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EXPERIMENTAL

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# Standardized lung recruitment during high frequency and conventional ventilation: similar pathophysiologic and inflammatory responses in an animal model of respiratory distress syndrome

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**Abstract** *Objective:* To evaluate standardized lung recruitment strategy during both high frequency oscillation (HFO) and volume-targeted conventional ventilation (CV+V) in spontaneously breathing piglets with surfactant washout on pathophysiologic and inflammatory responses. *Design:* Prospective animal study. *Setting:* Research laboratory. *Subjects:* Twenty-four newborn piglets. *Interventions:* We compared pressure support and synchronized intermittent mandatory ventilation, both with targeted tidal volumes, (PSV+V, SIMV+V) to HFO. Animals underwent saline lavage to produce lung injury, received artificial surfactant and were randomized to one of the three treatment groups (each $n$=8). After injury and surfactant replacement, lung volumes were recruited in all groups using a standard protocol. Ventilation continued for 6 h. *Measurements and main results:* Arterial and central venous pressures, heart rates, blood pressure and arterial blood gases were continuously monitored. At baseline, post lung injury and 6 h we collected serum and bronchoalveolar lavage samples for proinflammatory cytokines: IL 6, IL 8 and TNF-$\alpha$, and performed static pressure-volume (P/V) curves. Lungs were fixed for morphometrics and histopathologic analysis. No physiologic differences were found. Analysis of P/V curves showed higher opening pressures after lung injury in the HFO group compared to the SIMV+V group ($p$<0.05); no differences persisted after treatment. We saw no differences in change in proinflammatory cytokine levels. Histopathology and morphometrics were similar. Mean airway pressure ($P_{aw}$) was highest in the HFO group compared to SIMV+V ($p$<0.002). *Conclusions:* Using a standardized lung recruitment strategy in spontaneously breathing animals, CV+V produced equivalent pathophysiologic outcomes without an increase in proinflammatory cytokines when compared to HFO.

**Keywords** High frequency oscillation · Mechanical ventilation · Respiratory failure · Lung injury · Cytokines

## Introduction

Despite advances in the management of respiratory distress syndrome (RDS), neonatal chronic lung disease (CLD) is still a major problem in mechanically ventilated premature infants. Recruitment of collapsed alveoli results in improved lung compliance [1, 2] and gas exchange [3, 4]. However, mechanical ventilation itself can worsen acute lung injury. This has been demonstrated in clinical studies of neonates with RDS [5] and in animal studies [6, 7]. In adults with acute respiratory distress syndrome, the use of lung protective strategies has been shown to reduce mortality [8]. In children, high frequency ventilation has been advocated as a lung protective strategy. By delivering smaller tidal volumes (Vts) at very high rates and by recruiting and maintaining lung volumes, this type of ventilation has been reported to produce less acute and long-term lung injury [9].

These approaches may, in part, improve outcome by altering the endogenous pulmonary inflammatory response. Recent experimental studies have provided three lines of evidence suggesting that mechanical ventilation can initiate or exacerbate an inflammatory response: (1) pathologic evidence of neutrophil infiltration [10]; (2) increased cytokine levels in lung lavage [11] and (3) increased cytokine levels in the systemic circulation [7, 12].

These strategies have been incompletely studied in neonates. In addition, the ability of high frequency oscillation (HFO) to improve pulmonary outcomes has been far more evident in the laboratory than in clinical studies, perhaps in part due to the different lung recruitment strategies used for CV and HFO. To investigate these possibilities further, we hypothesized that the use of standardized lung volume recruitment techniques during volume-targeted conventional ventilation or high frequency oscillatory ventilation would produce equivalent pathophysiologic outcomes and inflammatory responses in a spontaneously breathing animal model of RDS.

## Materials and methods

### Animal preparation

The Institutional Animal Care and Use Committee of Children's Health Care–St.Paul, Minnesota, approved this study. Animals were cared for in accordance with National Institute of Health guidelines [13]. We studied 24 newborn piglets, weighing 800–1725 g (mean 1335±256 g). The animals were anesthetized with ketamine (50 mg/kg per dose) and intubated with neonatal cuffed "Hi Lo" endotracheal tubes (ETs) size 3.0–3.5 (Mallinckrodt, St. Louis, MO). All were initially ventilated with a pressure-limited volume-targeted infant ventilator (Dräger Babylog 8000, Dräger America, Telford, PA) with the following settings: rate 30, PEEP 5 $cmH_2O$, Vt 6 ml/kg, peak inspiratory pressure (PIP) automatically adjusted to deliver set Vt, inspiratory time adequate for end-expiratory gas flow to return to zero, and $FiO_2$ of 1.0. The animals were then paralyzed with pancuronium bromide (0.2 mg/kg) i.m. Catheters were placed in the internal carotid artery and external jugular vein. A tracheostomy was performed and secured to prevent air leaks. An intravascular pH/$PaO_2$/$PaCO_2$/temperature continuous monitoring sensor (Paratrend-7, Diametrics Medical, St. Paul, MN) was threaded into the carotid artery catheter. The animals were hydrated with $D_5$ 1/4 normal saline and 10 mEq KCl/l at 6 cc/kg per h. Analgesia and sedation were maintained with a continuous intravenous infusion of ketamine at 5 mg/kg per h (1 ml/kg per h). After instrumentation, the animals underwent saline lavage to produce lung injury, received artificial surfactant and were randomized to one of three treatment groups (each $n$=8). The piglets were allowed to breathe spontaneously during the entire 6-h study period.

