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Positive End-Expiratory Pressure and Tidal Volume During Initial Ventilation of Preterm Lambs

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ABSTRACT: Positive end-expiratory pressure (PEEP) protects the lung from injury during sustained ventilation, but its role in protecting the lung from injury during the initiation of ventilation in the delivery room is not established. We aimed to evaluate whether PEEP and/or tidal volume (V_T) within the first 15-min of ventilation are protective against lung injury. Operatively delivered preterm lambs (133 ± 1 d gestation) were randomly assigned to unventilated controls or to one of four 15 min ventilation interventions: 1) V_T15 mL/kg, PEEP 0 cm H_2O; 2) V_T15 mL/kg, PEEP 5 cm H_2O; 3) V_T8 mL/kg, PEEP 0 cm H_2O; and 4) V_T8 mL/kg, PEEP 5 cm H_2O. Each group was subsequently ventilated with V_T <10 mL/kg, PEEP 5 cm H_2O for 1 h 45 min. Lung function was assessed and measurements of lung injury were evaluated postmortem. After the 15 min ventilation maneuver, the V_T15 groups were hypocarbic, had higher oxygenation, and required lower pressures than the V_T8 groups; no consistent effect of PEEP was found. Markers of lung injury were significantly elevated in all ventilation groups compared with unventilated controls; no effect of PEEP was found. Ventilation resulted in localization of IL-6 to the small airways. Initial ventilation of preterm lambs with PEEP and/or V_T of 8 mL/kg did not prevent an inflammatory injury to the lung. **(*Pediatr Res* 64: 517–522, 2008)**

The initiation of breathing at birth is an essential but complex adaptation that must rapidly transition the fluid filled fetal lung to gas exchange (1). Critical components of this transition are clearance of fluid from the airways, establishment of a functional residual capacity (FRC), and increased blood flow to the lungs. This transition often requires assistance for term infants and is more difficult and ineffective for preterm infants. A poor transition to air breathing in preterm infants is frequently because of surfactant deficiency, decreased respiratory drive, and perhaps more lung fluid as the majority of preterm infants are now delivered via cesarean section (2). Despite the frequent need for ventilatory assistance after delivery, and numerous experimental demonstrations that the preterm lung can be easily injured by mechanical ventilation (3,4), there is minimal clinical information about how best to provide initial ventilatory assistance to the preterm lung. The equipment used for resuscitation of the preterm has not been standardized and does not allow for accurate control of tidal volume (V_T), positive end-expiratory pressure/continuous positive airway pressure (PEEP/CPAP), or inspiratory times (5,6).

Ventilation of the preterm lung for hours without PEEP will cause lung injury and high initial V_Ts further increase that lung injury (3,4,7). A brief period of high V_T ventilation without PEEP will injure the preterm lung and subsequent ventilation with PEEP will amplify that injury (8); this injury can be reduced by surfactant treatment before mechanical ventilation of the preterm lung (4,9). In preterm sheep and baboons, CPAP decreases injury compared with mechanical ventilation without PEEP (10,11).

Ventilation of the fluid filled preterm lung has similarities to the lung injury resulting from ventilation of saline lavaged adult lungs, in that there is surfactant deficiency and fluid in the airways with atelectasis or fluid filled alveoli (12). Ventilation over-distends and injures the airways proximal to the atelectatic or fluid filled alveoli (13,14). Atelectasis with nonuniform ventilation results in over-distension of the ventilated lung—the concept of the baby lung (15). Surfactant deficient fluid columns in small airways also cause fluid mechanical stresses that disrupt the airway epithelium (16). Volutrauma results from volumes that overstretch the lung regionally or from ventilation of collapsed lung units, and stretch induced injuries can occur in both the airways and the alveoli (12).

