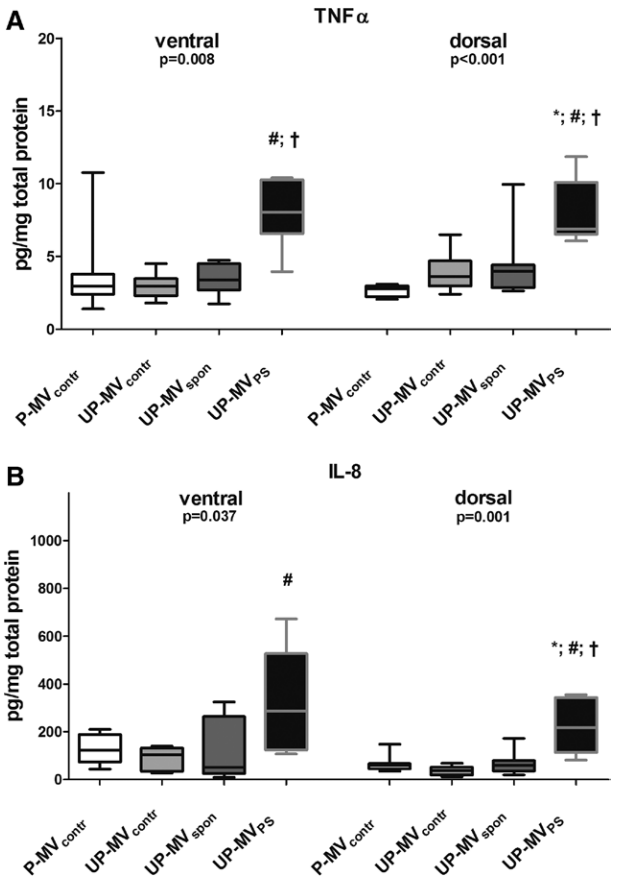

Fig. 4. Markers of inflammation in lung tissue. (*A*) Values are shown as median and interquartile range with whiskers indicating minimum and maximum, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Differences among groups were tested with Kruskal–Wallis test, followed by Mann–Whitney U test with Bonferroni–Holm adjustment for pairwise comparison. **Versus* P-$MV_{contr;}$ *#versus* UP-$MV_{contr;}$ †*versus* UP-MV_{spon}. *P* values in the figure represent group effect. *Post hoc* analysis: (*A*) ventral: P-MV_{contr} = *versus* UP-MV_{contr} $P = 0.902$, *versus* UP-MV_{spon} $P = 0.710$, *versus* UP-MV_{PS} $P = 0.026$; UP-MV_{contr} = *versus* UP-MV_{spon} $P = 0.383$, *versus* UP-MV_{PS} $P = 0.001$ and UP-MV_{spon} = *versus* UP-MV_{PS} $P = 0.002$; (*A*), dorsal: P-MV_{contr} = *versus* UP-MV_{contr} $P = 0.018$, *versus* UP-MV_{spon} $P = 0.038$, *versus* UP-MV_{PS} $P < 0.001$; UP-MV_{contr} = *versus* UP-MV_{spon} $P = 0.901$, *versus* UP-MV_{PS} $P = 0.001$ and UP-MV_{spon} = *versus* UP-MV_{PS} $P = 0.011$; (*B*), ventral: P-MV_{contr} = *versus* UP-MV_{contr} $P = 0.259$, *versus* UP-MV_{spon} $P = 0.620$, *versus* UP-MV_{PS} $P = 0.053$; UP-MV_{contr} = *versus* UP-MV_{spon} $P = 0.805$, *versus* UP-MV_{PS} $P = 0.007$ and UP-MV_{spon} = *versus* UP-MV_{PS} $P = 0.053$; (*B*), dorsal: P-MV_{contr} = *versus* UP-MV_{contr} $P = 0.097$, *versus* UP-MV_{spon} $P = 1.000$, *versus* UP-MV_{PS} $P = 0.002$; UP-MV_{contr} = *versus* UP-MV_{spon} $P = 0.165$, *versus* UP-MV_{PS} $P < 0.001$ and UP-MV_{spon} = *versus* UP-MV_{PS} $P = 0.002$. Dorsal = dorsal lung regions; IL-8 = interleukin 8 (*B*), and in ventral and, P-MV_{contr} = controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; TNF-α = tumor necrosis factor-α (A); UP-MV_{contr} = controlled ultraprotective mechanical ventilation; UP-MV_{PS} = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV_{spon} = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing.

