Tidal volume effects on surfactant treatment responses with the initiation of ventilation in preterm lambs

KAZUKO WADA, ALAN H. JOBE, AND MACHIKO IKEGAMI
Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California 90502

Wada, Kazuko, Alan H. Jobe, and Machiko Ikegami. Tidal volume effects on surfactant treatment responses with the initiation of ventilation in preterm lambs. J. Appl. Physiol. 83(4): 1054–1061, 1997.—We hypothesized that initiation of ventilation in preterm lambs with high volumes would cause lung injury and decrease the subsequent response to surfactant treatment. Preterm lambs were randomized to ventilation for 30 min after birth with 5 ml/kg (VT5), 10 ml/kg (VT10), or 20 ml/kg (VT20) tidal volumes and then ventilated with ~10 ml/kg tidal volumes to achieve arterial PCO2 values of ~50 Torr to 6 h of age. VT20 lambs had lower compliances, lower ventilatory efficiencies, higher recoveries of protein, and lower recoveries of surfactant in alveolar lavages and in surfactant that had decreased compliances when tested in preterm rabbits than VT5 or VT10 lambs. Other lambs randomized to treatment with surfactant at birth and ventilation with 6, 12, or 20 ml/kg tidal volumes for 30 min had no indicators of lung injury. An initial tidal volume of 20 ml/kg decreased the subsequent response to surfactant treatment, an effect that was prevented with surfactant treatment at birth.

METHODS
Experimental Strategy

We performed two separate protocols that were very similar except for the time of surfactant treatment: at birth and at 30 min of age. Preterm lambs were randomized to target tidal volumes for the first 30 min of ventilation, and then a standardized method of ventilation was used for the subsequent study period. For the surfactant treatment at 30 min protocol, animals were randomized to ventilation for the first 30 min with a low tidal volume of 5 ml/kg (VT5), a tidal volume of 10 ml/kg (VT10), or a high tidal volume of 20 ml/kg (VT20). Animals treated at birth and before the initiation of ventilation with surfactant received tidal volumes of 6 ml/kg (VT6), 12 ml/kg (VT12), or 20 ml/kg (VT20).

Delivery and Ventilation of Lambs

Preterm lambs were delivered by cesarean section, as previously described (11). Briefly, each pregnant ewe carrying twins or triplets was preanesthetized with 1 g ketamine im and given spinal-epidural anesthesia using 10 ml of a 1:1 (vol/vol) 2% lidocaine-0.5% bupivacaine. The anterior neck of the ewe was anesthetized with 2% lidocaine, and an endotracheal tube was tied into the trachea to facilitate mechanical ventilation. The fetal head was exposed through midline abdominal and uterine incisions, the superficial structures on the anterior neck were anesthetized with lidocaine, and a cross-clamped endotracheal tube was tied into the trachea. Ketamine (10 mg/kg) and acepromazine (0.1 mg/kg) were given by intramuscular injection. The fetal lung fluid that was easily aspirated was withdrawn. The lambs were then delivered and weighed, and the lambs assigned to surfactant treatment received 100 mg/kg (4 ml/kg) Survanta (Ross Products, Columbus, OH). All lambs were ventilated with