We examined how PEEP might moderate lung injury in the preterm lung to help develop recommendations for neonatal resuscitation. We hypothesized that initiation of ventilation in surfactant deficient preterm lambs using PEEP would decrease lung injury and that the injury caused by a large V_T might be decreased with the use of PEEP, by minimizing collapse at end expiration. We evaluated volume-targeted ventilation us-

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Abbreviations: BALF, bronchoalveolar lavage fluid; **BPD,** bronchopulmonary dysplasia; **CPAP,** continuous positive airway pressure; **FRC,** functcmh ional residual capacity; **PEEP,** positive end-expiratory pressure; **PIP,** peak inspiratory pressure; **V_T,** tidal volume

Table 1. *Description of animals*

	Nonventilated Controls	V_T15		V_T8	
		PEEP 0	PEEP 5	PEEP 0	PEEP 5
Number	7	7	7	7	7
Body wt (kg)	3.2 ± 0.2	3.5 ± 0.2	3.6 ± 0.3	3.4 ± 0.3	3.6 ± 0.7
Cord blood pH	—	7.27 ± 0.02	7.20 ± 0.03	7.19 ± 0.04	7.21 ± 0.06
Cord blood $PaCO_2$ (mm Hg)	—	61 ± 3	66 ± 3	69 ± 7	70 ± 7
Saturated phosphatidylcholine in BALF (μmol/kg)	2.0 ± 0.6	3.8 ± 0.8	3.2 ± 0.8	2.1 ± 0.6	2.8 ± 0.6

ing V_T of 8 or 15 mL/kg with and without PEEP for 15 min after birth and assessed lung injury 2 h after birth.

METHODS

Animal handling and the initial ventilation maneuver. Investigations were approved by the animal ethics committees of the Western Australian Department of Agriculture and Food, the University of Western Australia and Cincinnati Children's Hospital Medical Center. Lambs were randomized before delivery to an unventilated control group, or to one of four ventilation regimes for the first 15 min: 1) V_T15 mL/kg, PEEP of 0 cm H_2O; 2) V_T15 mL/kg, PEEP of 5 cm H_2O; 3) V_T8 mL/kg, PEEP of 0 cm H_2O; and 4) V_T8 mL/kg, PEEP of 5 cm H_2O. Preterm fetuses (133 ± 1 d gestational age; term 150 d gestational age) were exteriorized via cesarean section, a 4.5 mm endotracheal tube was inserted via a tracheotomy, and the assigned ventilation protocol was initiated immediately following delivery at 40 breaths/min using heated, humidified 40% oxygen in air and an inspiratory time of 0.7 s. Peak inspiratory pressures (PIP) were adjusted to achieve the target V_T by 10 min of age and maintained at that V_T until 15 min of age. Ventilated lambs were anesthetized by infusion of Remifentanil (Domitor® 0.1 mg/kg/min, Pfizer Animal Health, NSW, Australia) and Propofol (Repose®, 0.05 mg/kg/min, Norbrook Laboratories Ltd., Victoria, Australia). Control lambs (n = 5) were euthanased immediately after delivery. Lambs did not receive corticosteroids or surfactant treatment.

Ventilation after 15 min. After 15 min of age, the four groups received ventilation designed to minimally injure the lung during the remaining ventilation period, using a respiratory rate of 40 breaths/min and 5 cm H_2O PEEP. Blood gas measurements every 30 min guided the ventilator adjustments. The combined targets for PCO_2 in arterial blood ($PaCO_2$) of 50–60 mm Hg and a V_T <10 mL/kg were achieved by adjusting the PIP. The fractional inspired oxygen (FiO_2) was adjusted to target a PO_2 in arterial blood (PaO_2) of 40–100 mm Hg. Oxygenation index (OI) was calculated by the equation OI = mean airway pressure × FiO_2 × 100/PaO_2. V_T values were measured continuously with Florian Respiratory Monitors (Acutronic Medical Systems, Hirzel, Switzerland).

Lung processing. At 2 h of age, animals were ventilated with FiO_2 of one for 3 min at 2 h of age before clamping of the tracheal tube for 3 min to achieve atelactesis. The lambs received a lethal dose of pentobarbital (100 mg/kg). After opening the chest, a deflation pressure-volume curve was constructed after gas inflation to 40 cm H_2O pressure (17). Bronchoalveolar lavage fluid (BALF) samples were obtained from saline lavage of the left lung (17).