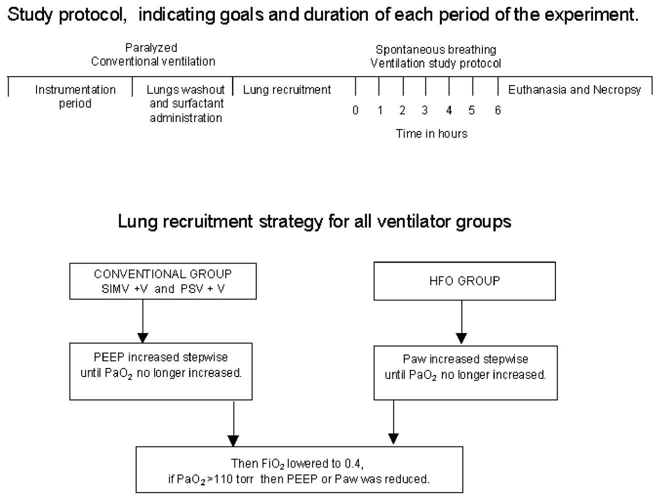


**Fig. 1** Study protocol and lung volume recruitment strategy

### Lung injury model

Pulmonary compromise was induced by repeated normal saline lavage [14]. The lungs were filled with normal saline until a meniscus was seen in the ET. Saline remained in the lungs for 3–5 min during ventilation with a Vt of 10 cc/kg. Animals qualified when: $PaO_2$ was less than 60 torr (8 kPa) in $FiO_2$ 1.0 and if there was a minimum 30% dynamic lung compliance reduction (Ventrak 1550, Novametrix Medical Systems, Wallingford, CT). During lung injury, the ventilator rate was adjusted to maintain $PaCO_2$ between 35 and 45 torr (5–6 kPa). All animals received surfactant, (Survanta, Ross Products Division, Abbott Laboratories, Chicago, IL) 4 ml/kg; the surfactant was administered according to the manufacturer's recommendations by manual ventilation with 100% oxygen through the pressure monitoring port of the "Hi Lo" ET. After surfactant instillation, the animals were randomized to one of the following groups:

1. HFO (Sensor Medics 3100A, Yorba Linda, CA).
2. Synchronized intermittent mandatory ventilation with Vt targeting (SIMV+V; Dräger Babylog 8000).
3. Pressure support with Vt targeting (PSV+V; Dräger Babylog 8000).

### Lung volume recruitment and ventilator adjustment

With HFO, the mean airway pressure ($P_{aw}$) was initially adjusted to 2 $cmH_2O$ higher than that set during conventional ventilation (CV) and increased in steps of 2 $cmH_2O$ increments until $PaO_2$ no longer increased. We used $PaO_2$ as a surrogate marker for lung volume recruitment during this study, as lung volumes were not directly measured [15]. When $PaO_2$ no longer increased or began to fall, we considered this evidence that the lung was fully inflated, with the possibility of beginning overdistention [16]. At this point, $P_{aw}$ was decreased by 2 $cmH_2O$. While maintaining $PaO_2$ between 90 and 110 torr (12–15 kPa), $FiO_2$ was weaned to 0.40. When $FiO_2$ was stable at 0.40, $P_{aw}$ was further decreased to keep the $PaO_2$ within the target range (Fig. 1, Table 1). Amplitude was adjusted to maintain $PaCO_2$ between 35 and 45 torr (5–6 kPa). Frequency was set at 10 Hz and I: E ratio at 1:2 throughout the experiment. Flow for HFO was set at 10 l/min.