recommendations for resuscitation of the newborn include the use of long inspiratory times for the first few breaths and then ventilation at 40–60 breaths/min using peak inspiratory pressures (PIP) of 20–40 cmH2O (3). However, the surfactant-deficient lung may require higher pressures to initiate ventilation (21). Clinical practice is to ventilate to achieve clearly visible chest excursions and a pink infant, as rapidly as possible without monitoring tidal volumes. Maximal lung gas volumes of full-term human lungs have been estimated to be 23–50 ml/kg body wt, and maximal lung gas volumes for infants with respiratory distress syndrome have been reported to be ~20 ml/kg (2, 8). The preterm lung has a small potential gas volume in part because of structural immaturity and partial alveolarization and in part because the lung will not open completely as a result of surfactant deficiency (18, 24). Dreyfuss and Saumon (6) demonstrated that the adult lung was injured by mechanical ventilation primarily when the sum of the functional residual capacity (FRC) and tidal volume approached or exceeded maximal lung volume. The preterm lung is particularly susceptible to injury by mechanical ventilation because of structural immaturity, and tidal volumes considered safe for the adult may approach the maximal lung gas volumes in the preterm lung (15). Tidal volumes and maximal lung volumes are seldom measured during ventilation of the surfactant-deficient preterm infant. The clinical indicator of ventilation and, indirectly, tidal volume is the arterial Pco2 (PaCO2), and retrospective clinical studies demonstrate that low PaCO2 values in preterm infants are associated with poor neurological outcomes (29) and an increased incidence of bronchopulmonary dysplasia (7, 17). Kraybill et al. (17) noted that PaCO2 values <40 Torr at 48–96 h of age were associated with an increased risk of bronchopulmonary dysplasia in ventilated infants with respiratory distress syndrome. Recently, Garland et al. (7) reported that ventilated low-birth-weight infants with low PaCO2 values before surfactant treatment also had an increased incidence of bronchopulmonary dysplasia, a counterintuitive association, because hyperventilation should occur in the infants with less severe lung disease. Our hypothesis was that ventilation of the preterm lung with high tidal volumes in the immediate newborn period would cause lung injury that would compromise the subsequent treatment response to surfactant. A second hypothesis was that surfactant treatment before the initiation of ventilation would protect the preterm lung from the injury.

RECOMMENDATIONS FOR resuscitation of the newborn include the use of long inspiratory times for the first few breaths and then ventilation at 40–60 breaths/min using peak inspiratory pressures (PIP) of 20–40 cmH2O (3). However, the surfactant-deficient lung may require higher pressures to initiate ventilation (21). Clinical practice is to ventilate to achieve clearly visible chest excursions and a pink infant as rapidly as possible without monitoring tidal volumes. Maximal lung gas volumes of full-term human lungs have been estimated to be 23–50 ml/kg body wt, and maximal lung gas volumes for infants with respiratory distress syndrome have been reported to be ~20 ml/kg (2, 8). The preterm lung has a small potential gas volume in part because of structural immaturity and partial alveolarization and in part because the lung will not open completely as a result of surfactant deficiency (18, 24). Dreyfuss and Saumon (6) demonstrated that the adult lung was injured by mechanical ventilation primarily when the sum of the functional residual capacity (FRC) and tidal volume approached or exceeded maximal lung volume. The preterm lung is particularly susceptible to injury by mechanical ventilation because of structural immaturity, and tidal volumes considered safe for the adult may approach the maximal lung gas volumes in the preterm lung (15). Tidal volumes and maximal lung volumes are seldom measured during ventilation of the surfactant-deficient preterm infant. The clinical indicator of ventilation and, indirectly, tidal volume is the
time-cycled, pressure-limited infant ventilators (Sechrist Industries, Anaheim, CA). Tidal volumes were monitored continuously (model CP-100, Biorec Monitoring System, Anaheim, CA). During the first 30 min, ventilatory settings were as follows: inspired O₂ fraction (Fᵢ₀₂), 1.0; positive end-expiratory pressure (PEEP), 4 cmH₂O; inspiratory time, 0.7 s; rate, 40 breaths/min; flow, 10 l/min. PIP values were adjusted to achieve the target tidal volumes for the lambs in two protocols.

Immediately after ventilation was started, a 5-Fr catheter was advanced into the aorta via an umbilical artery and a 10 ml/kg transfusion of filtered cord blood was given. This catheter was used for continuous blood pressure recording and frequent blood-gas measurements. Another 5-Fr catheter was passed into the left ventricle via the left carotid artery for injections of microspheres to measure cardiac outputs and brain blood flows. A constant infusion of 5% dextrose (100 ml·kg⁻¹·day⁻¹) was given, and rectal temperature was monitored and maintained at 38–39°C using radiant heat and heating pads.

