Increased airway liquid volumes at birth impair cardiorespiratory function in preterm and near-term lambs

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RESEARCH ARTICLE

Increased airway liquid volumes at birth impair cardiorespiratory function in preterm and near-term lambs

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Abstract

Respiratory distress is relatively common in infants born at or near-term, particularly in infants delivered following elective cesarean section. The pathophysiology underlying respiratory distress at term has largely been explained by a failure to clear airway liquid, but recent physiological evidence has indicated that it results from elevated airway liquid at the onset of air-breathing. We have investigated the effect of elevated airway liquid volumes at birth on cardiorespiratory function in preterm and near-term lambs. Preterm (130 ± 0 days gestation, term ~147 days gestation; *n* = 12) and near-term (139 ± 1 days gestation; *n* = 13) lambs were instrumented (to measure blood pressure, blood flow, and blood gas status) and, at delivery, airway liquid volumes were adjusted to mimic levels expected following vaginal delivery (Controls; ~7 mL/kg) or elective cesarean section with no labor (elevated liquid (EL); 37 mL/kg). Lambs were delivered, mechanically ventilated, and monitored for blood gas status, oxygenation, ventilator requirements, blood flows (carotid artery and pulmonary artery), and blood pressure during the first few hours of life. Preterm and near-term EL lambs had poorer gas exchange and required greater ventilatory support to maintain adequate oxygenation. Pulmonary blood flow was reduced and carotid artery blood flow, mean arterial blood pressure, and heart rate were reduced in EL near-term but not preterm lambs. These data provide further evidence that greater airway liquid volumes at birth adversely affect newborn cardiorespiratory function, with the effects being greater in near-term newborns.

NEW & NOTEWORTHY We provide evidence for adverse effects of elevated airway liquid volumes at birth on pulmonary blood flow and gas exchange in both preterm and near-term lambs, although the effects were greatest in near-term newborns. Our study is an important step toward understanding the fundamental physiology underlying the cardiorespiratory morbidity associated with near-term newborns with elevated airway liquid volumes leading to respiratory distress soon after birth.

airway liquid volume; cardiorespiratory transition; respiratory distress; respiratory function; transient tachypnea of the newborn

INTRODUCTION

Respiratory distress shortly after birth in near-term newborns is an increasing clinical problem, now accounting for 53% of infants admitted into intensive care for respiratory support (1). Transient tachypnea of the newborn (TTN) is the most common diagnosis, but this likely represents only a subset of afflicted infants (1, 2). TTN is characterized by rapid and labored breathing that develops within hours of birth and most commonly affects babies born near-term by elective cesarean section without labor (3, 4). Although the underlying pathophysiology is unclear, it was assumed to result from airway (alveoli and/or conducting airways) liquid retention (5) caused by a failure to activate Na^+ reabsorption (6). However, as the majority (>95%) of airway liquid is cleared after birth via pressure gradients generated by inspiration (7, 8), an absence of Na^+ reabsorption does not readily explain the pathophysiology. This finding is consistent with the evidence that airway liquid clearance can occur in the absence of Na^+ reabsorption (9) and that treatments targeting the stimulation of Na^+ reabsorption have not been effective at treating or preventing TTN in infants (2).

Although fetal lung liquid plays a vital role in regulating normal lung growth and development before birth, after birth, this liquid must be cleared to enable the entry of air and the onset of pulmonary gas exchange (10, 11). In pregnancies with normal amniotic fluid volumes, liquid loss from the respiratory tract primarily occurs during labor and is thought to exit via the nose and mouth (8, 12). However, following birth and the onset of air-breathing, all liquid present within the alveoli/airways must be cleared into lung tissue (7, 13), irrespective of whether the underlying mechanism is Na^+

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reabsorption or hydrostatic pressures generated by inspiration. This liquid then takes 3–4 h to clear from lung tissue (14, 15). During this time, the chest wall must expand to accommodate both the preexisting liquid and the incoming air that comprises the new functional residual capacity (FRC) (13). Fortunately, the newborn chest wall is highly compliant and can readily expand (14), necessitating only a small increase in interstitial tissue pressure (~6 cmH_2O) as a result of lung aeration (14). Logically, therefore, larger volumes of airway liquid at birth require larger volumes of liquid to be accommodated in lung tissue leading to greater chest wall expansion and higher interstitial tissue pressures. Indeed, higher airway liquid volumes at birth increase airway resistance, reduce lung compliance, and reduce FRC levels following lung aeration, particularly in the mature lung when lung airspace to tissue space volumes have greatly increased (16). More recently, we have shown that elevated airway liquid volumes at birth reduce lung aeration rates, reduce FRC at lower positive end-expiratory pressures, expand the chest wall, and flatten the diaphragm in near-term newborn rabbits (17).

The movement of airway liquid into lung tissue increases interstitial tissue pressures that reduce the transmural pressure across the interstitial tissue and alveolar capillary wall, leading to capillary collapse and an increase in pulmonary vascular resistance (PVR) as originally described by Hughes et al. (18). In this study, our aim was to determine the effect of elevated airway liquid volumes (akin to the volume expected after elective cesarean section with no labor compared with vaginal delivery) on respiratory function and the increase in pulmonary blood flow (PBF) after birth in near-term and preterm lambs. We hypothesized that increased airway liquid volumes at birth will increase PVR and decrease PBF following lung aeration after birth. Furthermore, as interalveolar interstitial tissue markedly thins with increasing gestational age, we hypothesized that the reduction in PBF will be greater in near-term lambs than in preterm lambs due to reduced capacity of lung tissue to accommodate elevated liquid volumes after birth.

MATERIALS AND METHODS

Ethical Approval

All experimental procedures were approved by the Monash Medical Center Animal Ethics Committee and Monash University. All experiments were conducted in accordance with the National Health and Medical Research Council (NHMRC) of Australia code of practice for the care and use of animals for scientific purposes. Methodological reporting is provided as per the relevant ARRIVE guidelines (19).