**Table 1** Gas exchange and hemodynamics, before and after lung injury, during recruitment and treatment stages. Data as means ± SD. To convert torr to kpA, multiply the value by 0.1333

| | Groups | $FiO_2$ | PIP | PEEP | $P_{aw}$ | HR | MBP | pH | $PCO_2$ | $PO_2$ | a/A | OI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Healthy | – | 0.21 | 17±5 | 5 | 6.7±1.7 | 173±35 | 60±11 | 7.40±0.04 | 42±5 | 81±20 | 0.86±0.23 | 1.86±0.7 |
| Post lavage | – | 1.0 | 27±6 | 5 | 12.7±2.1 | 159±23 | 62±12 | 7.32±0.08 | 43±9 | 50±7 | 0.07±0.01 | 26.00±6.6 |
| Recruited | HFO | 1.0 | 29±6 | – | 24±6[a] | 183±31 | 54±4 | 7.39±0.04 | 36±2 | 472±140 | 0.72±0.21 | 5.5±2.5 |
| | SIMV+V | 1.0 | 24±4 | 14±2 | 17±3 | 166±22 | 66±12 | 7.38±0.07 | 42±5 | 467±162 | 2.8±1.4 | 1.6±1.3 |
| | PSV+V | 1.0 | 22±6 | 13±3 | 16±4 | 157±20 | 61±12 | 7.36±0.06 | 41±5 | 461±151 | 2.05±1.5 | 2.35±1.77 |
| 1 hour | HFO | 0.44±0.15 | 18±5 | – | 15.3±3.1 | 176±25 | 54±6 | 7.38±0.07 | 40±4 | 104±5 | 0.47±0.22 | 6.75±3.34 |
| | SIMV+V | 0.37±0.11 | 22±10 | 11±5 | 13.7±5.7 | 168±42 | 60±8 | 7.37±0.07 | 40±5 | 101±7 | 0.54±0.19 | 5.3±3.58 |
| | PSV+V | 0.42±0.11 | 20±7 | 12±3 | 14.4±4.5 | 163±32 | 62±11 | 7.40±0.06 | 39±6 | 104±5 | 0.46±0.14 | 5.95±2.41 |
| 3 hours | HFO | 0.37±0.11 | 18±5 | – | 15±2.8 | 166±16 | 54±6 | 7.39±0.08 | 41±3 | 104±4 | 0.57±0.23 | 5.54±2.42 |
| | SIMV+V | 0.37±0.09 | 22±11 | 11±5 | 13±4.9 | 167±36 | 58±9 | 7.42±0.05 | 38±4 | 105±5 | 0.56±0.19 | 4.70±2.25 |
| | PSV+V | 0.44±0.11 | 19±5 | 12±3 | 14±2.8 | 151±20 | 57±9 | 7.38±0.06 | 41±6 | 103±7 | 0.44±0.15 | 6.08±2.71 |
| 6 hours | HFO | 0.31±0.09 | 17±5 | – | 14±2.8 | 141±16 | 55±9 | 7.40±0.09 | 40±3 | 106±4 | 0.76±0.28 | 4.17±2.06 |
| | SIMV+V | 0.41±0.16 | 21±7 | 11±5 | 11.5±5 | 143±18 | 54±15 | 7.38±0.08 | 41±5 | 104±4 | 0.54±0.26 | 4.51±2.53 |
| | PSV+V | 0.44±0.15 | 21±6 | 11±3 | 13.5±2.6 | 141±25 | 52±10 | 7.39±0.09 | 42±8 | 102±5 | 0.47±0.19 | 5.76±1.98 |

*HFO* high frequency oscillation, *SIMV+V* synchronized intermittent mandatory ventilation + volume, *PSV+V* pressure support ventilation + volume, *PIP* peak inspiratory pressure ($cmH_2O$), *PEEP* positive end-expiratory pressure, $P_{aw}$ mean airway pressure ($cmH_2O$), *HR* heart rate (beats/min), *MBP* mean arterial blood pressure (mmHg), $PCO_2$ and $PO_2$ arterial measurements of $CO_2$ and $O_2$ in torr, *OI* oxygenation index, *a/A* arterial/alveolar oxygenation ratio

[a] $p<0.01$ HFO versus CV+VV

## Conventional ventilation

Two volume-targeted conventional ventilation strategies were used: PSV+V and SIMV+V. Vt for both was set at 6 ml/kg [16] and maintained by automatically varying PIP using the ventilator software. We then used stepwise 2 $cmH_2O$ increases in PEEP at $FiO_2$ of 1.0 until $PaO_2$ no longer increased or began to fall [17]. As with HFO, this was considered to reflect full lung inflation. PEEP was then dropped to the previous level; while maintaining $PaO_2$ between 90 and 110 torr (12–15 kPa), $FiO_2$ was weaned to 0.40. When $FiO_2$ was stable at 0.40, $P_{aw}$ was decreased further (Fig. 1, Table 1) to keep $PaO_2$ within the target range. The respiratory rate was set at 40/min and adjusted only if the animal's spontaneous respiratory efforts were inadequate to maintain $PaCO_2$ between 35 and 45 torr (5–6 kPa). Inspiratory time was adjusted so that end-expiratory flow returned to zero.

## Physiologic measurements

We continuously monitored arterial blood gases, oxygen saturations, intravascular pressures and vital signs (Space Labs, Redmond, WA). Respiratory system compliance and resistance were measured before and after the induction of lung injury. Chest X-rays were obtained after lung washout, after optimization of the therapies applied and at study end.