Possible Clinical Implications

Our results support the hypothesis that UP-MV and $ECCO_2$-R without spontaneous breathing may improve lung protection in the early phase of severe ARDS, as compared to conventional P-MV. This hypothesis is in line with a prospective cohort study showing that V_T less than 6 ml/kg predicted body weight and extracorporeal decarboxylation improved markers of lung protection.[5] Furthermore, a recent randomized controlled trial suggested that the use of UP-MV combined with $ECCO_2$-R has the potential to further reduce ventilator-associated lung injury in severe ARDS.[6] It is worth noting that, despite potential beneficial effects on oxygenation, a relative worsening of lung damage or inflammation occurred with spontaneous breathing. These results suggest that spontaneous breathing should be used cautiously during UP-MV in the early phase of severe ARDS, even when the patient shows low RR and inspiratory effort.

Limitations

The current study has several limitations. First, the double-hit model does not reproduce all features of the more complex human severe ARDS. Second, the therapy period was limited to 6 h, and we cannot exclude that results can differ in the long term. Theoretically, complications other than VILI could arise from atelectasis mainly with UP-MV_{contr}, for example hypoxemia, right heart failure due to an increase in mean pulmonary arterial pressure, pneumonia, and difficult weaning, among others. Third, we used an F_{IO_2} of 1.0 in all groups. Although comparability among ventilation modes was enhanced, the higher F_{IO_2} level during a relatively long time period may have led to reabsorption atelectasis, possibly increasing collapse and reopening of mid-dorsal and dorsal lung zones. Fourth, the spontaneous breathing during UP-MV was resumed with time-cycled and flow-cycled modes, and our results cannot be directly extrapolated to other assisted ventilation modes. Fifth, our data were obtained in the early phase of severe ARDS. Thus, different findings are possible when spontaneous breathing is applied later in the course of ARDS. In fact, spontaneous breathing during extracorporeal lung support has been successfully used in the late phase of severe ARDS[34] and other forms of lung disease.[35] Sixth, to avoid derecruitment and maintain comparability among groups, PEEP was kept constant during the therapy period in all groups, contributing to values of $P_{aw,plat}$ greater than 30 cm H_2O in some animals.

Conclusion

In the current model of severe ARDS in pigs, UP-MV with $ECCO_2$-R and without spontaneous breathing slightly reduced histologic lung damage, but not inflammation, as compared to P-MV with low V_T. During UP-MV, spontaneous breathing improved gas exchange and distribution of ventilation, but pressure support increased lung inflammation.

Table 4. Gene Expression of Proinflammatory Mediators and Cell Stress Markers Stress in Lung Tissue