Experimental Procedure

Pregnant Merino X Border-Leicester ewes at 130 ± 0 (preterm group; equivalent to ~32 wk gestation in terms of human lung development) or 139 ± 1 (near-term group; equivalent to ~37 wk human lung development) days gestational age (term ~147 days) were anesthetized intravenously with sodium thiopentone (Pentothal; 1 g in 20 mL). Following intubation, anesthesia was maintained with 1.5%–2.5% isoflurane (Isoflow, Abbot Pty. Ltd., Australia) in room air/oxygen as previously described (20, 21). The fetal head and neck were exteriorized via cesarean section and a blocked endotracheal tube (3.5 mm for preterm or 4.5 mm for near-term lambs cuffed ET tube; Covidien, Dublin, Ireland) was inserted to prevent loss of fetal lung liquid before delivery of the lamb. Vascular catheters were implanted into the right internal jugular vein for anesthetic administration following delivery and the right common carotid artery to obtain blood gas samples and measure blood pressure. A Precision S 4 mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the left pulmonary artery to measure PBF and a saline-filled latex balloon was inserted into the thoracic cavity to measure intrathoracic pressure as previously described (22). A Precision S 3 mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was also placed around the left common carotid artery to measure carotid blood flow. A near-infrared spectroscopy optode (NIRS; Casmed Foresight, CAS Medical Systems Inc., Branford, CT) sensor was placed over the left frontal cortex to measure cerebral tissue oxygen saturation ($SctO_2$). Core body temperature was measured using a probe inserted into the lamb's rectum and used to temperature-adjust blood gas values. All physiological values were recorded digitally, along with ventilation parameters (tidal volume, Vt; mean airway pressure, Paw; peak inflation pressure, PIP; fraction of inspired oxygen, FI_{O_2}), using a data acquisition system running LabChart 7 software (ADInstruments, Castle Hill, Australia).

Delivery and Ventilation

Following exteriorization, lambs were dried and baseline physiological data recordings and blood gas samples were collected. Immediately before umbilical cord clamping, fetal lung liquid was passively drained from the endotracheal tube until liquid ceased exiting the airways (10–20 s). Following lung liquid drainage, both preterm and near-term lambs were randomly subdivided into one of two groups as previously described in newborn rabbits (16, 17). The Control group had no liquid returned to their airways to mimic natural liquid clearance after vaginal delivery (Preterm, n = 6; near-term, n = 7 lambs), despite being delivered by cesarean section. The elevated liquid (EL preterm, n = 6; EL near-term, n = 6 lambs) group had 30 mL/kg (estimated body weight) of Hartmann's solution [similar ionic composition to fetal lung liquid (23)] returned to their airways. This volume simulates the volume of airway liquid (35–45 mL/kg) expected in singleton lambs with normal amniotic fluid volumes before the onset of labor (i.e., the volume expected after an elective cesarean section) (8, 24). As the volume of residual liquid remaining in the distal airways after drainage is ~7 mL/kg (24), at ventilation onset, we estimate that lambs in the Control group had ~7 mL/kg and EL lambs had ~37 mL/kg of airway liquid.

After the umbilical cord was clamped and cut, lambs were immediately transferred to an infant warmer (CosyCot, Fisher and Paykel, Auckland, New Zealand). Lambs were mechanically ventilated to control for differences in airway liquid clearance caused by differences in breathing effort associated with spontaneous breathing. Mechanical ventilation was commenced as soon as possible

Table 1. *Equations for measures derived from physiological recordings obtained during experiments*

Measure	Equation
Dynamic lung compliance	[Vt/kg]/(PIP − PEEP)
Alveolar-arterial difference in oxygen ($AaDO_2$)	$(P_{barometric} - P_{H2O}) \times FI_{O_2} - (Pa_{CO_2}/RQ) - Pa_{O_2}$
Cerebral tissue oxygen delivery (cerebral DO_2)	$CBF \cdot [(1.39 \cdot Hb \times Sa_{O_2}/100) + (0.003 \cdot Pa_{O_2})]/100$

V_t, tidal volume; PIP, peak inflation pressure; PEEP, positive end expiratory pressure; FI_{O_2}, fraction of inspired O_2 (%); Pa_{O_2}, partial pressure of arterial oxygen; $p_{barometric}$, barometric pressure (760 mmHg); P_{H2O}, water vapor pressure at body temperature (47 mmHg at 39°C); Pa_{CO_2}, partial pressure of arterial carbon dioxide; RQ, respiratory quotient (0.8); CBF, carotid artery blood flow; Hb, hemoglobin concentration (g/dL); Sa_{O_2}, arterial oxygen saturation.

with a single 30-s sustained inflation (35 cmH_2O, 21% O_2) delivered by a Neopuff (Fisher and Paykel Healthcare, Panmure, Auckland, New Zealand) to establish FRC and uniform lung aeration (25). Following the sustained inflation, pressure limited (40 cmH_2O) assist/control volume guarantee ventilation (Babylog 8000+, Draeger, Luebeck, Germany) was applied with a set tidal volume (7 mL/kg) based on estimated lamb body weight. Initial ventilator settings were as follows: peak inflation pressure (PIP), 40 cmH_2O; positive end-expiratory pressure (PEEP), 5 cmH_2O; inspiratory time, 0.4 s; expiratory time, 0.6 s; and fraction of inspired oxygen (FI_{O_2}), 21%. During the ventilation period, FI_{O_2} and/or ventilator settings were adjusted to maintain Sp_{O_2} (90%–95%) and the partial pressure of carbon dioxide in arterial blood (Pa_{CO_2}; >40 and <60 mmHg) within target ranges. Lambs were ventilated for a total period of either 1 h (preterm groups) or 2 h (near-term groups). Arterial blood gas samples (0.25 mL) were collected at regular intervals following ventilation onset; 5, 10, 15, 20, 30, 40, 50, and 60 min (preterm and near-term) in addition to 70, 80, 90, 110, and 120 min (near-term group only) to measure Pa_{CO_2}, pH, hemoglobin, partial pressure of arterial oxygen (Pa_{O_2}), and arterial oxygen saturation (Sa_{O_2}).

Postmortem Examination

Following delivery of the lambs, ewes were euthanized with an intravenous overdose of sodium pentobarbitone (Virbac Pty. Ltd., Peakurst, Australia). At the conclusion of the experiment, all lambs were euthanized using an intravenous overdose of sodium pentobarbitone (Virbac Pty. Ltd., Peakurst, Australia). Lamb body and organ weights were recorded. The lungs were excised and dissected to remove surrounding connective tissue and fat before total wet lung weight was recorded. A section of left lung tissue was collected to determine wet/dry weight ratios.

Lung Wet/Dry Weight Ratio

Lung tissue was weighed immediately after postmortem (wet lungs) and then placed in an oven at 60°C to dehydrate. The samples were weighed daily to observe a change in mass and once a constant mass was maintained over 3 days, the dry weight of the tissue was recorded. The ratio of wet lung weight to dry lung weight was calculated as previously described (20, 26).

Analysis of Physiological Recordings

Physiological measures (10–20 s epochs) of arterial blood pressure, blood flow (cerebral and pulmonary), oxygenation (cerebral and systemic), intrathoracic pressure, and ventilation parameters (PIP, PEEP, Paw, Vt) obtained using Labchart software were analyzed to coincide with the timing of arterial blood gas samples. To assess newborn respiratory function, lung compliance, minute volume, and alveolar-arterial difference in oxygen ($AaDO_2$) were derived from the physiological measurements obtained using equations in Table 1, as was cerebral oxygen delivery (cerebral DO_2).