Measurements on lung tissue. Protein (18), protein carbonyls (19), saturated phosphatidylcholine (20), and myeloperoxidase (21) were measured in BALF. Total RNA was isolated from the right lower lobe of the lung and liver, and 10 μg of total RNA was used for IL-1β, IL-6, IL-8, TNF-α, TGF-β1, and TLR-2 quantitation using RNAse protection assays (22). The right upper lobe of the lung was inflation fixed (Formalin) and *in situ* hybridization for IL-6 was performed (22).

Data analysis and statistics. Results are shown as mean (±SEM). Statistics were analyzed using SigmaStat 3.5 (Systat Software Inc., San Jose, CA). For normally distributed data a one-way or two-way analysis of variance with the Holm-Sidak multiple comparison procedure were used for comparisons between groups. A Kruskal–Wallis analysis of variance on Ranks was used for non-normalized data. Significance was accepted as $p < 0.05$.

RESULTS

Initial ventilation maneuver. The seven animals randomized to each group had similar body weights and cord blood gas values (Table 1). High cord blood $PaCO_2$ values resulted from the anesthesia and positioning of the ewe for delivery. The V_Ts achieved at 5, 10, and 15 min within the respective groups were similar with or without PEEP (Table 2). The use of PEEP also did not change the ventilatory pressures needed to achieve the V_T except for the V_T15 groups at 5 min and V_T8 groups at 10 min. All animals required relatively high pressures to achieve the V_T targets by 10 min of age. At 15 min, the V_T15 groups were hypocarbic and the V_T8 groups were hypercarbic. The OI was significantly lower in the V_T15 groups compared with V_T8 groups (p = 0.012) irrespective of PEEP (Table 2). Therefore, there was no consistent effect of 5 cm H_2O PEEP on ventilatory pressure, $PaCO_2$ or OI at 15 min of age using volume targeted ventilation at either V_T.

Maintenance ventilation to 2 h of age. The ventilatory pressures required to maintain V_T <10 mL/kg in the subsequent ventilation period were significantly higher for the V_T8 groups compared with V_T15 groups (p = 0.006) irrespective of PEEP (Fig. 1). However, V_T was higher in V_T8 groups for the remainder of the study compared with V_T15 groups (p = 0.067; Fig. 1). $PaCO_2$ values were not different between groups after 30 min of age (Fig. 2). In contrast, OI were lower for the groups receiving the initial V_T15 ventilation maneuver relative to the V_T8 groups ($p < 0.001$), irrespective of PEEP (Fig. 2). Therefore, the use of PEEP had no consistent effect on lung physiology over 2 h. The initial 15 min of V_T15 ventilation improved lung function as measured by ventilatory pressures and oxygenation over the entire subsequent period of ventilation.

Table 2. *Ventilation maneuver after delivery*

	V_T15		V_T8	
	PEEP 0	PEEP 5	PEEP 0	PEEP 5
V_T (mL/kg)				
5 min	11.2 ± 0.9	10.2 ± 1.7	6.3 ± 0.7§	7.8 ± 1.4
10 min	14.9 ± 0.6	14.0 ± 1.8	7.9 ± 0.4§	7.9 ± 0.8§
15 min	16.4 ± 0.7	15.4 ± 1.5	7.9 ± 0.5§	8.4 ± 0.5§
Ventilatory Pressures (cm H_2O)				
5 min	36 ± 1	31 ± 1*	31 ± 1	28 ± 1
10 min	40 ± 2	37 ± 1	34 ± 1	28 ± 2*
15 min	42 ± 2	39 ± 2	32 ± 3	29 ± 2
$PaCO_2$ (mm Hg) at 15 min	25 ± 2	23 ± 2	71 ± 6§	60 ± 6§*
Oxygenation Index at 15 min	7.6 ± 1.6	5.5 ± 0.5	15.0 ± 2.3§	10.6 ± 3.0§

Ventilatory pressures are peak inspiratory pressures minus positive end expiratory pressures (PEEP). Oxygenation Index = mean airway pressure × FiO_2 × 100/PaO_2.