Physiologic data recording points were:

1. Prior to lung injury
2. Immediately following lung injury
3. 30 min following surfactant administration
4. Every hour during the 6-h study.

## Pressure-volume curves

Static inspiratory pressure-volume (P/V) curves were obtained before, after lung injury, following surfactant replacement and at the study end. Airway pressures were measured using a pressure transducer (Endevco, Meggitt, CA) attached to a side port of the ET's proximal end, and tracings were recorded on a calibrated multi-channel recorder. Animals were given neuromuscular blockade with pancuronium bromide (0.1 mg/kg i.v.) while obtaining P/V curves. The animals exhaled to resting volume during ventilator disconnect before each measurement. Volume aliquots of oxygen were delivered using a graduated syringe. The animals were given at least three tidal ventilator breaths between the P/V measurements.

## Cytokine analysis

Serum samples for pro-inflammatory cytokines IL6, IL8 and TNF-$\alpha$ were obtained before, after lung injury and at termination of the study, with simultaneous bronchoalveolar lavages (BALs). BAL samples were obtained by lung washout with two aliquots of 10 cc of normal saline. Washout samples were suctioned and collected for analysis. Serum samples were initially collected in microtainer vials with no additives and stored at 4°C overnight. The samples were spun at 6,600 G for 2 min and the serum was stored at −70°C. BAL samples were handled similarly at 4°C after initial filtration through a 100 $\mu$m cell strainer. The filtered aspirate was spun at 400 G for 15 min at 4°C. The supernatant was subsequently spun at 8,000 G for 30 min at 4°C. Sample aliquots were stored at −70°C for cytokine analysis later. Both serum and BAL samples were analyzed by ELISA technique (IL6, R & D Systems, MN; IL8, TNF-$\alpha$, BioSource, CA). Samples were run in duplicate and assay analysis was blinded.

## Histologic analysis

At study end the animals were killed and their lungs were removed en bloc. The lungs were inflated to 30 $cmH_2O$ pressure. The left main stem bronchus was clamped. The right lung was lavaged with two aliquots of 10 cc of normal saline and inflated by hand bagging five times with a PIP of 22 $cmH_2O$ and PEEP of 5 $cmH_2O$. The BAL fluid was then aspirated.

The clamped left lung was removed and immersed in 10% formalin. This was sent for morphometric analysis and lung histopathologic studies. Slides from the craniodorsal (non-dependent) and caudal ventral (dependent) lobes were stained with hematoxylin and eosin. A pediatric pathologist blinded to treatment groups assessed a ratio of total cellular tissue to airspace as previously described using a computer-assisted morphometrics analyzer [18]. Variables for histopathology scoring included alveolar and interstitial inflammation, alveolar and interstitial hemorrhage, edema, atelectasis and necrosis [18]. These variables were scored on a scale from 0 (no injury), 1 (25% injury), 2 (50% injury), 3 (75% injury) to 4 (maximum area of injury) by the pathologist.

Statistical analysis

Primary outcome variables were changes in proinflammatory cytokine levels from post-injury to end of study. Physiologic variables were heart rate, mean blood pressure, central venous pressure (CVP), pH, $pCO_2$, oxygenation index (OI = Paw x [FiO2 x 100])/ PaO2), arterial/alveolar (a/A) ratio ($PaO_2 \div [(700 \times FiO_2) - (PaCO_2/0.8])$, mean airway pressure ($P_{aw}$) and histopathologic changes. The data were analyzed using statistical software (Statview, SAS Institute, Cary, NC). Continuous data were analyzed using paired and unpaired *t*-tests or ANOVA; inflammatory cytokine and histopathologic lung injury scores were analyzed using the non-parametric Kruskall Wallis test. Probability values of less than 0.05 were accepted as statistically significant.

## Results

There were no physiologic differences among the groups at baseline, after injury and throughout the 6-h experiment protocol (Table 1). The set ventilator rates from baseline to end of study for SIMV+V animals ranged from 55±12 to 30±10, and for the PSV+V group from 55±26 to 43±31; total respiratory rates for SIMV+V were 77±30 to 86±36 and for PSV+V from 99±31 to 87±29. During SIMV+V, the percent of spontaneous breaths rose from 22±7.6% initially to 55±19% at the end; 100% of PSV+V breaths were spontaneously initiated and supported. $P_{aw}$ for lung volume recruitment during HFO was significantly higher than that required during CV+V, and was higher than that required during SIMV+V throughout ($p<0.01$; Table 1). Analysis of P/V curves showed a higher inflation limb inflection point, or opening pressure of the pressure-volume curve, after lung injury in the HFO group compared to the SIMV+V group ($p<0.05$); no