Statistical Analysis

Our study specifically aimed to investigate the effect of greater airway liquid volumes at birth on PBF and newborn respiratory function in preterm and near-term lambs. Therefore, the differences have been compared within each age group to most appropriately address our research question. Data were tested for normality and transformed if required. All data are represented as mean ± standard error of the mean (SE). Statistical analysis was performed with Prism v7 (GraphPad Software, San Diego, CA) and $P \leq 0.05$ was considered statistically significant. The sex of lambs was analyzed as a categorical variable using Fisher's exact test. Fetal blood gas values and body/organ weights and PBF area under the curve were analyzed using a Student's unpaired *t* test for comparison between the Control and EL groups within each age group. Blood gas values and physiological data were analyzed by two-way repeated-measures ANOVA or mixed-effects model (if there were missing values in the data set indicated by outliers at > ± 2 standard deviations from the group mean) for treatment (i.e., Control vs. EL) and time with Holm–Sidak's post hoc analysis to account for multiple comparisons. When there was an interaction between the two factors (treatment and time; $P < 0.05$), data are presented as the difference between the Control and EL groups at individual time points during the ventilation period.

RESULTS

Baseline Characteristics and Fetal Blood Gas Values

There were no significant differences in sex, gestational age, fetal blood gas values, or body weight between Control and EL lambs in the preterm (Table 2) or near-term (Table 3) groups. The mean volume of lung liquid drained was 43.8 ± 7.7 mL in preterm lambs and 58.3 ± 7.5 mL in near-term lambs. In EL lambs, the mean volume of liquid returned to the lungs was 104 ± 5.6 mL in preterm and 130 ± 7.2 mL in near-term lambs.

Effect of Greater Airway Liquid Volume at Birth on Intrathoracic Pressure

During the ventilation period, intrathoracic pressures in both preterm and near-term lambs were not significantly different between Control and EL lambs (Fig. 1, *A* and *B*), although pressures tended to be lower in Control lambs. During the ventilation period in near-term lambs, intrathoracic pressures gradually decreased over time (Fig. 1*B*), at an average rate of −6.4 and −7.9 cmH_2O/h in Control and EL lambs, respectively, with pressures gradually

Table 2. *Baseline characteristics, fetal blood gas values, and measures of lung weight in preterm lambs*

	Preterm Control	Preterm Elevated Liquid
Lambs, *n*	6	6
Male:Female	6:0	3:3
Gestational age at delivery, days	130 ± 0	130 ± 0
Pa_{CO_2}, mmHg	54.8 ± 2.4	52.3 ± 3.1
pH	7.27 ± 0.01	7.25 ± 0.02
Sa_{O_2}, %	53.5 ± 1.8	46.5 ± 5.5
Body weight, kg	3.6 ± 0.1	3.7 ± 0.2
Relative wet lung weight, g/kg	40.0 ± 2.1	49.5 ± 4.0
Lung wet/dry weight ratio	8.2 ± 0.5	9.3 ± 0.5

Data are presented as means ± SE. Sex was analyzed as a categorical variable using Fisher's exact test. All other measurements were analyzed by the Student's unpaired *t* test. $P \leq 0.05$ was considered significant.

becoming more subatmospheric despite positive pressure ventilation with PEEP. In preterm lambs (Fig. 1*A*), the rate of decrease was significantly ($P < 0.001$) greater in Control preterm lambs (−4.3 cmH_2O/h) than in EL preterm lambs (−0.15 cmH_2O/h).

Effect of Greater Airway Liquid Volume at Birth on Newborn Blood Gas Status

In preterm lambs, Sa_{O_2} (Fig. 2*A*) values were similar in Control and EL lambs, but EL lambs required significantly higher FI_{O_2} levels during the entire ventilation period to maintain adequate oxygenation levels (Fig. 2*C*; average FI_{O_2}, Control = 31.5 ± 1.3% vs. EL = 67.8 ± 2.8%, $P < 0.001$). As a result, the $AaDO_2$ (Fig. 2*E*) was significantly higher in EL than in Control preterm lambs throughout the ventilation period. Similarly, the Pa_{CO_2} (Fig. 3*A*) was significantly higher and pH significantly lower (Fig. 3*C*) in EL than in Control preterm lambs throughout the ventilation period.

In near-term lambs, Sa_{O_2} (Fig. 2*B*) was significantly lower in EL than in Control lambs throughout the entire ventilation period, despite receiving a significantly higher FI_{O_2} (Fig. 2*D*; average FI_{O_2}, Control = 21.0 ± 0.04% vs. EL = 50.0 ± 2.5%, $P < 0.001$). As a result, the $AaDO_2$ (Fig. 2*F*) was significantly higher in EL than in Control near-term lambs. In contrast to preterm lambs, in near-term lambs, the Pa_{CO_2} (Fig. 3*B*) and pH (Fig. 3*D*) levels were similar in Control and EL lambs throughout the entire ventilation period.

Effect of Greater Airway Liquid Volume at Birth on Newborn Cardiovascular Function

In preterm lambs, PBF corrected for body weight (Fig. 4*A*) tended to lower in EL and Control lambs from 15–30 min after ventilation onset, which was significant when corrected for wet lung weight (Fig. 4*C*). Calculated over the entire ventilation period, PBF corrected for lung weight was 24% lower in EL than in Control preterm lambs, but this difference was not significant (area under the curve: Control = 11.8 ± 1.2; EL = 9.0 ± 1.3 mL/g; $P = 0.14$). Carotid artery blood flow (CBF; Fig. 5*A*), mean arterial blood pressure (Fig. 5*C*), and heart rate (Fig. 5*E*) were similar in EL and Control preterm lambs. However, significant time-related changes were observed in both groups over time.

In near-term lambs, while PBF corrected for body weight tended to be lower in EL than in Control lambs (Fig. 4*B*), when corrected for lung weight, PBF was significantly lower at 5–15 min after ventilation onset (Fig. 4*D*). Over the entire ventilation period, PBF corrected for lung weight was 38% lower in EL than in Control lambs (area under the curve: Control = 24.7 ± 3.6; EL = 15.2 ± 1.09 mL/g, $P = 0.04$). Mean arterial blood pressure (Fig. 5*D*) and heart rate (Fig. 5*F*) were significantly lower in EL than in Control near-term lambs throughout the ventilation period. Similarly, CBF was significantly lower in EL than in Control lambs at 30 min after ventilation onset (Fig. 5*B*).

Effect of Greater Airway Liquid Volume at Birth on Cerebral Oxygen Delivery and Oxygenation

In preterm lambs, cerebral DO_2 (Fig. 6*A*) and $SctO_2$ (Fig. 6*C*) were not significantly different between EL and Control preterm lambs. Both cerebral DO_2 and $SctO_2$ increased with time following ventilation onset in preterm lambs (Fig. 6, *A* and *C*).

In near-term lambs, cerebral DO_2 tended to be lower in EL than in Control lambs during the entire ventilation period; however, this difference was only significant at 30 min after ventilation onset (Fig. 6*B*). $SctO_2$ increased over time and was significantly lower in EL than in Control near-term lambs during the entire ventilation period (Fig. 6*D*).