At 30 min of age the lambs not treated at birth were given 100 mg/kg Survanta containing 1.2 µCi of [¹⁴C]choline-labeled dipalmitoylphosphatidylcholine (DPC). Radiolabeled Survanta was prepared by the addition of [¹⁴C]DPC (New England Nuclear, Boston, MA) to Survanta that had been extracted into 2:1 chloroform-methanol. The radiolabeled surfactant was then resuspended into a saline-based lipid suspension with glass beads to a concentration of 25 mg/ml. Radiolabeled surfactant (1 ml) was added to unradiolabeled surfactant. Each animal received 2 ml/kg surfactant while in the left lateral position, then 30 s of ventilation and 2 ml/kg surfactant in the right lateral position, then 30 s of ventilation before being returned to the prone position (26). After 30 min, PIP values for all groups were changed to target PaCO₂ values between 45 and 55 Torr and tidal volumes of 8–10 ml/kg. FIO₂ was lowered sequentially to 20, 15, 10, 5, and 0 cmH₂O, with lung volumes recorded 30 s after each pressure was reached. Volumes were corrected for the compliance of the system. The lungs were removed from the chest and divided into left lung and right lung. The left lung was lavaged and then used to prepare a lung homogenate. The lung was filled with cold saline until fully dis tended, and the alveolar lavage saline was recovered by syringe (16). This procedure was repeated five times, and the recovered volumes were pooled. Aliquots from the pooled alveolar lavage were used to measure ¹²⁵I-albumin radioactivity, saturated phosphatidylcholine (Sat-PC), and protein content. Sat-PC was recovered from 2:1 chloroform-methanol extracts by neutral alumina column chromatography after exposure to osmium tetroxide and was quantified by phosphorus assay (1, 20). Protein was determined using the method of Lowry et al. (19).

After an initial centrifugation of alveolar lavage at 140 g for 10 min, large- and small-aggregate surfactant fractions were separated by centrifugation at 40,000 g for 15 min (13). The supernatant that contained small-aggregate surfactant was used for Sat-PC and [¹⁴C]DPC content measurements. The pellet containing large-aggregate surfactant was resuspended in saline and centrifuged at 40,000 g for 15 min over 0.8 M sucrose. The interface was aspirated, diluted with saline, and again centrifuged at 40,000 g for 15 min. The large-aggregate surfactant then was resuspended in a small amount of saline, and aliquots were used for Sat-PC and [¹⁴C]DPC content measurements. The large-aggregate surfactants were frozen and saved for the studies of surfactant function. After alveolar lavage the left lung was homogenized in saline, and aliquots were used to measure ¹²⁵I-albumin radioactivity, Sat-PC, and hemoglobin content (16). Two weighed pieces from standardized locations in each right lung were dried at 70°C for 72 h and reweighed for calculation of dry-to-wet weight ratios.

Testing Surfactant Function in Preterm Rabbits

Pooled large-aggregate surfactants from lambs treated with surfactant at 30 min of age and the surfactant used for treatment were adjusted to a concentration of 12.5 mg/ml. Surfactant function was tested by evaluating the treatment responses of 27-day ± 2-gestation preterm rabbits (13, 25). After sequential cesarean delivery, an 18-gauge stainless steel tube was secured into the trachea, and each rabbit was treated with 50 mg/kg of one of the surfactants. One or two rabbits of each litter were untreated controls. After lung inflation with 100% O₂, the rabbits were transferred to a 37.0°C temperature-controlled ventilator-plethysmography system and ventilated with an Fᵢ₀₂ of 1.0, a rate of 30 breaths/min, and a PEEP of 3 cmH₂O. The PIP was adjusted to a tidal volume of 8 ml/kg. After 15 min of ventilation, the tracheal tube was plugged for 5 min to allow absorption of O₂, and a quasi-static pressure-volume curve was performed by inflating the lungs in 5-cmH₂O pressure increments to 35 cmH₂O (25). Lung volumes were recorded after 30 s at each pressure, then the lungs were deflated to the same pressure increments. The values were corrected for system compliance.

Data Analysis

Values are means ± SE. Differences between groups were tested by analysis of variance followed by Student-Newman-
Keuls multiple-comparison procedure. Student’s t-tests were used only when indicated. Significance was accepted at $P < 0.05$.