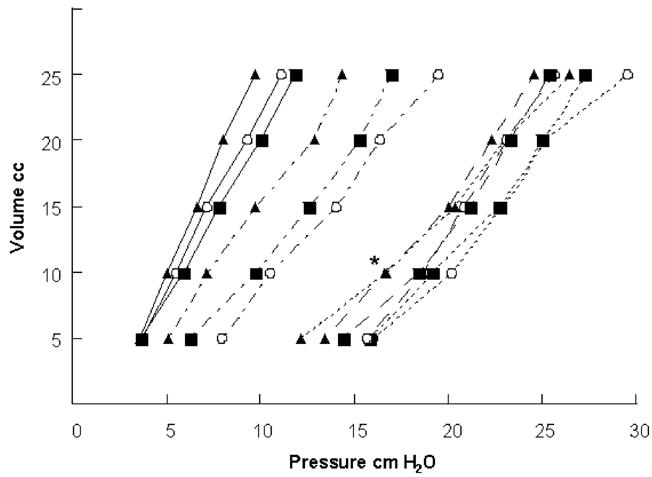


**Fig. 2** Static inspiratory pressure-volume relationships of the total respiratory system for the experimental groups. *Solid triangles* synchronized intermittent mandatory ventilation + volume, *solid squares* pressure support + volume, *open circles* high frequency oscillation. Measurements obtained after lung injury but before randomization (*dotted lines*) show a significant shift to the right in comparison to the measurements from the uninjured animals (*straight line*). The measurements obtained at the end of the experiment (*interrupted* and *dotted lines*) show improved compliance for all the groups. The surfactant replacement (*interrupted lines*) states were similar to the post-injury state. Post lung injury, the animals in the synchronized intermittent mandatory ventilation + volume group showed lower opening pressure (*$p<0.05$ versus high frequency oscillation) The curves have not been started from zero for reasons of simplicity

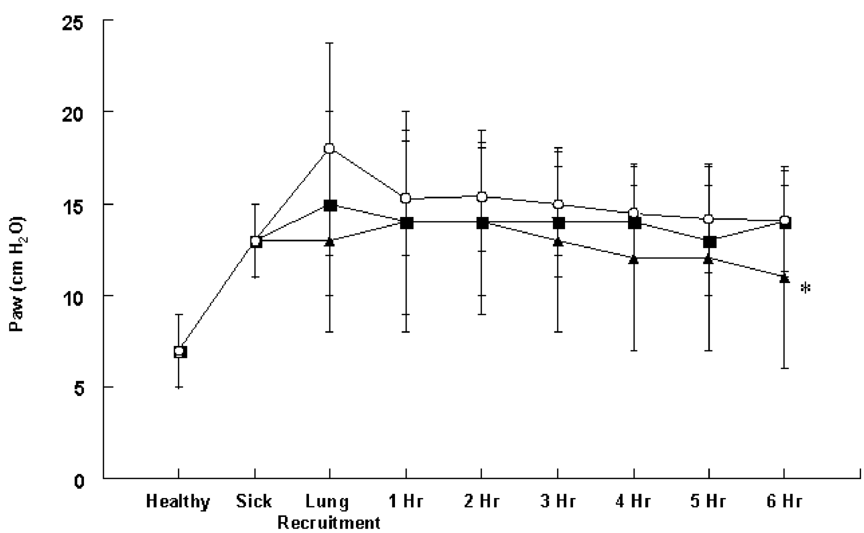


**Fig. 3** Mean airway pressure ($P_{aw}$) throughout the study. *Solid squares* pressure support + volume, *solid triangles* synchronized intermittent mandatory ventilation + volume, *open circles* high frequency oscillation. The $P_{aw}$ was lowest in the synchronized intermittent mandatory ventilation + volume group (*$p<0.002$ versus high frequency oscillation)

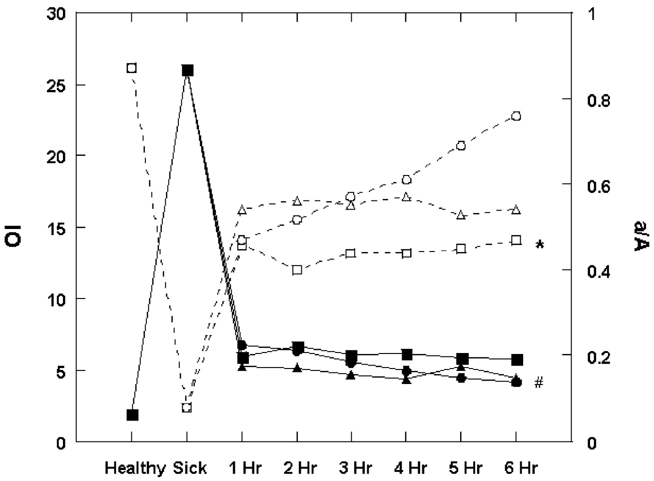


**Fig. 4** Oxygenation Index (*OI*) and arterial/alveolar oxygenation ratio (a/A) throughout the study. All the groups had similar effects on oxygenation. *Solid squares* OI pressure support + volume, *open squares* a/A pressure support + volume, *solid triangles* OI synchronized intermittent mandatory ventilation + volume, *open triangles* a/A synchronized intermittent mandatory ventilation + volume, *solid circles* OI high frequency oscillation, *open circles* a/A high frequency oscillation. The OI was lowest in the synchronized intermittent mandatory ventilation + volume group (#$p<0.05$ versus other groups) and the a/A was lowest in the pressure support + volume group (*$p<0.05$ versus other groups)