Effect of Greater Airway Liquid Volume at Birth on Respiratory Function

In preterm lambs, the PIP required to achieve a set Vt of 7 mL/kg was not significantly different between EL and Control lambs (Fig. 7*A*), but EL lambs did have a significantly higher mean airway pressure throughout the entire ventilation period (Fig. 7*C*). There was no significant difference in lung compliance between EL and Control preterm lambs, and lung compliance increased in both groups over time (Fig. 7*G*). Minute volumes were also similar between groups (Fig. 7*E*).

In near-term lambs, the PIP required to achieve a set Vt of 7 mL/kg was significantly higher in the EL than in Control lambs during the entire experimental period (Fig. 7*B*). As a result, mean airway pressures were also significantly higher in EL than in Control lambs during the entire ventilation period (Fig. 7*D*). Lung compliance increased over time but was significantly lower in EL than in Control near-term lambs

Table 3. *Baseline fetal characteristics, fetal blood gas values and measures of lung weight in near-term lambs*

	Near-Term Control	Near-Term Elevated Liquid
Lambs, *n*	7	6
Male:Female	3:4	4:3
Gestational age at delivery, days	139 ± 1	139 ± 1
Pa_{CO_2}, mmHg	64.6 ± 5.3	58.7 ± 3.9
pH	7.21 ± 0.04	7.28 ± 0.02
Sa_{O_2}, %	47.7 ± 11.1	53.6 ± 5.6
Body weight, kg	4.1 ± 0.3	4.4 ± 0.3
Relative wet lung weight, g/kg	33.4 ± 1.4	44.2 ± 2.8*
Lung wet/dry weight ratio	8.0 ± 0.3	8.9 ± 0.6

Data are presented as means ± SE. Sex was analyzed as a categorical variable using Fisher's exact test. All other measurements were analyzed by the Student's unpaired *t* test. *$P \leq 0.05$ was considered significant.

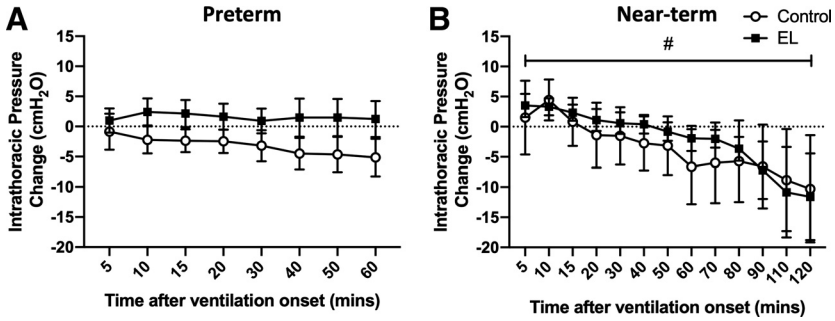

Figure 1. Effect of greater airway liquid volume at birth on intrathoracic pressure. Changes in intrathoracic pressure from fetal baselines values in Control (white circles) and elevated liquid (EL; black squares) preterm (*A*) and near-term lambs (*B*). $P < 0.05$, #effect of time following ventilation onset.

during the entire ventilation period (Fig. 7*H*). There was no difference in the minute volumes between groups (Fig. 7*F*), despite the tendency for Control lambs to require lower inflation rates to maintain blood gas values within the physiological target range during the last half of the ventilation period (Control = 40 ± 2.5 vs. EL = 48 ± 4.67 inflations per minute at 40 min).

Effect of Greater Airway Liquid at Birth Volumes on Lung Tissue

In preterm lambs, there was an increase in relative wet lung weight in EL lambs, however, this did not reach statistical significance ($P = 0.06$; Table 2). Near-term lambs with EL had significantly greater relative wet lung weight than Controls ($P = 0.004$; Table 3). There was no significant difference in wet/dry lung weight ratios in EL compared with Control in preterm or near-term lambs (Tables 2 and 3). When comparing the wet/dry lung weight ratio of the EL group between preterm and near-term lambs, there was no significant effect ($P = 0.49$) despite the difference in ventilation duration between the age groups.

DISCUSSION

These data demonstrate that the presence of greater airway liquid volumes at birth adversely affects respiratory

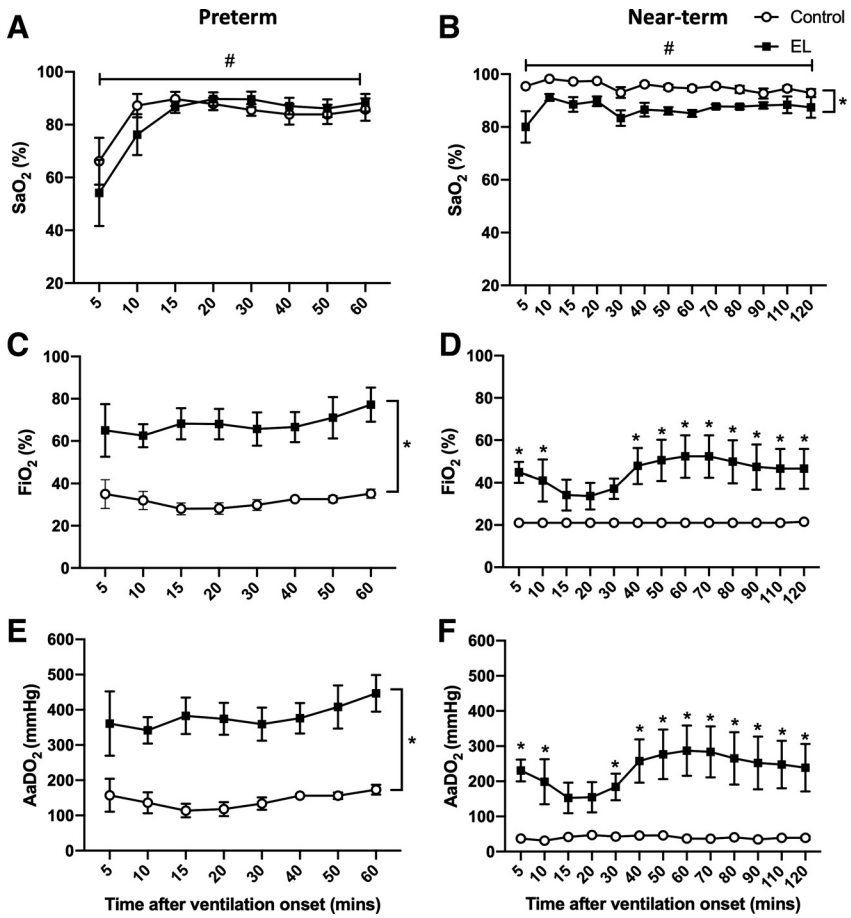

Figure 2. Effect of greater airway liquid volume at birth on oxygenation. Arterial oxygen saturation (Sa_{O_2}; *A* and *B*), fraction of inspired oxygenation (FI_{O_2}; *C* and *D*) and alveolar-arterial difference in oxygen ($AaDO_2$; *E* and *F*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A, C,* and *E*) and near-term lambs (*B, D,* and *F*). $P < 0.05$, *effect of treatment (i.e., control vs. elevated liquid), #effect of time following ventilation onset.