* $p < 0.05$ vs same V_T target group; § $p < 0.05$ *vs* V_T15.

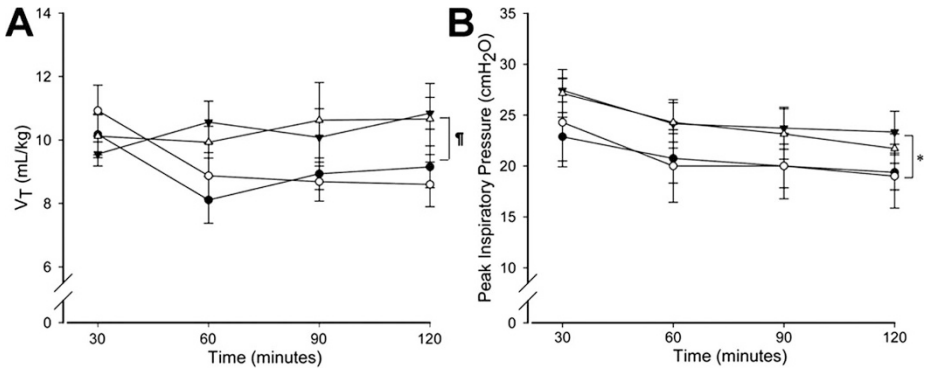

Figure 1. $PaCO_2$ (*A*) and OI, (*B*) in ventilation groups following the 15 min initial ventilation period up to 90 min. Values at 120 min are not included because the lambs were ventilated with 100% oxygen to facilitate lung collapse. ▼; V_T8 PEEP 0, △: V_T8 PEEP 5, ●: V_T15 PEEP 5, ○: V_T15 PEEP 0. Groups receiving V_T of 15 mL/kg during initial ventilation had lower $PaCO_2$ before normalization after 30 min, and a lower OI throughout subsequent ventilation, compared with groups that received V_T of 8 mL/kg. Group means ± SEM shown. *$p < 0.05$ V_T15 mL/kg *vs* V_T8 mL/kg. ¶$p = 0.067$ V_T15 mL/kg *vs* V_T8 mL/kg.

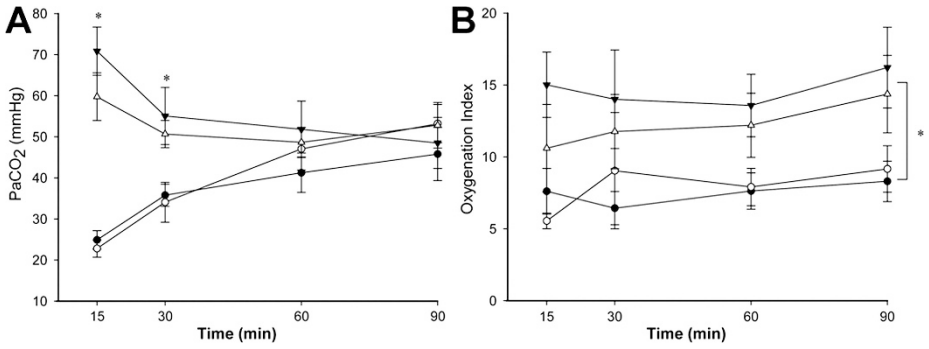

Figure 2. Tidal volume V_T(*A*) and PIP (*B*) in ventilatory groups after the initial ventilation period. ▼: V_T8 PEEP 0, △: V_T8 PEEP 5, ●: V_T15 PEEP 5, ○: V_T15 PEEP 0. *$p < 0.05$ V_T15 mL/kg *vs* V_T8 mL/kg. Groups receiving high V_T during the initial 15 min ventilation period required lower ventilatory pressures to maintain a V_T of 8 mL/kg during subsequent ventilation.