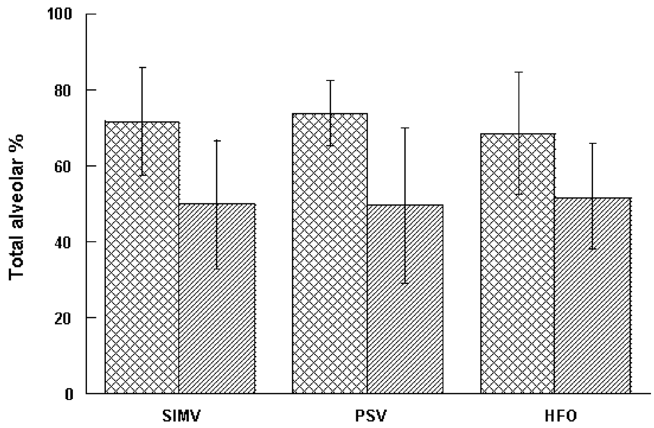


**Fig. 5** Lung morphometric scoring plotted as total alveolar percentage. *Cross-hatched columns* non-dependent area, *hatched columns* dependent area of the lung

differences persisted after surfactant treatment (Fig. 2). The mean airway pressure was lowest in the SIMV+V group compared to HFO ($p<0.002$, Fig. 3); arterial blood gases were not different at any time (Table 1). OI was lowest in the SIMV+V group ($p<0.05$); a/A was lowest in the PSV+V group (Fig. 4). Chest X-rays taken in the different groups were similar.

We observed no significant differences in inflammatory cytokines IL6, IL8 and TNF-$\alpha$ responses at any time (Table 2). All ventilation groups had similar baseline cytokine levels; both serum and BAL cytokines were elevated post lung injury ($p<0.05$) without differences among the groups (Table 2). We also saw a trend towards reduction in inflammatory cytokine response with treatment in all groups. There were no significant differences in responses across the different treatment groups or in the change in inflammatory cytokines from time of injury to end of study. Morphometrics scores and histopathology were not significantly different at 6 h (Figs. 5, 6).

## Discussion

In this study, we evaluated the impact of lung volume recruitment and ventilator treatment strategy on physiologic, pathologic and inflammatory outcomes in an animal model of RDS. Unlike previous laboratory studies of HFO and CV, we found no evidence that the mode of ventilation altered outcomes when a standardized technique initially to maximize oxygenation, and presumably lung volume, was used [19, 20]. In fact, there were no important differences in any studied outcome among the groups. These findings suggest, at least over the short

**Table 2** Changes in serum and bronchoalveolar lavage (*BAL*) cytokines (IL6, IL8, TNF-$\alpha$ response in pg/ml) before (*healthy*) and after lung injury (*sick*) and end of treatment stages. Data as means ± SEM

| | IL6 | | | IL8 | | | TNF $\alpha$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Sick | End | Healthy | Sick | End | Healthy | Sick | End |
| Serum cytokines | | | | | | | | | |
| HFO | 131±53.1 | 377±123 | 300±60.5 | 66±49 | 81±69 | 61±53 | 15±18 | 10±14 | 9±12 |
| SIMV+V | 35±14.1 | 264±96 | 139±41.6 | 33±35 | 37±35 | 27±31 | 40±52 | 45±60 | 14±17 |
| PSV+V | 144±77.6 | 515±129 | 374±124 | 46±50 | 51±58 | 44±54 | 32±58 | 62±115 | 16±19 |
| BAL cytokines | | | | | | | | | |
| HFO | 15±3.1 | 29±3.4 | 106±25.6 | 286±75 | 442±117 | 94±21 | 15±1.5 | 10±1.9 | 10±1.5 |
| SIMV+V | 14±4.3 | 55±8.9 | 90±15.5 | 185±43.4 | 468±229.3 | 138±32 | 40±1.2 | 45±2.8 | 14±1 |
| PSV+V | 13±3.5 | 43±10.8 | 85±27.1 | 211±35.9 | 222±33.5 | 327±230 | 32±1.5 | 62±1.9 | 16±5.5 |

*HFO* high frequency oscillation, *SIMV+V* synchronized intermittent mandatory ventilation + volume, *PSV+V* pressure support ventilation + volume