Gene	Region	P-MV$_{contr}$	UP-MV$_{contr}$	UP-MV$_{spon}$	UP-MV$_{PS}$	Group Effect, *P* Value
TNF-α	Ventral	2.5 [1.6–4.0]	2.1 [1.0–6.9]	4.3 [2.0–7.3]	1.8 [1.3–4.1]	0.5408
	Dorsal	3.8 [1.8–8.0]	2.8 [1.3–3.8]	4.3 [2.7–30.8]	1.3 [1.1–5.5]	0.1243
IL-6	Ventral	1.6 [1.1–2.6]	1.2 [0.5–1.4]	1.3 [0.9–2.5]	1.2 [0.8–2.7]	0.5274
	Dorsal	0.8 [0.5–2.2]	0.8 [0.7–1.0]	1.2 [0.4–3.4]	1.5 [0.6–3.9]	0.8383
IL-8	Ventral	0.0 [0.0–0.2]	0.0 [0.0–0.1]	0.1 [0.0–0.1]	0.4 [0.0–0.7]	0.1141
	Dorsal	0.0 [0.0–0.1]	0.0 [0.0–0.1]	0.1 [0.0–0.1]	0.1 [0.1–0.7]	0.0348*
Tenascin-c	Ventral	8.4 [4.3–16.3]	9.7 [3.6–14.8]	5.7 [2.5–12.4]	8.4 [2.4–13.6]	0.9620
	Dorsal	5.7 [4.9–7.9]	6.4 [4.2–12.7]	6.5 [2.8–11.0]	5.6 [3.0–10.1]	0.8655
Amphiregulin	Ventral	7.1 [5.4–15.4]	4.7 [1.8–8.1]	10.7 [5.5–66.5]	4.07 [2.8–33.1]	0.2433
	Dorsal	7.7 [4.6–39.3]	7.3 [3.6–16.8]	15.0 [6.4–125.8]	2.5 [1.9–6.5]	0.0653

Values represent x-fold expression of the respective gene normalized to housekeeping genes cyclophilin A and ß2-microglobulin. Values are shown as median and interquartile range, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. Statistical significance was accepted at $P < 0.05$. Differences among groups were tested with Kruskal–Wallis test (results are shown in column "Group effect.") followed by Mann–Whitney U test and Bonferroni–Holm adjustment for pairwise comparison. **post hoc* analysis: P-MV$_{contr}$ = *versus* UP-MV$_{contr}$ $P = 0.797$, *versus* UP-MV$_{spon}$ $P = 0.901$, *versus* UP-MV$_{PS}$ $P = 0.026$; UP-MV$_{contr}$ = *versus* UP-MV$_{spon}$ $P = 0.443$, *versus* UP-MV$_{PS}$ $P = 0.030$ and UP-MV$_{spon}$ = *versus* UP-MV$_{PS}$ $P = 0.011$.

Dorsal = dorsal lung regions; IL-6 = interleukin 6; IL-8 = interleukin 8; P-MV$_{contr}$ = protective controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; TNF-α = tumor necrosis factor α; UP-MV$_{contr}$ = controlled ultraprotective mechanical ventilation; UP-MV$_{PS}$ = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV$_{spon}$ = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing; ventral = ventral lung regions.

Table 5. Markers of Inflammation in Bronchoalveolar Lavage Fluid

Protein	P-MV$_{contr}$	UP-MV$_{contr}$	UP-MV$_{spon}$	UP-MV$_{PS}$	Group Effect, *P* Value
Total protein (μg/μl)	1.9 [1.4–2.1]	1.2 [0.9–1.9]	1.7 [1.0–2.5]	1.2 [0.8–1.7]	0.6265
TNF-α (pg/mg total protein)	13.0 [12.9–26.4]	14.4 [6.6–58.6]	17.4 [9.0–108.4]	46.6 [21.9–56.7]	0.4398
IL-6 (pg/mg total protein)	519.8 [230.1–661.7]	322.6 [175.1–890.0]	543.9 [148.9–578.8]	916.3 [294.0–1621.0]	0.6687
IL-8 (pg/mg total protein)	60.5 [9.5–121.4]	380.5 [53.5-11154.0]	1657.0 [44.1–5131.0]	2402.0 [117.4–4502.0]	0.2189
MPO	1.3 [0.6–2.0]	1.6 [0.6–2.2]	1.4 [0.7–1.6]	0.9 [0.5–1.3]	0.5787