Indicators of injury. Deflation pressure-volume curves were similar for the ventilated groups: lung volumes measured at 40 cm H_2O were about 50 mL/kg (Fig. 3). The amounts of surfactant in the BALF were similar for the ventilation groups (Table 1). BALF from ventilated lungs contained more protein than did the BALF of the unventilated lungs reflecting an epithelial permeability abnormality (Fig. 4). There were no differences between the ventilated groups and no significant protection with PEEP ($p = 0.088$). Expression of the pro-inflammatory cytokine mRNA IL-1β, IL-6, and IL-8 was greatly elevated in all ventilation groups compared with unventilated controls, with no differences between the ventilation groups (Fig. 4). Protein Carbonyls, a measure of oxidative injury, and TLR-2 mRNA were higher in all ventilation groups relative to unventilated controls (Table 3). Myeloperoxidase, a marker of neutrophil activity, was not different between ventilation groups and unventilated controls. TNFα and TGF-β1 mRNA in the lungs were similar between all groups (Table 3). IL-6 was expressed highly in terminal bronchiolar and alveolar duct epithelium of the ventilated

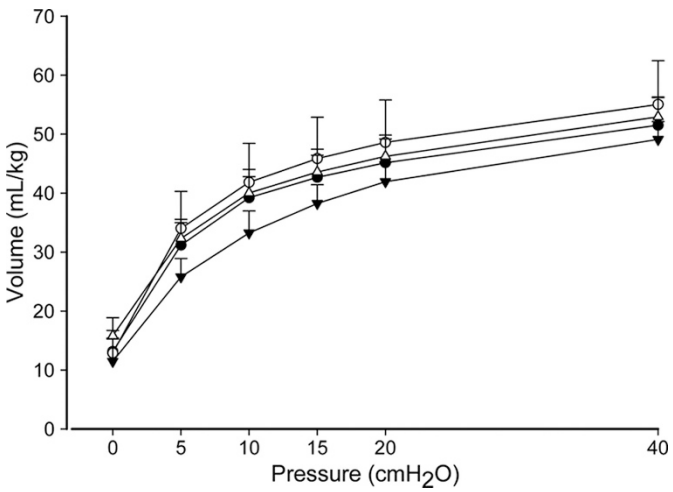

Figure 3. Deflation pressure-volume curves. ▼: V_T8 PEEP 0, △: V_T8 PEEP 5, ●: V_T15 PEEP 5, ○: V_T15 PEEP 0. Volumes at 15, 20 and 40 cm H_2O were similar between ventilation groups. Group means ± SEM shown.

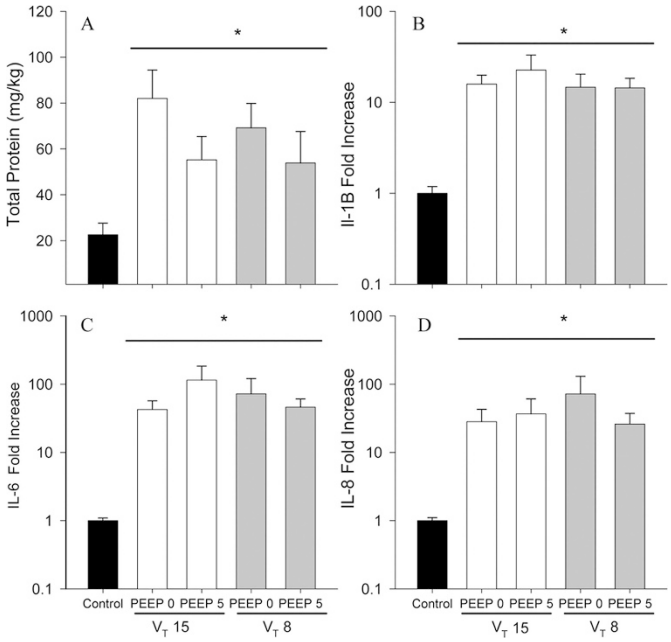

Figure 4. Total protein (*A*), IL-1β (*B*), IL-6 (*C*), and IL-8 (*D*) mRNA from lung tissue in lambs receiving V_T15 mL/kg (□) or V_T8 mL/kg (▨) receiving either 0 cm H_2O or 5 cm H_2O PEEP during the initial resuscitation period; expressed as fold increase relative to control (nonventilated) lambs (■). Group means ± SEM shown. *$p < 0.05$ *vs* controls.