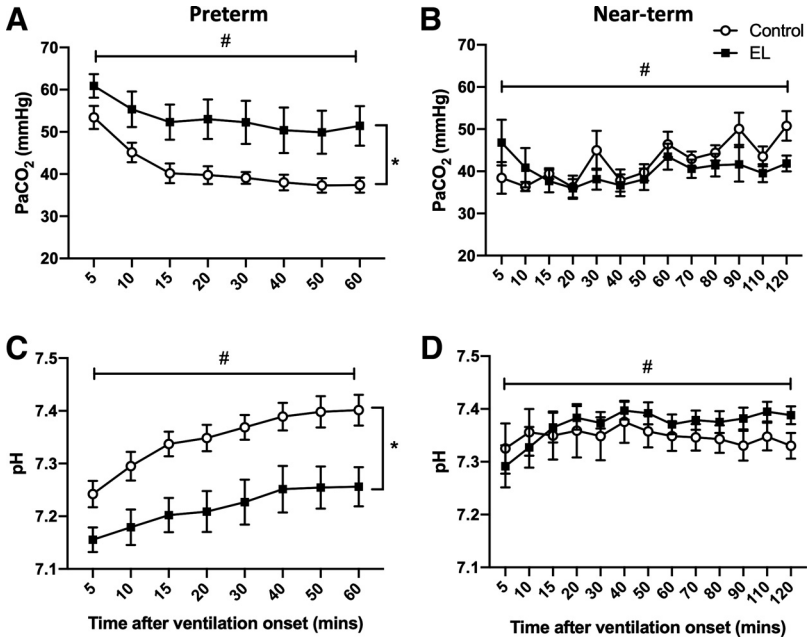

Figure 3. Effect of greater airway liquid volume at birth on blood gas status. Partial pressure of carbon dioxide (Pa_{CO_2}; *A* and *B*) and pH (*C* and *D*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A* and *C*) and near-term lambs (*B* and *D*). $P < 0.05$, #effect of time following ventilation onset.

function, PBF, and other cardiovascular parameters, particularly in lambs born near term. EL lambs at both ages had poorer gas exchange requiring significantly more respiratory support and supplemental oxygen to maintain similar arterial blood gases. Furthermore, despite similar levels of oxygenation, near-term EL lambs also had greater difficulty maintaining adequate systemic and cerebral oxygenation. Similarly, although both respiratory and cardiovascular functions were impaired by EL in near-term lambs, EL only impaired respiratory function in preterm lambs. These data provide further evidence that elevated airway liquid volumes at birth adversely affect both the cardiovascular and respiratory transition at birth and these adverse effects are greater in more mature newborns.

Irrespective of whether airway liquid is cleared via Na^+ reabsorption (6) or via inspiration (7, 13), all of the liquid present in the airways at the onset of air-breathing must be cleared into lung tissue (14, 15). Liquid accommodated in the lung at birth transiently (over 3–4 h) increases lung interstitial tissue pressures, which gradually decreases as the liquid is cleared from lung tissue (14) via the blood vessels and lymphatics. Our finding of a gradually decreasing intrapleural pressure over time is consistent with this process. Logically, therefore, at birth, the presence of greater airway liquid volumes at breathing onset means that more liquid has to be accommodated in lung tissue and as such, the increase in interstitial tissue pressure will be greater. Furthermore, as the lung matures,

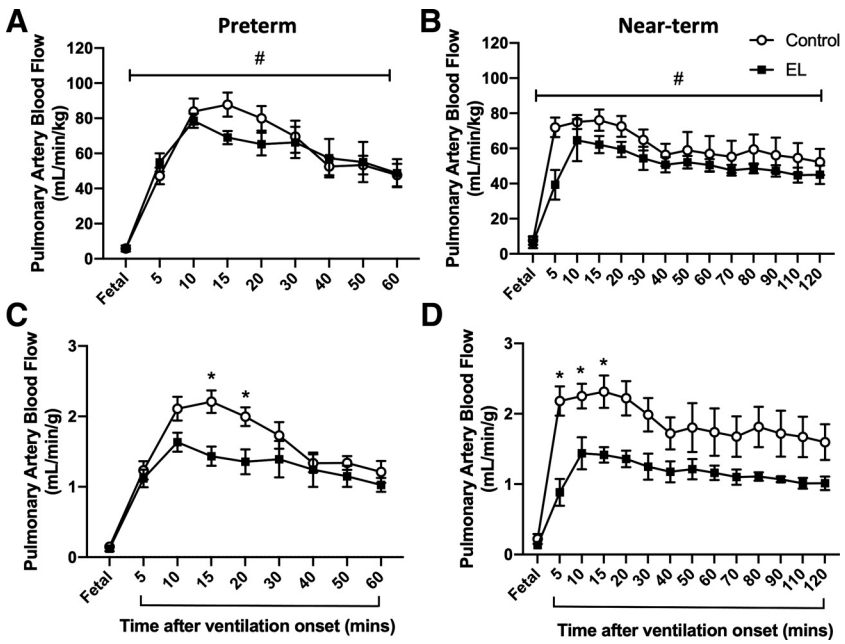

Figure 4. Effect of greater airway liquid volume at birth on pulmonary blood flow during the transition from fetal to newborn life. Pulmonary artery blood flow expressed per kg body weight (PBF; *A* and *B*) and per gram of wet lung weight (*C* and *D*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A* and *C*) and near-term lambs (*B* and *D*). $P < 0.05$, *effect of treatment (i.e. control vs. elevated liquid), #effect of time during the transition.