RESULTS

Lambs Treated With Surfactant at 30 Min of Age

Respiratory outcomes. Twenty-one lambs at 129 ± 1 day gestational age were randomized such that each target tidal volume group contained seven lambs. The lambs weighed 2.38 ± 0.12 kg, and all lambs had normal cord pH values at delivery. Respiratory variables and blood-gas values for these lambs are given in Figs. 1 and 2. The VT10 lambs had $P_{aCO_2}$ values of ~60 Torr for the 30 min before surfactant treatment. This tidal volume was achieved using a mean ventilatory pressure, defined as PIP minus 4 cmH2O PEEP, of 30 ± 2 cmH2O. The VT5 group had a mean $P_{aCO_2}$ for the first 30 min of 90 Torr and was ventilated with 23 ± 2 cmH2O pressures. The VT20 lambs required ventilatory pressures of 35 ± 3 cmH2O to achieve $P_{aCO_2}$ values of ~30 Torr. The VT20 lambs had higher arterial $P_{aO_2}$-to-$F_{IO_2}$ ratios, higher compliances, and higher VEI values than did the lower-tidal volume groups for the first 30 min. However, after surfactant treatment and adjustment of the tidal volume to ~10 ml/kg for all lambs, the VT20 lambs required higher ventilatory pressures and had lower compliances and VEI values for the duration of the 6-h period of ventilation.

FRC values were 11 ml/kg for the VT5 lambs, 20 ml/kg for the VT10 lambs, and 30 ml/kg for the VT20 lambs at 15 min of age (Fig. 3A). Subsequent measurements of FRC were ~30 ml/kg and were similar for the three groups of lambs. Maximal lung volumes for the groups of lambs measured after 6 h of ventilation were ~55 ml/kg (Fig. 3B). The deflation limbs of the pressure-volume curves also were similar except for the higher retained volume at 0 cmH2O pressure for the VT20 lambs.

Cardiac outputs and blood flows. The mean blood pressure for these lambs was 50 mmHg for the first 30 min and gradually decreased to 39 mmHg at 6 h, and there were no differences between groups. The lambs also had arterial pH values that reflected $P_{aCO_2}$ values for the initial 30-min period of ventilation, and the average pH was 7.30 for the three groups for the subsequent period of ventilation. For all groups, car-

![Fig. 1. Sequential measurements of tidal volume, arterial $P_{aCO_2}$ ($P_{aCO_2}$), and arterial $P_{aO_2}$-to-inspired $O_2$ fraction ($P_{aO_2}/F_{IO_2}$) ratios for lambs treated with surfactant at 30 min of age. A: groups were ventilated with target tidal volumes of 5 ml/kg (VT5), 10 ml/kg (VT10), and 20 ml/kg (VT20) for 30 min. After surfactant treatment, all groups were ventilated with similar tidal volumes of ~10 ml/kg to 360 min. B: $P_{aCO_2}$ values were significantly different between groups at 5, 20, and 40 min. After 60 min, $P_{aCO_2}$ values were maintained at ~50 Torr for all groups. C: $P_{aO_2}/F_{IO_2}$ ratios were similar for all 3 groups. *$P < 0.05$ vs. VT5 and VT10. **$P < 0.05$ vs. VT10 and VT20.

![Fig. 2. Sequential measurements of ventilatory pressure (peak inspiratory pressure $-$ positive end-expiratory pressure), dynamic compliance, and ventilatory efficiency index (VEI) for lambs treated with surfactant at 30 min of age. Tidal volumes for first 30 min were 5 ml/kg (VT5), 10 ml/kg (VT10), and 20 ml/kg (VT20). A: ventilatory pressures were significantly higher for VT20 group than for VT5 and VT10 groups after 120 min. B: after surfactant treatment, dynamic compliances increased for VT5 and VT10 groups and decreased for VT20 group. Dynamic compliances were significantly lower for VT20 group than for other groups after 120 min. C: VEI were higher for VT20 group than for other groups before surfactant treatment. After surfactant treatment, VEI increased for VT5 and VT10 groups and decreased for VT20 group. VEI were lower for VT20 group than for other groups after 120 min. *$P < 0.05$ vs. VT5 and VT10. **$P < 0.05$ vs. VT10. ***$P < 0.05$ vs. VT10 and VT20.](http://jap.physiology.org/).
Diac outputs at 20 min of age were higher than the outputs at 240 min of age (Fig. 4A). The lambs with the higher PaCO2 values had the higher cardiac outputs. By 240 min, all lambs had similar cardiac outputs. Blood flow to the brain and to three brain regions was higher in the VT5 lambs at 20 min than in the other groups (Fig. 4, B and C). Blood flows to the brain were also higher for the VT10 than for the VT20 lambs at 20 min. By 240 min, all brain blood flows were similar for the three groups of lambs. Blood flow to the kidneys was 5–8 ml·kg⁻¹·min⁻¹ for all lambs at 20 and 240 min.