Table 3. *Assessment of injury/inflammation*

	Nonventilated Controls	V_T15		V_T8	
		PEEP 0	PEEP 5	PEEP 0	PEEP 5
TNF-α (mRNA fold increase)	1.0 ± 0.2	1.1 ± 0.2	1.8 ± 0.4	1.6 ± 0.1	1.7 ± 0.1
TGF-β1 (mRNA fold increase)	1.0 ± 0.02	1.1 ± 0.03	1.1 ± 0.02	1.0 ± 0.05	1.0 ± 0.07
TLR2 (mRNA fold increase)	1.0 ± 0.1	2.7 ± 0.3*	3.1 ± 1.0*	2.8 ± 0.9*	2.3 ± 0.4*
Protein Carbonyl (nmol/mg)	0.06 ± 0.02	0.42 ± 0.02*	0.38 ± 0.07*	0.38 ± 0.20*	0.29 ± 0.09*
Myeloperoxidase (nmol/g)	2.3 ± 0.6	3.7 ± 1.1	3.0 ± 0.8	5.0 ± 2.4	2.4 ± 0.6

* $p < 0.05$ *vs* nonventilated controls.

lambs, with sparing of large bronchi (Fig. 5). The pattern and quantity of expression was similar in all ventilated groups.

Histology for lung injury. The tissue from the ventilated lungs showed minimal inflammation, some atelectasis and over-distended regions without bleeding or remarkable airway injury. There were no differences in histology between groups, and no evidence of severe lung injury.

DISCUSSION

Recent Cochrane Reviews concluded that there is insufficient evidence to recommend CPAP/PEEP for resuscitation in term or preterm infants because there were no studies that were adequate for review (23,24). Our studies were designed to represent a controlled version of what occurs during resuscitation of the preterm with the variables being V_T and PEEP. In practice, 76% of neonatology units provide CPAP or PEEP during resuscitation, and the preferred pressure level is 5 cm H_2O (25). The 15 min resuscitation interval approximates the time required for neonatal resuscitations (6).