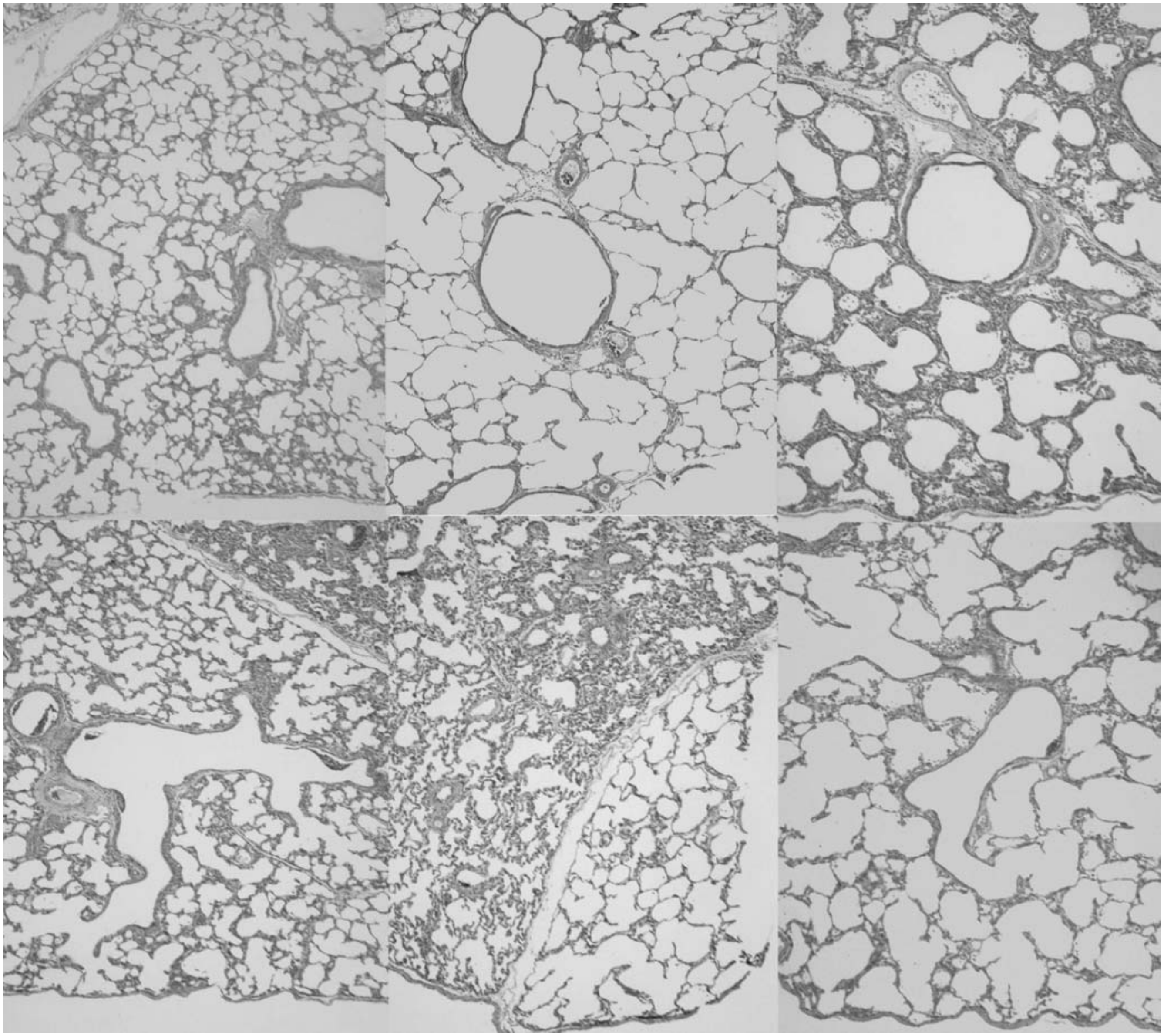

**Fig. 6** Sections of lung from non-dependent lobes (*top panels*) and dependent lobes (*bottom panels*). Animals treated with SIMV (*left pair*), PSV (*middle pair*) and HFO (*right pair*) all show minimal pathologic change. The non-dependent PSV and both HFO-treated lobes, however, show patchy acute hyperinflation causing rounded air spaces and the false impression of interstitial thickening. Histologic scores, however, were not different. (All sections stained with H&E and photographed at 50X magnification)

duration of this study, that a conventional ventilation strategy that limits Vt while maintaining overall lung volumes may be just as effective as high frequency techniques in protecting the surfactant-treated lung.

For lung recruitment we used a standardized stepwise, rapid adjustment sequence of $P_{aw}$. This was accomplished by altering PEEP during CV and $P_{aw}$ directly during HFO. Arterial oxygenation changes were our surrogate for lung volume changes, as changes in oxygenation and lung volume have been previously correlated [15]. The optimal use of PEEP during mechanical ventilation has been extensively studied [4]. Clearly, either high or low PEEP (and $P_{aw}$) can significantly increase lung injury patterns in terms of both inflammatory cytokine response and pathologic lung injury. We used a $P_{aw}$ of 13±2 $cmH_20$ for conventional groups and 16±1 $cmH_2O$ in the HFO group during this study. The requirement of a higher $P_{aw}$ during HFO than that seen during CV to produce equivalent gas exchange is consistent with previous studies [9, 21].