Values are shown as median and interquartile range, and were obtained from 28 animals in total (n = 7 per group). There were no missing values. However, a variable number of measurements yielded values less than the detection limit of the respective ELISA kit and were excluded from the analysis. The remaining number of values were: for TNF-α = P-MV$_{contr}$ 3, UP-MV$_{contr}$ 6, UP-MV$_{spon}$ 4 and UP-MV$_{PS}$ 5 animals and for IL-8 = P-MV$_{contr}$ 4, UP-MV$_{contr}$ 4, UP-MV$_{spon}$ 4 and UP-MV$_{PS}$ 7 animals. Statistical significance was accepted at $P < 0.05$. Differences among groups were tested with Kruskal–Wallis test (Results are shown in column "Group effect.") followed by Mann–Whitney U test and Bonferroni–Holm adjustment for pairwise comparison.

IL-6 = interleukin 6; IL-8 = interleukin 8; MPO = activity of myeloperoxidase; P-MV$_{contr}$ = protective controlled mechanical ventilation according to the Acute Respiratory Distress Syndrome network; TNF-α = tumor necrosis factor α; UP-MV$_{contr}$ = controlled ultraprotective mechanical ventilation; UP-MV$_{PS}$ = continuous positive airway pressure combined with pressure supported spontaneous breathing; UP-MV$_{spon}$ = ultraprotective mechanical ventilation with mandatory cycles and superposed unassisted spontaneous breathing.

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Competing Interests

The authors declare no competing interests.

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References

1. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. N Engl J Med 2000; 342:1301–8
2. Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, Schoenfeld D, Thompson BT; National Heart, Lung, and Blood Institute ARDS Clinical Trials Network: Higher *versus* lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. N Engl J Med 2004; 351:327–36
3. Gattinoni L, Protti A, Caironi P, Carlesso E: Ventilator-induced lung injury: The anatomical and physiological framework. Crit Care Med 2010; 38(suppl 10):S539–48

4. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, Gandini G, Herrmann P, Mascia L, Quintel M, Slutsky AS, Gattinoni L, Ranieri VM: Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. Am J Respir Crit Care Med 2007; 175:160–6
5. Bein T, Weber-Carstens S, Goldmann A, Müller T, Staudinger T, Brederlau J, Muellenbach R, Dembinski R, Graf BM, Wewalka M, Philipp A, Wernecke KD, Lubnow M, Slutsky AS: Lower tidal volume strategy (≈3 ml/kg) combined with extracorporeal CO_2 removal *versus* 'conventional' protective ventilation (6 ml/kg) in severe ARDS: The prospective randomized Xtravent-study. Intensive Care Med 2013; 39:847–56
6. Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, Faggiano C, Quintel M, Gattinoni L, Ranieri VM: Tidal volume lower than 6 ml/kg enhances lung protection: Role of extracorporeal carbon dioxide removal. Anesthesiology 2009; 111:826–35
7. Dembinski R, Hochhausen N, Terbeck S, Uhlig S, Dassow C, Schneider M, Schachtrupp A, Henzler D, Rossaint R, Kuhlen R: Pumpless extracorporeal lung assist for protective mechanical ventilation in experimental lung injury. Crit Care Med 2007; 35:2359–66
8. Güldner A, Braune A, Carvalho N, Beda A, Spieth PM, Zeidler S, Uhlig C, Koch T, Andreef M, Pelosi P, Kotzerke J, Gama de Abreu MG: Higher levels of spontaneous breathing induce lung recruitment and reduce global stress/strain in experimental lung injury. Anesthesiology 2014; 120:673–82.
9. Yoshida T, Uchiyama A, Matsuura N, Mashimo T, Fujino Y: The comparison of spontaneous breathing and muscle paralysis in two different severities of experimental lung injury. Crit Care Med 2013; 41:536–45
10. de Abreu MG, Quelhas AD, Spieth P, Bräuer G, Knels L, Kasper M, Pino AV, Bleyl JU, Hübler M, Bozza F, Salluh J, Kuhlisch E, Giannella-Neto A, Koch T: Comparative effects of vaporized perfluorohexane and partial liquid ventilation in oleic acid-induced lung injury. Anesthesiology 2006; 104:278–89
11. Spieth PM, Güldner A, Beda A, Carvalho N, Nowack T, Krause A, Rentzsch I, Suchantke S, Thal SC, Engelhard K, Kasper M, Koch T, Pelosi P, de Abreu MG: Comparative effects of proportional assist and variable pressure support ventilation on lung function and damage in experimental lung injury. Crit Care Med 2012; 40:2654–61
12. Spieth PM, Güldner A, Huhle R, Beda A, Bluth T, Schreiter D, Ragaller M, Gottschlich B, Kiss T, Jaber S, Pelosi P, Koch T, Gama de Abreu M: Short-term effects of noisy pressure support ventilation in patients with acute hypoxemic respiratory failure. Crit Care 2013; 17:R261
13. Hübler M, Souders JE, Shade ED, Hlastala MP, Polissar NL, Glenny RW: Validation of fluorescent-labeled microspheres for measurement of relative blood flow in severely injured lungs. J Appl Physiol (1985) 1999; 87:2381–5
14. Spieth PM, Carvalho AR, Pelosi P, Hoehn C, Meissner C, Kasper M, Hübler M, von Neindorff M, Dassow C, Barrenschee M, Uhlig S, Koch T, de Abreu MG: Variable tidal volumes improve lung protective ventilation strategies in experimental lung injury. Am J Respir Crit Care Med 2009; 179:684–93
15. Rich PB, Reickert CA, Sawada S, Awad SS, Lynch WR, Johnson KJ, Hirschl RB: Effect of rate and inspiratory flow on ventilator-induced lung injury. J Trauma 2000; 49:903–11
16. Peterson BT, Brooks JA, Zack AG: Use of microwave oven for determination of postmortem water volume of lungs. J Appl Physiol Respir Environ Exerc Physiol 1982; 52:1661–3
17. Spieth PM, Knels L, Kasper M, Domingues Quelhas A, Wiedemann B, Lupp A, Hübler M, Neto AG, Gianella Neto A, Koch T, Gama de Abreu M: Effects of vaporized perfluorohexane and partial liquid ventilation on regional distribution of alveolar damage in experimental lung injury. Intensive Care Med 2007; 33:308–14
18. Wang HM, Bodenstein M, Duenges B, Ganatti S, Boehme S, Ning Y, Roehrig B, Markstaller K: Ventilator-associated lung injury superposed to oleic acid infusion or surfactant depletion: Histopathological characteristics of two porcine models of acute lung injury. Eur Surg Res 2010; 45:121–33
19. Matute-Bello G, Frevert CW, Martin TR: Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol 2008; 295:L379–99
20. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: Acute respiratory distress syndrome: The Berlin Definition. JAMA 2012; 307:2526–33
21. Protti A, Andreis DT, Monti M, Santini A, Sparacino CC, Langer T, Votta E, Gatti S, Lombardi L, Leopardi O, Masson S, Cressoni M, Gattinoni L: Lung stress and strain during mechanical ventilation: Any difference between statics and dynamics? Crit Care Med 2013; 41:1046–55
22. Chiumello D, Carlesso E, Cadringher P, Caironi P, Valenza F, Polli F, Tallarini F, Cozzi P, Cressoni M, Colombo A, Marini JJ, Gattinoni L: Lung stress and strain during mechanical ventilation for acute respiratory distress syndrome. Am J Respir Crit Care Med 2008; 178:346–55
23. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D, Brochard L, Richard JC, Lamontagne F, Bhatnagar N, Stewart TE, Guyatt G: Higher *vs* lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: Systematic review and meta-analysis. JAMA 2010; 303:865–73
24. Nielsen ND, Andersen G, Kjaergaard B, Staerkind ME, Larsson A: Alveolar accumulation/concentration of nitrogen during apneic oxygenation with arteriovenous carbon dioxide removal. ASAIO J 2010; 56:30–4
25. Broccard AF, Hotchkiss JR, Suzuki S, Olson D, Marini JJ: Effects of mean airway pressure and tidal excursion on lung injury induced by mechanical ventilation in an isolated perfused rabbit lung model. Crit Care Med 1999; 27:1533–41
26. Pelosi P, Goldner M, McKibben A, Adams A, Eccher G, Caironi P, Losappio S, Gattinoni L, Marini JJ: Recruitment and derecruitment during acute respiratory failure: An experimental study. Am J Respir Crit Care Med 2001; 164:122–30
27. Gama de Abreu M, Spieth PM, Pelosi P, Carvalho AR, Walther C, Schreiber-Ferstl A, Aikele P, Neykova B, Hübler M, Koch T: Noisy pessure support ventilation: A pilot study on a new assisted ventilation mode in experimental lung injury. Crit Care Med 2008; 36:818–27
28. Carvalho AR, Spieth PM, Güldner A, Cuevas M, Carvalho NC, Beda A, Spieth S, Stroczynski C, Wiedemann B, Koch T, Pelosi P, de Abreu MG: Distribution of regional lung aeration and perfusion during conventional and noisy pressure support ventilation in experimental lung injury. J Appl Physiol (1985) 2011; 110:1083–92
29. Güldner A, Braune A, Carvalho N, Beda A, Zeidler S, Wiedemann B, Wunderlich G, Andreeff M, Uhlig C, Spieth PM, Koch T, Pelosi P, Kotzerke J, de Abreu MG: Higher levels of spontaneous breathing induce lung recruitment and reduce global stress/strain in experimental lung injury. Anesthesiology 2014; 120:673–82
30. Grasso S, Stripoli T, Mazzone P, Pezzuto M, Lacitignola L, Centonze P, Guarracino A, Esposito C, Herrmann P, Quintel M, Trerotoli P, Bruno F, Crovace A, Staffieri F: Low respiratory rate plus minimally invasive extracorporeal CO_2 removal decreases systemic and pulmonary inflammatory mediators in experimental Acute Respiratory Distress Syndrome. Crit Care Med 2014; 42:e451–60
31. Spieth PM, Carvalho AR, Güldner A, Kasper M, Schubert R, Carvalho NC, Beda A, Dassow C, Uhlig S, Koch T, Pelosi P, Gama de Abreu M: Pressure support improves oxygenation and lung protection compared to pressure-controlled