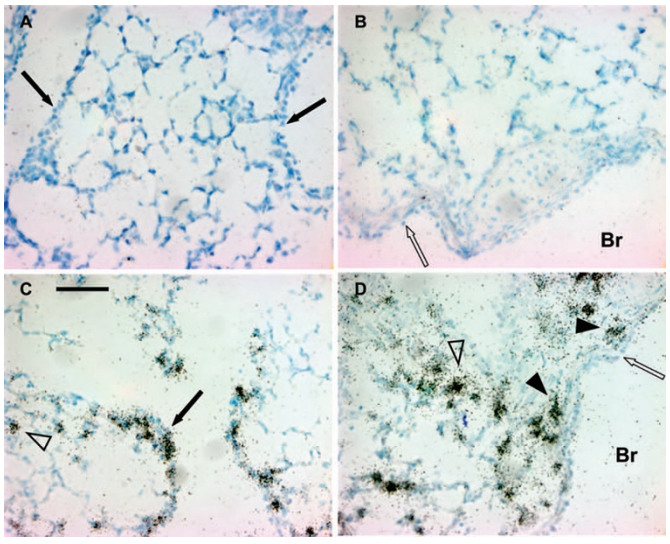

Figure 5. Localization of IL-6 mRNA expression in control (*A,B*), a lamb ventilated with V_T15 mL/kg, PEEP 0 cm H_2O (*C*), and a lamb ventilated with V_T8 mL/kg, PEEP 5 cm H_2O (*D*). IL-6 expression is depicted in black silver grains. No IL-6 expression was detected in control lambs (n = 2–3/group). Mechanical ventilation robustly induced IL-6 expression in the terminal bronchiolar/alveolar duct epithelium (*bold arrows; see panel C*), inflammatory cells (*open arrowhead*) (*panels C and D*) and bronchial smooth muscle (*dark arrowhead; see panel D*). Note the absence of IL-6 expression in the large bronchial epithelium (*open arrow in panel D*) (Bar denotes 50 μm, Br = Bronchus).

We anticipated that the V_T15, PEEP 0 group would have lung injury and investigated if a PEEP of 5 cm H_2O might minimize that injury. The 15 mL/kg volume was chosen because many infants receive large V_T inadvertently and this volume was used previously in preterm lamb models to cause severe lung injury (4,8). The group given V_T8, PEEP 5 was selected to represent an optimal initial ventilation as clinicians most frequently use 5 cm H_2O PEEP (25), and 8 mL/kg is the normal V_T for spontaneously breathing preterm lambs on CPAP (26). We hypothesized that the recruitment of a V_T of 8 mL/kg over the first 10 min with the use of 5 cm H_2O PEEP would be less-injurious compared with the other groups. However, all groups had similar increases in BALF protein, lung tissue cytokine expression, and the other biochemical indicators of injury. The conclusion is that neither the V_T of 8 mL/kg nor the use of PEEP protected these preterm lungs.

The amount of lung injury during the initiation of ventilation depends on variables that differ with each experimental design. Surfactant treatment before the use of similar V_T and PEEP did protect the lungs (4,9). Probyn *et al.*(27), found that initiation of ventilation in more preterm lambs (125 d) required high PIP and PEEP improved oxygenation, although they did not evaluate lung injury. V_T of 15 mL/kg, PEEP 0 for 15 min was evaluated in 128 d gestation lambs either returned to the intrauterine environment or delivered, surfactant treated, and ventilated for 3 h (8); all groups had lung injury, but ventilation following the injury greatly amplified the expression of IL-6 and IL-8 in the lungs. Thus, the injury caused by the initial ventilation maneuver was masked, in part, by the subsequent ventilation. Other studies compared spontaneously breathing lambs at 130–134 d gestation given CPAP with lambs ventilated comparably to the V_T8, PEEP 5 group, and neither group had large increases in biochemical markers of lung injury (10). The caveat to those studies was treatment of the ewes with β-methasone and epostane (a progesterone antagonist) to induce spontaneous breathing in the lambs at delivery. In the ventilated newborn mouse, alterations in lung architecture and septation can occur without overt inflammation (28). The injury response is developmental stage specific and may differ by species studied.

We interpret our results within the context of the effects of V_T on the fluid filled surfactant-deficient lung. We chose animals at 133 d gestation because we wanted animals with surfactant deficiency that could be ventilated without severe lung injury. Small surfactant pool sizes, total lung volumes of

about 50 mL/kg and ventilation pressures at 2 h of age of about 20 cm H_2O pressure all indicate lung immaturity. Of interest, the animals initially ventilated with higher V_T of 15 mL/kg required less pressure and had better oxygenation over the subsequent 1 h 45 min than the V_T 8 mL/kg animals. This suggests that these preterm lungs needed a high V_T to "open up" or recruit the lung. The initial recruitment did not result in more injury in our animals.

Surfactant has important effects on the fetal lung during transition to air breathing. The term fetal lung secretes surfactant into the fetal lung fluid and concurrently absorbs fetal lung fluid during the terminal phases of delivery (29). As surfactant pool sizes are 10- to 20-fold higher at term than at any other time during life, the concentration of surfactant in the residual fetal lung fluid is high (30). Surfactant facilitates clearance of fluid from the airways and strikingly lowers the opening pressures of the lung (31). Fluid movement in small airways can injure the epithelium by fluid mechanical stress (16). The fetuses we studied did not experience labor, were surfactant deficient, and had fluid removed only from the large airways before ventilation. The high PIP of up to 40–50 cm H_2O for the first 15 min were needed to clear the airways of fluid. Ventilation without surfactant results in over distention of the open lung because of the nonuniform surface tensions throughout the lung (32).