Lung and alveolar lavage measurements. The lung dry-to-wet weight ratios were 0.140 ± 0.005, 0.145 ± 0.005, and 0.130 ± 0.006 for the VT5, VT10, and VT20 lambs, respectively, and these ratios were not different. The amount of protein in alveolar lavages was higher for VT20 lambs than for VT5 or VT10 lambs (Fig. 5A). However, the recoveries at 6 h of 125I-albumin given at 5 h were similar in alveolar lavages and the total lungs for the three groups of lambs that were not treated with surfactant until 30 min of age (Fig. 5, B and C). Surfactant pool sizes as assessed by measurements of Sat-PC were similar for the VT5, VT10, and VT20 groups, as was the recovery of surfactant in a large-aggregate fraction (Table 1). Recovery of radiolabeled DPC was decreased from the alveolar lavages and lung.
tissues of the VT20 lambs relative to the VT5 animals (Fig. 6).

Function of surfactant recovered from the lambs. Preterm rabbits were treated with the large-aggregate surfactants recovered by differential centrifugation from the alveolar lavages of the lambs. The numbers of rabbits studied were as follows: seven treated with the Survanta used to treat the lambs, eight or nine for the VT5, VT10, and VT20 lambs, and five controls. The rabbits were ventilated to achieve a tidal volume of 8 ml/kg, and compliances at 15 min were higher for rabbits treated with the large-aggregate surfactant from VT5 lambs than for rabbits treated with other surfactants (Fig. 7A). Compliance values for large-aggregate surfactants from VT10 lambs also were higher than for VT20, Survanta-treated, or control animals. Lung volumes on the deflation limbs of the pressure-volume curves for the rabbits treated with large-aggregate surfactants from VT5 lambs were higher than for Survanta-treated rabbits, and the VT10 and VT20 surfactants were intermediate in effect (Fig. 7B).

Lambs Treated With Surfactant at Birth

Respiratory outcomes. Eight lambs each were randomized to initial target tidal volume groups of 6, 12, or 20 ml/kg. These lambs had a mean birth weight of 2.12 ± 0.09 kg and were 126 or 127 days gestational age. All lambs had normal cord pH values at delivery. The VT6 lambs had \( \text{P}_{\text{aco}} \) values of ~75 Torr for the first 30 min with ventilatory pressures of ~25 cmH\(_2\)O. The VT12 lambs had \( \text{P}_{\text{aco}} \) values of ~40 Torr. To achieve \( \text{P}_{\text{aco}} \) values of ~25 Torr, the VT20 lambs required ventilatory pressures of ~40 cmH\(_2\)O. The arterial \( \text{Po}_{2} \)-to-ratios were higher for the VT12 and VT20 lambs for the first 30 min than for the VT6 lambs. There were no differences in ventilatory pressures, compliances, or VEI values for times to 6 h after the tidal volumes were adjusted to ~10 ml/kg at 30 min.

Cardiac outputs and blood flows. Cardiac outputs at 20 and 240 min were similar for all groups at ~180 ml·kg\(^{-1}\)·min\(^{-1}\), except for a higher output of 220 ml·kg\(^{-1}\)·min\(^{-1}\) for the VT6 lambs at 20 min. Total and regional brain flows were also similar, except for an increase at 20 min for the VT6 lambs that had \( \text{P}_{\text{aco}} \) values of ~75 Torr.

Lung and alveolar wash measurements. The dry-to-wet weight ratios were 0.120 ± 0.006, 0.132 ± 0.006, and 0.146 ± 0.009 for the VT6, VT12, and VT20 lambs, respectively. This ratio for VT6 lambs was significantly lower than that for the VT20 group (\( P < 0.05 \), by t-test). Also, the ratio for the VT6 lambs was significantly lower than that for VT5 lambs treated with surfactant at 30 min of age (\( P < 0.05 \)).