ventilation and is further improved by random variation of pressure support. Crit Care Med 2011; 39:746–55

32. Saddy F, Oliveira GP, Garcia CS, Nardelli LM, Rzezinski AF, Ornellas DS, Morales MM, Capelozzi VL, Pelosi P, Rocco PR: Assisted ventilation modes reduce the expression of lung inflammatory and fibrogenic mediators in a model of mild acute lung injury. Intensive Care Med 2010; 36:1417–26
33. Yoshida T, Torsani V, Gomes S, De Santis RR, Beraldo MA, Costa EL, Tucci MR, Zin WA, Kavanagh BP, Amato MB: Spontaneous effort causes occult pendelluft during mechanical ventilation. Am J Respir Crit Care Med 2013; 188:1420–7
34. Mauri T, Bellani G, Grasselli G, Confalonieri A, Rona R, Patroniti N, Pesenti A: Patient-ventilator interaction in ARDS patients with extremely low compliance undergoing ECMO: A novel approach based on diaphragm electrical activity. Intensive Care Med 2013; 39:282–91
35. Nosotti M, Rosso L, Tosi D, Palleschi A, Mendogni P, Nataloni IF, Crotti S, Tarsia P: Extracorporeal membrane oxygenation with spontaneous breathing as a bridge to lung transplantation. Interact Cardiovasc Thorac Surg 2013; 16:55–9