We measured and limited Vt in addition to initially recruiting the lung. Studies have shown that both low and high Vts can exacerbate lung injury [22, 23, 24]. Low Vts result in micro-atelectasis and re-inflation injury, while high Vts overdistend alveolar regions. We used low normal Vt in this animal model to avoid such problems [16]. In addition, by allowing the animals to breathe spontaneously, we potentially avoided inadvertent over- or under-ventilation. As HFO operates using different mechanisms and tiny Vts, a similar regulation of Vt is not necessary.

We assessed lung mechanics in our experimental protocol by measuring total respiratory system P/V relationships. The significant right shift of the P/V relationships after lung lavage in the experimental groups, in comparison with the uninjured states, indicates that all injured groups experienced similar reductions in lung compliance. Although all animals met our physiologic criteria for lung injury and group comparisons showed no significant differences, we did find lower opening pressures in the SIMV+V group initially, which were no longer present after surfactant administration or subsequent treatment ($p<0.05$; Fig. 2). At the completion of the experiment, all groups exhibited similar improvements in lung compliance in comparison to post-injury measurements. Other investigators have observed these ventilation strategy-dependent changes in P/V relationships [24, 25].

We did not study a control group of animals ventilated using conventional ventilatory techniques without synchronization, volume targeting or PEEP adjustment to improve lung volumes. Numerous previous studies have shown such techniques to be ineffective in preventing lung injury and maintaining gas exchange when compared to HFO [15, 19, 20, 22]. Most recently, van Kaam and colleagues, in a similar newborn piglet model, compared conventional pressure-controlled ventilation using modest PEEP settings to CV and HFO, both with standardized volume recruitment techniques [26]. Like others, they found increased lung injury and worsened gas exchange during CV without lung recruitment when compared to HFO; they also found that CV with lung recruitment produced gas exchange and pathologic outcomes similar to those seen during HFO and superior to standard CV. Our study expands this observation in spontaneously breathing animals, using two newer modes of CV with Vt-targeting techniques.

Ventilator-induced lung injury increases proinflammatory cytokines in adult lungs and alters proinflammatory mediators and cytokine mRNA expression in the preterm lung [27, 28, 29]. We asked if volume-targeted conventional ventilation with a standardized lung recruitment strategy produced similar inflammatory responses when compared to HFO. We saw significant cytokine elevations following lung injury and varying responses to our ventilatory treatment. However, we found no significant differences across the groups, suggesting equivalent lung injury. Previous studies have shown that high frequency oscillatory ventilation, when used in animal models with a strategy of lung recruitment, improved gas exchange and lung mechanics, promoted uniform inflation, reduced air leak and decreased the concentration of inflammatory mediators in the lung, as compared with conventional mechanical ventilation without recruitment [29, 30]. In contrast, with standardized recruitment, our study showed no differences in physiologic, pathologic or cytokine response, supporting the contention that ventilation strategy rather than ventilator type impacts the development of injury.

Our inability to demonstrate significant differences in inflammatory response may be due to a number of causes. There may, in fact, be no real differences. The lung injury itself may not be severe enough to allow differences to appear. As in all small animal studies, sample size may play a role. If we define a reduction in proinflammatory cytokine response of 1 standard deviation as clinically important, our results suggest that a total sample size of at least 16–32 animals per group would be necessary to detect this change with a power of 80% and two-sided alpha of 0.05. In addition, our cytokine responses showed no significant trend, suggesting that a larger sample still might not show differences. Finally, this neonatal animal model, while similar in size to preterm newborns, is not a true model of naturally occurring surfactant deficiency. Still, our conclusion that ventilation strategy may be more important that ventilator type seems worthy of continued investigation.

Other recent works support our findings. In a comparison of HFO to CV in paralyzed surfactant-deficient rabbits with and without surfactant treatment, HFO did not produce better gas exchange, lung deflation stability or pathologic lung injury when lung expansion was preserved. The authors concluded that achieving and maintaining alveolar expansion was more important than ventilator type per se [31]. Similarly, in surfactant-deficient rats, lung volume recruitment during CV and HFO produced no improvement in lung mechanics or protein influx during HFO, again suggesting a critical role for lung volume recruitment in physiologic and pathologic outcome [32]. Finally, a study of sustained inflation as a recruiting technique during CV and HFO in surfactant-deficient rabbits showed similar oxygenation, lung pathology and myeloperoxidase content in the two groups [22]. Taken together, these studies support the concept that lung recruitment and the maintenance of lung volumes, with any ventilator type, may be critical in the ultimate pulmonary pathologic and inflammatory outcomes.

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