Therefore, the likely sequence that resulted in large increases in multiple indicators of lung injury in this experiment is as follows: the initial breaths given at about 35 cm H_2O pressure using a volume targeted strategy delivered V_T of 6–8 mL/kg at 5 min primarily to the airways. The FRC of the airways may have been maintained with the PEEP of 5 cm H_2O. Each V_T then over distended the compliant preterm airways resulting in the airway injury indicated by expression of IL-6 in the small airways and bronchi. As the lungs progressively opened, the more compliant open lung regions became over-distended. At autopsy, some lung parenchyma was not inflated with a static pressure of 40 cm H_2O. This sequence of lung injury has been described with ventilation of the saline-lavaged adult lung (12–14).

The beneficial effects of PEEP may not be as evident in the transition of the fluid-filled lung to air breathing as in the aerated lung. PEEP can improve oxygenation (27,33), but it did not over the first 15 min in our study. The small airways may not collapse because of foam or fluid pockets which can splint the lung during the transition to air breathing (34), while surfactant deficient fluid may promote lung injury (16). Perhaps a higher PEEP would have decreased injury and improved oxygenation in this trial and in surfactant deficient lungs. A recent study by the authors showed that conventional mechanical ventilation with 10 cm H_2O PEEP resulted in increased lung injury (Schulzke *et al.* Submitted). However, no indications were found that PEEP decreased injury during the transition to air breathing. Indeed, IL-6 expression in the bronchi and alveolar epithelium was not different between ventilated groups.

In clinical practice, V_T is an unregulated and nonmeasured variable in the delivery room. V_T is likely to be large and cause hyperventilation (6). Some delivery rooms use ventilators to control V_T and pressures, and V_T targeted ventilation is thought to be ideal for ventilation of infants to minimize the risk of BPD (35). Our results demonstrate the risks of V_T targeted ventilation of the fluid filled lung. These lungs required high PIP to deliver a V_T of 8 mL/kg to lungs with total lung capacities of 50 mL/kg. Furthermore, using a rate of 40 breaths/min and a long inspiratory time to achieve the V_T, the lambs were hypercarbic with $PaCO_2$ values of 60–70 mm Hg at 15 min of age. These results suggest that lung injury will be the inevitable consequence of attempts to achieve normocarbia quickly in a surfactant deficient and fluid-filled lung.

The lack of PEEP and the use of V_T15 as the target volume for the first 15 min did not consistently increase indices of injury at 2 h. The assessment at 2 h is very early in the progression of lung injury, but the high expression of IL-1β and IL-8 mRNA and the increase in protein carbonyls suggest that more inflammation would be apparent later. The lack of differences between the experimental groups could result from a stretch injury, with the V_T8 being sufficient to initiate a maximum response that was replicated by the other groups. Also, the need for subsequent ventilation may have amplified the initial injuries and masked any differences (8). Other variables of ventilation, such as the inspiratory flow and achievement of plateau pressure have not been adequately studied. Also, oxygen exposure may also contribute to the injury although we minimized oxygen exposure for these experiments. There could be substantial differences for injury markers that we did not measure. Our experimental conditions were controlled relative to the human experience and thus the translation of these observations to the preterm infant must be cautious. Very low birth-weight preterm infants deliver because of an abnormality with the pregnancy, most receive some antenatal corticosteroid exposure, and many are exposed to some preterm labor. Nevertheless, the frequent use of cesarean section assures that many of these infants have large amounts of fluid in their lungs. These studies provide a clear example of how V_T targeted ventilation can injure the preterm lung during the initiation of ventilation.

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