The amount of protein in alveolar

---

**Table 1. Saturated phosphatidylcholine pool sizes**

<table>
<thead>
<tr>
<th></th>
<th>Alveolar Lavage</th>
<th>Lung Tissue</th>
<th>Total (Alveolar + Tissue)</th>
<th>% Large Aggregate Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT5</td>
<td>20.7 ± 3.1</td>
<td>86.4 ± 7.1</td>
<td>107.0 ± 8.5</td>
<td>79.5 ± 3.0</td>
</tr>
<tr>
<td>VT10</td>
<td>22.3 ± 1.6</td>
<td>75.0 ± 2.5</td>
<td>97.2 ± 3.7</td>
<td>78.7 ± 1.8</td>
</tr>
<tr>
<td>VT20</td>
<td>18.1 ± 2.2</td>
<td>76.2 ± 6.5</td>
<td>94.3 ± 6.1</td>
<td>76.8 ± 1.4</td>
</tr>
<tr>
<td>VT6</td>
<td>27.8 ± 1.1</td>
<td>71.3 ± 4.9</td>
<td>99.1 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>VT12</td>
<td>22.9 ± 1.8</td>
<td>69.7 ± 6.5</td>
<td>92.6 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>VT20</td>
<td>22.1 ± 2.1</td>
<td>78.9 ± 6.9</td>
<td>101.7 ± 7.7</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in µmol/kg. VT5, VT10, and VT20, ventilation with 5, 10, and 20 ml/kg tidal volumes; VT6, VT12, and VT20, ventilation with 6, 12, and 20 ml/kg tidal volumes.

---

**Fig. 6. Percent recovery of \(^{14}\)C[dipalmitoylphosphatidylcholine](DPC) in lambs treated with surfactant at 30 min of age. Percent recoveries of \(^{14}\)C[DPC in alveolar lavage, lung tissue, and total lung (alveolar lavage + lung tissue) were significantly lower for VT20 lambs than for VT5 lambs. \* \( P < 0.05 \) vs. VT5 (by t-test).**

---

**Fig. 7. Dynamic compliances and deflation limbs of pressure-volume curves for preterm rabbits treated with large-aggregate surfactant recovered from preterm lambs and Survanta used to treat lambs. A: dynamic compliances were significantly higher for VT5 lambs than for all other groups. \* \( P < 0.05 \). Dynamic compliances were higher for VT10 group than for VT20, Survanta-treated, and control groups. \# \( P < 0.05 \). B: lung volumes on deflation limbs of pressure-volume curves were significantly higher for VT5 group than for Survanta-treated group at all pressures. \* \( P < 0.05 \) (by t-test).**
washes was higher for the VT12 and VT20 lambs than for the VT6 lambs (Fig. 5A). The total proteins in alveolar lavages also were less for lambs treated with surfactant at birth than for the similar VT groups treated with surfactant at 30 min of age (VT6 vs. VT5, VT12 vs. VT10, and VT20 vs. VT20). The recoveries of $^{125}$I-albumin in alveolar lavages and total lungs were similar for all the lambs treated with surfactant at birth (Fig. 5, B and C). These recoveries were less than the recoveries for the lambs ventilated initially with similar VT but treated with surfactant at 30 min of age.

DISCUSSION

Our hypothesis was that initiation of ventilation in the surfactant-deficient preterm lamb with tidal volumes that might normally be considered safe would cause injury, because the preterm lung could not accommodate the volume without being overstretched. We thought that this initial injury would compromise the subsequent surfactant response. We did not know what volume might be injurious; however, empirically, we have found that tidal volumes < 10 ml/kg decrease the transvascular leak of albumin into the lungs of preterm lambs (12). In preterm lambs at 129 days gestation, ventilation with a tidal volume of 20 ml/kg without surfactant treatment for 30 min resulted in lower compliances, lower VEI values, higher alveolar protein, and more loss of surfactant-associated DPC from the alveolar lavages and lungs than in lambs initially ventilated with a tidal volume of 5 ml/kg.