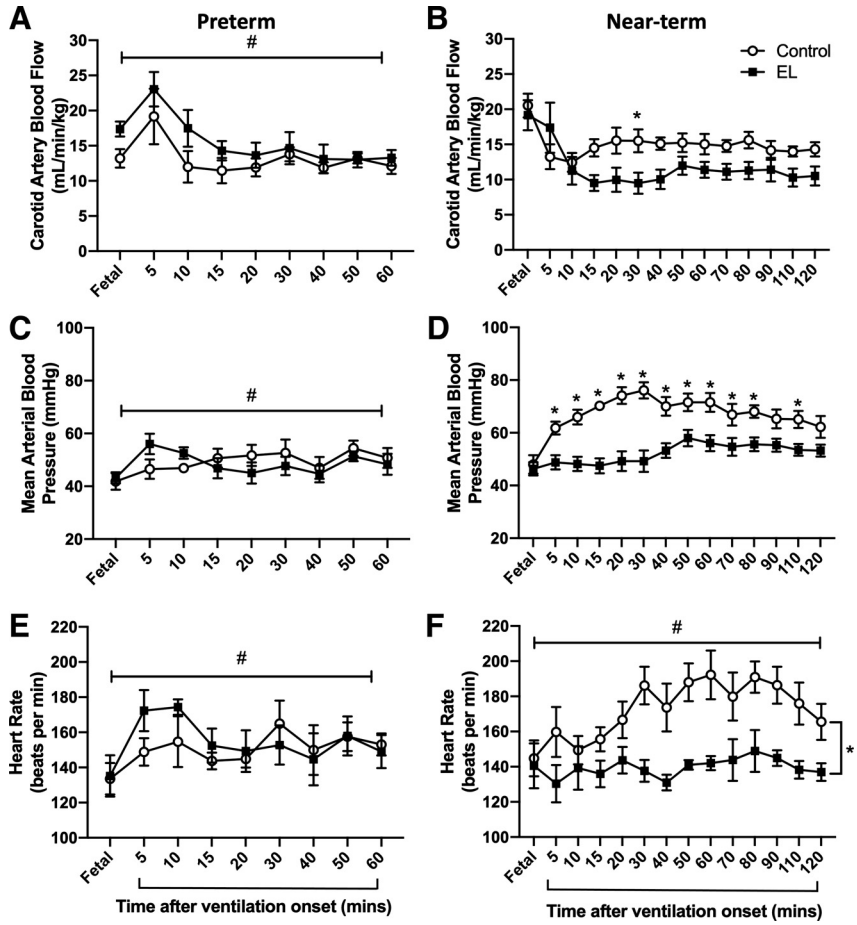

Figure 5. Effect of greater airway liquid volume at birth on hemodynamics during the transition from fetal to newborn life. Carotid artery blood flow expressed per kg of body weight (CBF; *A* and *B*), mean arterial blood pressure (*C* and *D*), and heart rate (*E* and *F*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A, C,* and *E*) and near-term lambs (*B, D,* and *F*). $P < 0.05$, *effect of treatment (i.e. control vs. elevated liquid), #effect of time during the transition.

perialveolar tissue volumes, relative to airspace volumes, decrease exponentially, leading to thinner interalveolar tissue and greatly reduced air/blood gas barriers. As such, larger volumes of airway liquid must be accommodated within a relatively smaller tissue volume as newborns mature and are delivered near term. The greater pulmonary edema would be expected to cause larger increases in interstitial tissue pressures and potentially explains the increased adverse effects of elevated liquid at older gestational ages (16, 17).

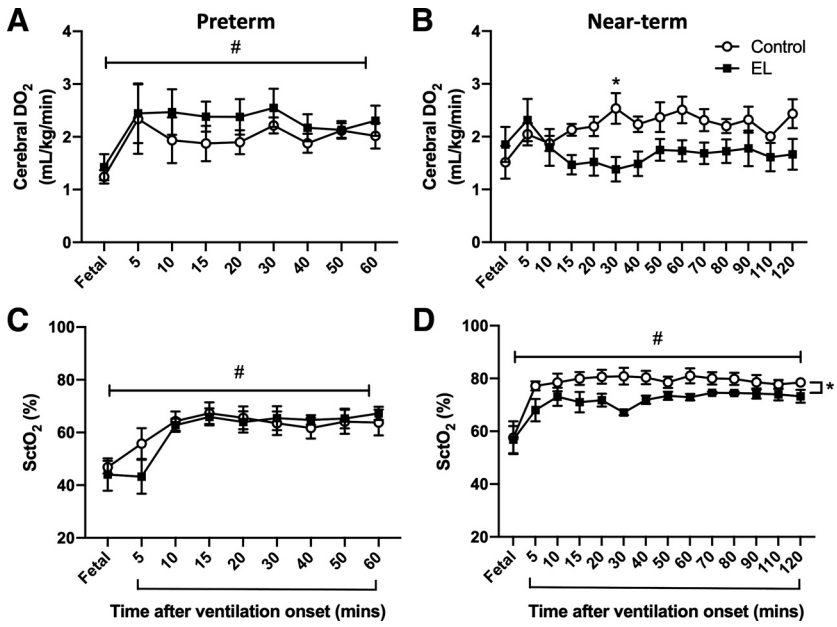

Figure 6. Effect of greater airway liquid volume at birth on cerebral oxygen delivery and oxygenation during the transition from fetal to newborn life. Cerebral tissue oxygen delivery (cerebral DO_2; *A* and *B*) and cerebral tissue oxygen saturation ($SctO_2$; *C* and *D*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A* and *C*) and near-term lambs (*B* and *D*). $P < 0.05$, *effect of treatment (i.e. control vs. elevated liquid), #effect of time during the transition.