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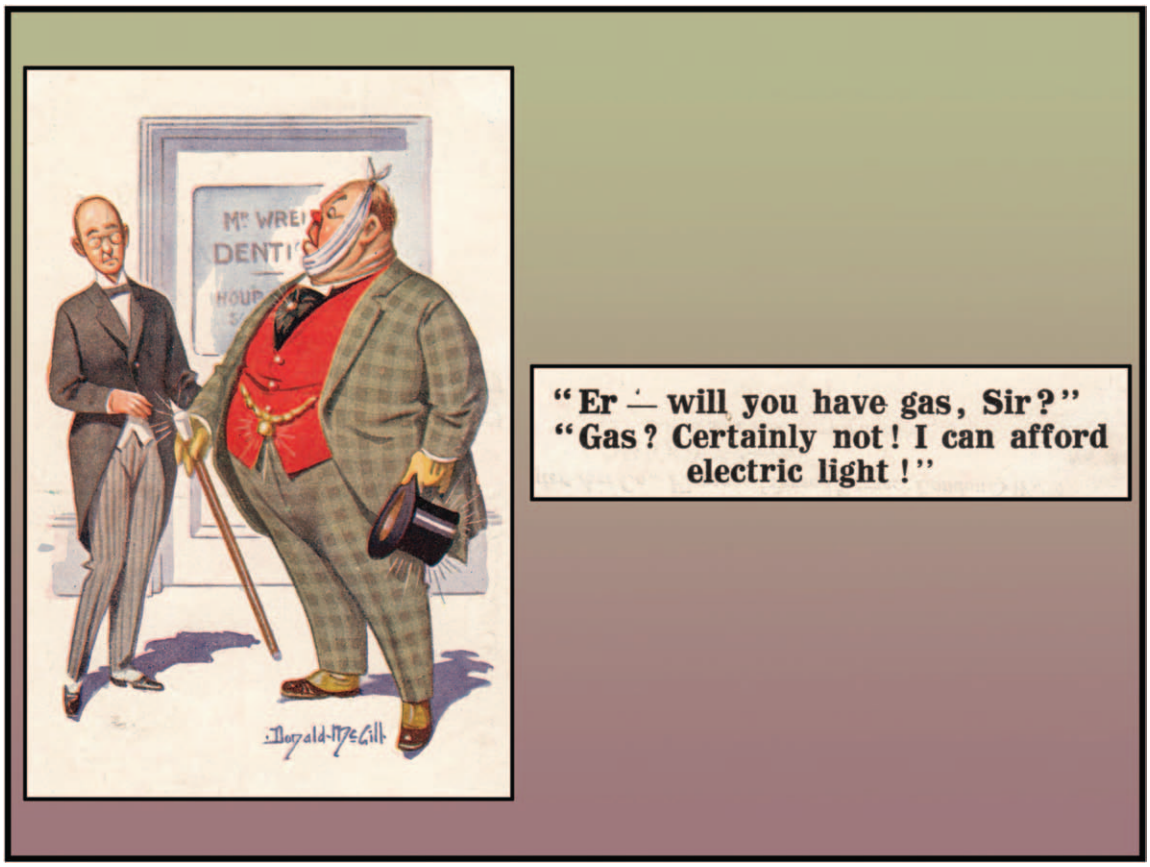

Mailed in 1920, this postcard featured the humorous artistry of British cartoonist Donald McGill (1875–1962). Perhaps aptly named "Mr. Wrench," McGill's slim dentist (*left*) in this pictorial enquires whether his portly patient wants [laughing] gas. The latter indignantly countered (*right*) with "Gas? Certainly not! I can afford electric light!" As he often did, McGill fully exploited the ambiguity of the word "gas" for both illuminating ("natural gas") and anesthetic ("laughing gas") purposes. This postcard is part of the Wood Library-Museum's Ben Z. Swanson Collection. (Copyright © the American Society of Anesthesiologists, Inc.)

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