Large-aggregate surfactant recovered from alveolar lavages of lambs ventilated with 5 or 10 ml/kg tidal volumes improved compliance responses in preterm rabbits relative to the surfactant used to treat the lambs. This effect on surfactant, previously referred to as activation (13), did not occur after ventilation with 20 ml/kg. The larger residual volume at 0 cmH2O on the deflation pressure-volume curve for the VT20 lambs probably is explained by tissue stretch that is not reversible (24). Lambs ventilated with 10 ml/kg had physiological and surfactant variables more similar to the 5 ml/kg group than to the 20 ml/kg group. Therefore, ventilation with 20 ml/kg for 30 min interfered

![Fig. 8. Sequential measurements of tidal volume, PaCO2, and PaO2/FIO2 ratios for lambs treated with surfactant at birth. A: lambs were ventilated with 6 ml/kg (VT6), 12 ml/kg (VT12), or 20 ml/kg (VT20) tidal volumes for 30 min, and then all groups were ventilated with similar tidal volumes of ~10 ml/kg to 360 min. B: PaCO2 values were significantly different for initial 40 min of ventilation. After 60 min, PaCO2 values were ~50 Torr for all groups. C: PaO2/FIO2 ratios were significantly higher for VT20 lambs than for other groups at 20 min. Subsequently, there were no differences except for a higher PaO2/FIO2 ratio for VT20 lambs at 360 min. *P < 0.05 vs. VT6 and VT12. **P < 0.05 vs. VT12 and VT20.](http://jap.physiology.org/)

![Fig. 9. Sequential measurements of ventilatory pressures (peak inspiratory pressure – positive end-expiratory pressure), dynamic compliance, and VEI for lambs treated with surfactant at birth. Lambs were initially ventilated with tidal volumes of 6 ml/kg (VT6), 12 ml/kg (VT12), or 20 ml/kg (VT20). A: at 40 and 60 min, ventilatory pressures were significantly lower for VT20 lambs than for other groups. After 120 min there were no differences. B: dynamic compliances of 3 groups were significantly different until 60 min. After 120 min there were no differences. C: VEI of 3 groups were significantly different until 60 min. After 120 min there were no significant differences. *P < 0.05 vs. VT6 and VT12. **P < 0.05 vs. VT12 and VT20.](http://jap.physiology.org/)
with the treatment response to surfactant and interfered with surfactant function after 6 h of ventilation. Initiation of ventilation in the fluid-filled fetal lung is unique in terms of the relationship between the potential gas volume of the lung and the volume being delivered. Ingimarsson et al. (14) demonstrated that six breaths with tidal volumes of 35–40 ml/kg resulted in lung injury in preterm lambs. This result would be anticipated, because the unventilated fetal lung with a high fluid volume would not have time to recruit volumes that approach the maximal lung volumes of ~50 ml/kg that we measured after 6 h of ventilation. Carlton et al. (5) found that tidal volumes of ~50 ml/kg resulted in increased lung microvascular permeability after 4–8 h of mechanical ventilation of full-term lambs, and Hernandez et al. (9) reported that the increased microvascular permeability was prevented by binding the chest. At 15 min the preterm lambs reported here had FRC values of 30 ml/kg, and addition of the 20 ml/kg tidal volume yielded maximal lung volumes at end inspiration of ~50 ml/kg, which are the same volumes measured as maximal lung volumes at 6 h. Therefore, the injury resulting from the 20 ml/kg tidal volume is consistent with the concept of lung injury resulting from ventilation to volumes that approach or exceed maximal lung volumes (6) and extends the results of Dreyfuss and Saumon (6) to the preterm lung not treated at birth with surfactant. However, maximal lung volumes probably were smaller in the period immediately after birth when the lung had not been expanded and remained partially fluid filled. The more subtle indications of some injury in the lambs ventilated with 10 ml/kg (somewhat higher alveolar protein and less activation of the large-aggregate surfactant) are consistent with that idea.