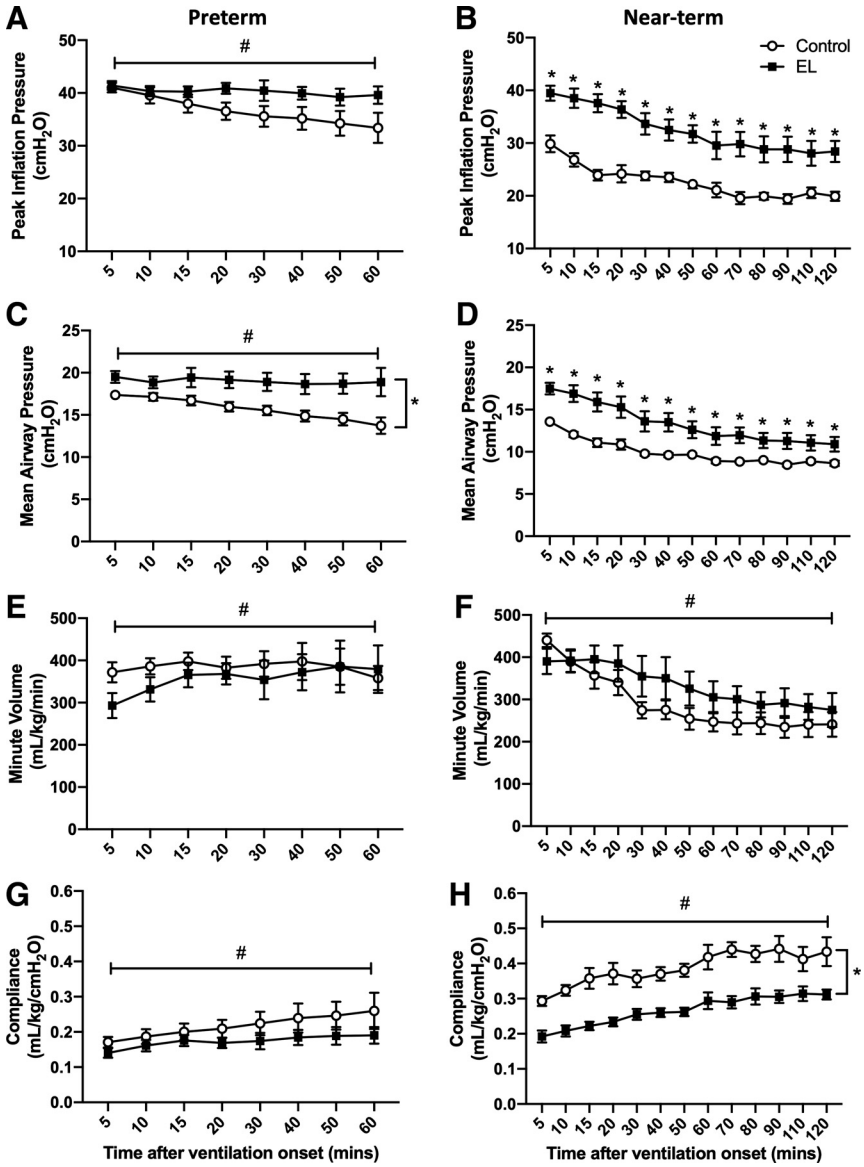

Figure 7. Effect of greater airway liquid volume at birth on respiratory support requirements and lung mechanics. Peak inflation pressure (*A* and *B*), mean airway pressure (*C* and *D*), minute volume (*E* and *F*), and lung compliance (*G* and *H*) in control (white circles) and elevated liquid (EL; black squares) preterm (*A, C, E,* and *G*) and near-term lambs (*B, D, F,* and *H*). $P < 0.05$, *effect of treatment (i.e. control vs. elevated liquid), #effect of time following ventilation onset.

Our finding that wet lung weights (per kg body weight) were higher in EL lambs at autopsy in both age groups (Preterm EL vs. Control, $P = 0.06$; near-term EL vs. Control, $P = 0.004$) confirms that some liquid is retained within the chest for at least the first 2 h after delivery. However, we would expect that a significant amount of this liquid would still have been cleared from the tissue by the time of autopsy. Furthermore, the suggestion that this increases interstitial tissue pressure and, through expansion of the lung, intrathoracic pressures, is consistent with the finding that intrathoracic pressures tended to be higher in EL than in Control lambs (Fig. 1). Although the differences were not statistically different, this was due to insufficient statistical power caused by the high variability in pressure measurements within and between animals.

Effect of Greater Airway Liquid Volume at Birth on PBF and Respiratory Function

We hypothesized that an increase in lung interstitial tissue pressure would reduce the transmural pressure across the alveolar capillary wall (i.e., between capillary and tissue), causing collapse or a reduction in caliber of alveolar capillaries, thereby increasing PVR and decreasing PBF. Our finding that PBF was reduced in EL lambs compared with Control lambs is consistent with this suggestion. As such, it is possible that elevated airway liquid at birth may contribute to persisting pulmonary hypertension of the newborn (PPHN) in near-term infants that develop respiratory distress after birth. In contrast, the effect of elevated airway liquid volumes on PBF was relatively smaller and nonsignificant in preterm lambs when PBF was adjusted for lung weight. As explained in the discussion above, we suggest that this is a result of a less mature lung structure and relatively greater lung tissue-to-airspace volume ratios in the preterm lung, leading to less pronounced increases in interstitial tissue pressure and reductions in capillary wall transmural pressure in response to elevated airway liquid volumes.

We have previously shown that the effects of elevated liquid on lung compliance and airway resistance is gestational

age dependent in newborn rabbits, which was explained by the differences in lung structural maturity at the different gestational ages (16). Thus, our finding in this study that the effect of elevated liquid on respiratory function, particularly lung compliance, is relatively less in the immature preterm lung is consistent with our previous finding in rabbits. Indeed, we found that the difference in PIP required to generate a target Vt of 7 mL/kg in EL lambs was substantially greater in older lambs (Fig. 7; average PIP in Control = 22.6 ± 0.43 vs. EL = 32.6 ± 0.69 cmH_2O; $P < 0.001$), who required PIPs that were not too dissimilar from Control preterm lambs (average PIP = 36.9 ± 0.72 cmH_2O). When combined with our previous studies in rabbits (16, 17), these data provide compelling evidence that elevated liquid volumes at the onset of air-breathing increase the volumes of liquid accommodated in lung tissue, which alters lung tissue mechanics. The presence of elevated liquid volume in the lung causes both the chest wall to expand and diaphragm to flatten further (17). As the chest wall is not infinitely compliant, it must come to a point where it cannot expand further, which likely greatly reduces respiratory compliances and increases interstitial tissue pressures. This would be expected to make expansion and therefore aeration of the lung considerably more difficult.

The Effect of Greater Airway Liquid Volume at Birth on Pulmonary Gas Exchange

Relative to age-matched controls, the effects of elevated liquid volumes on PBF and lung mechanics were greater in near-term than in preterm lambs, whereas the reductions in pulmonary gas exchange appeared relatively greater in preterm lambs than in near-term lambs. Indeed, although the differences in oxygen exchange were relatively similar between ages, as indicated by the significant $AaDO_2$ differences required to achieve similar arterial oxygen saturations, elevated liquid was associated with a significant hypercapnia and acidosis in preterm lambs (main effect of EL; Pa_{CO_2} P = 0.03 and pH P = 0.01), but not in near-term lambs (main effect of EL; Pa_{CO_2} P = 0.54 and pH P = 0.60). This finding is not what we predicted, as hypoxemia concurrent with mild hypercapnia and acidosis are frequently seen in infants with TTN (27). Nevertheless, in hindsight, it is possible that maturational changes in lung microstructure may explain these differences. As the lung matures, capillary walls fuse with alveolar walls to form a very narrow (~200 nm) air/blood-gas barrier in the mature lung that has no interstitial tissue between the capillary and alveolus (28). As such, it is difficult to envisage how elevated liquid in lung tissue could affect gas exchange in the mature lung except by causing alveolar collapse, but this was opposed by ventilation with PEEP in our study. Alternatively, alveolar surface area may have been reduced by excess alveolar liquid to a greater extent in preterm lambs. Indeed, we have previously shown that elevated liquid reduces FRC, causing alveolar reflooding between breaths, particularly in smaller alveoli (17). As CO_2 is considerably more soluble (~30×) than O_2, CO_2 exchange is far more robust than O_2 exchange and so a reduction in both O_2 and CO_2 indicates a severe reduction in gas exchange (29). As PEEP is very effective at maintaining FRC, it is possible that insufficient PEEP was used in preterm lambs compared with near-term lambs, causing a greater reduction in gas exchange in preterm lambs. This raises the very important question as to the role of end-expiratory pressure levels (PEEP or continuous positive airway pressure) in assisting gas exchange in newborns with elevated lung liquid volume at birth. Similarly, while all lambs received 5 cmH_2O of PEEP from birth, most newborns who develop TTN, breathe spontaneously after birth and are not supported by an end-expiratory pressure from birth. Thus, it is possible that the adverse cardiorespiratory effects we observed will be exacerbated in spontaneously breathing newborns.