Nilsson et al. (22) reported that surfactant treatment of preterm rabbits decreased histological indicators of lung injury, and measurements of the transvascular leak of albumin also were strikingly decreased in ventilated preterm rabbits treated with surfactant (10). Similar effects on vascular injury and protein edema also were demonstrated in ventilated preterm lambs (4, 16). The lambs treated with surfactant at birth in this protocol were 3 days more preterm and were ventilated with slightly higher tidal volumes of 6 and 12 ml/kg to try to achieve more normal PaCO2 values. The lower gestational age and higher tidal volumes should tend to increase indicators of lung injury. However, total protein in alveolar lavages and recoveries of radiolabeled albumin in alveolar lavages and total lungs were decreased in lambs treated at birth relative to the lambs ventilated with similar tidal volumes and treated at 30 min of age. This result demonstrates the remarkable effect of surfactant treatment before the initiation of ventilation on these indicators of injury. Maximal lung volumes at 6 h were also ~50 ml/kg for lambs treated at birth with surfactant, and the FRC of the Vt20 group was ~30 ml/kg. These volumes suggested that the lung should have been injured, but it was not if it had been treated with surfactant. This outcome may be explained by the effect of surfactant treatment to make alveolar inflation more uniform and to better distribute gas volume throughout the lung to minimize regional overinflation (24).

The FRC values and lung volumes measured in these preterm lambs are larger than estimates for preterm infants. The tidal volumes of 20 ml/kg that we used for the lambs were higher than those recommended for ventilating infants, because we wanted to evaluate tidal volumes in lambs that approached maximal lung volumes. Maximal lung volumes for full-term infants were recently reported to be 43–52 ml/kg (2). Infants at 10 h of age with respiratory distress syndrome had FRC values of ~11 ml/kg and maximal lung volumes of only 19 ml/kg. These values suggest that the preterm human lung may be susceptible to injury with tidal volumes of ~8 ml/kg. Resuscitation of the preterm infant normally is considered successful if the chest is visibly moving and the initial PaCO2 value is in the normal range. However, visible chest movements and normal PaCO2 values may require sufficient ventilation to injure the surfactant-deficient preterm lung (15). Retrospective studies associated PaCO2 values <40 Torr with an increased incidence of bronchopulmonary dysplasia (7, 17), PaCO2 values less than ~25 Torr also were associated with an increase in periventricular leucomalacia and poor neurological outcomes (29). The potentially negative result of a strategy for the initiation of ventilation that minimizes tidal volumes is that PaCO2 values will be higher than normal. For lambs not treated with surfactant until 30 min of age, a 5 ml/kg tidal volume at a rate of 40 breaths/min yielded PaCO2 values of ~95 Torr and an 8.2-fold increase in total brain blood flow relative to the value measured at 240 min when PaCO2 was ~50 Torr. Although this is the extreme situation, ventilation with 10 ml/kg yielded a PaCO2 of 60 Torr and a 2.7-fold increase in brain blood flow. For comparison, in lambs treated with surfactant at birth and ventilated with 10 ml/kg, PaCO2 values were ~40 Torr and brain blood flows were the same at 20 and 240 min. The cerebral vasculature of the ventilated preterm lamb is capable of vasodilation with large increases in blood flow. The preterm infant often is born with elevated PaCO2 values because of placental compromise or other obstetric-related problems. The risk of high PaCO2 values at birth is not known. In several studies reviewed by Volpe (28), high PaCO2 was associated with an increased incidence of intraventricular hemorrhage, and in others it was not. Vannucci et al. (27) found that high PaCO2 values protected the newborn rat brain from hypoxia.

These studies in preterm lambs demonstrate that there are two ways to minimize lung injury in the preterm lamb with the initiation of ventilation: ventilate with low tidal volumes, which may result in higher-than-normal PaCO2 values, or treat with surfactant, which will minimize the injury from inadvertent high tidal volumes. However, without monitoring tidal volumes, extremely high tidal volumes could occur in surfactant-treated infants and could cause injury (5). An issue that has not been adequately studied in the...
than-normal PaCO2 values.

The authors thank Judith Purcell and Kathy To for excellent technical assistance.

This work was supported by National Institute of Child Health and Human Development Grant HD-12714.

Address for reprint requests: M. Ikegami, Div. of Pulmonary Biology, Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229-3039.

Received 7 March 1997; accepted in final form 15 May 1997.

REFERENCES