Effect of Elevated Airway Liquid Volume at Birth on Cardiovascular Function and Cerebral Oxygen Delivery

In near-term, but not preterm lambs, elevated airway liquid volumes had a significant impact on cardiovascular function that is difficult to explain. In near-term lambs, elevated liquid caused a reduction in CBF from 10 min after ventilation onset, which could be explained by either a reduction in blood pressure or a reduction in cardiac output. Indeed, a reduction in PBF and pulmonary venous return will reduce left ventricular preload. In any event, the lower CBF significantly reduced cerebral oxygen delivery in EL near-term lambs. Although the magnitude of effect on $SctO_2$ was lower, these data are consistent with the finding of lower cerebral tissue oxygenation in near-term newborns delivered by elective cesarean section who subsequently develop mild respiratory distress after birth (30).

The substantial reduction in mean arterial blood pressure associated with elevated liquid in near-term, but not preterm, lambs is likely a consequence of the bradycardia. We have previously suggested that the increase in PBF at birth is in part mediated by the movement of liquid from the airways into lung tissue, causing "pulmonary edema" and activation of juxta-pulmonary capillary receptors (J-receptors) (31, 32). J-receptors respond to pulmonary edema and signal via afferent nerve fibers within the vagal trunk to stimulate tachypnea and the sensation of dyspnea (33), which are two major symptoms of TTN. As activation of these receptors also stimulates a reflex bradycardia (33), it is possible that J-receptor activation is also responsible for the bradycardia we observed. If so, activation of these receptors appears to override other reflexes such as the baroreceptor reflex, leading to sustained lower blood pressures. This observation may have clinical implications as low blood pressure in the newborn may prompt the use of inotropes or the addition of plasma volume, both of which could contribute further to pulmonary congestion. Nevertheless, the finding that bradycardia and hypertension were not observed in preterm EL lambs suggests that the level of pulmonary edema and increased interstitial tissue pressure suffered by these lambs was less than in near-term lambs. Alternatively, it is possible that J-receptors do not develop until late in gestation and so were not activated in the preterm lambs.

Clinical Considerations of Greater Airway Liquid Volume at Birth Underlying TTN Morbidity

We have shown that greater airway liquid volumes at birth reduce lung function, expand the chest wall, flatten the diaphragm (17), reduce PBF, and alter lung mechanics. When

these findings are combined with the observations that pulmonary edema directly stimulates tachypnea, bradycardia, and the sensation of dyspnea, it is difficult to not draw the conclusion that respiratory distress in near newborn babies (e.g., TTN) is due to enhanced pulmonary edema. Indeed, these findings readily explain all of the symptoms displayed by infants diagnosed with TTN. Furthermore, studies have also reported higher incidence of PPHN in neonates delivered by elective cesarean section compared with vaginal delivery, although the etiology of PPHN delivered by cesarean section was unclear (34–36). Apparently, these infants may initially appear normal or have mild respiratory symptoms requiring minimal oxygen supplementation, but have some lung consolidation on their chest X-rays (37, 38), However, a small subset of these infants may then deteriorate, requiring increased oxygen supplementation that does not respond to surfactant administration and they gradually develop PHHN (37).

We suggest that the majority of respiratory distress suffered by term infants (excluding abnormalities, infections, and meconium aspiration) arises from the presence of elevated airway liquid when air-breathing first commenced. However, it is likely that this common mechanism has been difficult to pinpoint due to the huge heterogeneity in clinical scenarios that may or may not lead to elevated levels of airway liquid at birth. While the absence of labor and vaginal delivery (due to elective cesarean section) deprives the lung of the only mechanism that results in the complete loss of airway liquid from the respiratory system, there is a large range of scenarios that can influence airway liquid volumes at birth. These include *1*) mode and timing of delivery in relation to labor onset, *2*) volume of amniotic fluid immediately before delivery, *3*) the presence of a twin, *4*) maturity of the infant, and *5*) use of antenatal steroids. These are important considerations given the substantially increasing rate of cesarean section globally (1, 39) and the need to reduce the neonatal morbidity associated with elective cesarean section delivery in near-term newborns.

Animal Models of Elevated Airway Liquid Volumes at Birth

Our animal (lambs and rabbits) models (17, 40) were designed to replicate the liquid volumes present in the airways of infants with normal amniotic fluid volumes before birth and whom are delivered by elective cesarean section without labor. As our aim was to understand how elevated liquid volumes affect pulmonary physiology, we did not replicate what occurs clinically. For instance, although most infants spontaneously breathe after birth, we sedated, intubated, and mechanically ventilated our lambs to avoid the large variability in airway liquid clearance associated with spontaneous breathing. A limitation of this study is that preterm and near-term lambs were ventilated for different durations (1 h in preterm lambs vs. 2 h in near-term lambs). This is largely because the EL preterm lambs had highly incompliant lungs that required high inflation pressures, with an associated high risk of lung injury.

Taken together with our previous findings and those of others, these data indicate that elevated airway liquid at birth leads to *1*) increased intrathoracic liquid and pulmonary edema for at least 2 h after birth, *2*) further expansion of the chest wall and flattening of the diaphragm, *3*) a reduction in lung compliance, *4*) a reduction in gas exchange potential, and *5*) the induction of tachypnea and dyspnea. Added to this, it causes a reduction in PBF, bradycardia, and a mild hypotension, the mechanisms for which are currently unknown.

Conclusions

This study increases our understanding of the effect of elevated airway liquid volumes at birth on cardiorespiratory physiology in preterm and near-term lambs. Importantly, it provides physiological evidence for factors underlying symptoms observed clinically in newborns who develop respiratory distress shortly after birth due to elevated airway liquid volumes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.Y., K.J.C., A.B.t.P., E.V.M., and S.B.H. conceived and designed research; S.Y., K.J.C., A.R.A.M., K.R., V.A.Z., A.M., E.V.M., and S.B.H. performed experiments; S.Y., E.V.M., and S.B.H. analyzed data; S.Y., A.R.A.M., E.V.M., and S.B.H. interpreted results of experiments; E.V.M. prepared figures; S.Y., K.J.C., E.V.M., and S.B.H. drafted manuscript; S.Y., K.J.C., A.R.A.M., K.R., V.A.Z., A.M., A.B.t.P., E.V.M., and S.B.H. edited and revised manuscript; S.Y., K.J.C., A.R.A.M., K.R., V.A.Z., A.M., A.B.t.P,, E.V.M., and S.B.H. approved final version of manuscript.

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