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RESEARCH ARTICLE

Increased end-expiratory pressures improve lung function in near-term newborn rabbits with elevated airway liquid volume at birth

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Abstract

Approximately 53% of near-term newborns admitted to intensive care experience respiratory distress. These newborns are commonly delivered by cesarean section and have elevated airway liquid volumes at birth, which can cause respiratory morbidity. We investigated the effect of providing respiratory support with a positive end-expiratory pressure (PEEP) of 8 cmH_2O on lung function in newborn rabbit kittens with elevated airway liquid volumes at birth. Near-term rabbits (30 days; term = 32 days) with airway liquid volumes that corresponded to vaginal delivery (∼7 mL/kg, control, n = 11) or cesarean section [∼37 mL/kg; elevated liquid (EL), n = 11] were mechanically ventilated (tidal volume = 8 mL/kg). The PEEP was changed after lung aeration from 0 to 8 to 0 cmH_2O (control, n = 6; EL, n = 6), and in a separate group of kittens, PEEP was changed after lung aeration from 8 to 0 to 8 cmH_2O (control, n = 5; EL, n = 5). Lung function (ventilator parameters, compliance, lung gas volumes, and distribution of gas within the lung) was evaluated using plethysmography and synchrotron-based phase-contrast X-ray imaging. EL kittens initially receiving 0 cmH_2O PEEP had reduced functional residual capacities and lung compliance, requiring higher inflation pressures to aerate the lung compared with control kittens. Commencing ventilation with 8 cmH_2O PEEP mitigated the adverse effects of EL, increasing lung compliance, functional residual capacity, and the uniformity and distribution of lung aeration, but did not normalize aeration of the distal airways. Respiratory support with PEEP supports lung function in near-term newborn rabbits with elevated airway liquid volumes at birth who are at a greater risk of suffering respiratory distress.

NEW & NOTEWORTHY Term babies born by cesarean section have elevated airway liquid volumes, which predisposes them to respiratory distress. Treatments targeting molecular mechanisms to clear lung liquid are ineffective for term newborn respiratory distress. We showed that respiratory support with an end-expiratory pressure supports lung function in near-term rabbits with elevated airway liquid volumes at birth. This study provides further physiological understanding of lung function in newborns with elevated airway liquid volumes at risk of respiratory distress.

newborn; lung aeration; lung liquid; positive end-expiratory pressure; term respiratory distress

INTRODUCTION

Respiratory distress (RD) in the newborn period most commonly occurs in very preterm infants and is largely due to the combined effect of lung immaturity and surfactant deficiency. However, RD in the newborn period is not unique to very preterm infants as a growing proportion of term and near-term newborns require admission into intensive care due to RD (1). Indeed, ∼53% of term/near-term infants admitted into intensive care shortly after birth are admitted for RD (2), but the underlying cause is likely to be very different from the lung immaturity and surfactant deficiency suffered by very preterm infants. These infants are mature, born at or near-term, and are usually otherwise healthy. Many (81%) of these admissions are characterized as either "nonspecific" RD or transient tachypnea of the

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newborn (TTN), with the latter being a transient and less severe form of RD in term newborns (2). However, the true clinical burden of RD in term and near-term infants is difficult to quantify as the definition is inconsistent and the underlying pathology is not well understood. Nevertheless, more severe forms of term RD are associated with significant morbidity and can be complicated by persistent pulmonary hypertension of the newborn (PPHN) (3, 4).

RD in infants born at or near-term was historically considered to result from delayed airway liquid clearance (5). However, overall treatments that induce airway liquid clearance by stimulating Na^+ reabsorption have had little success in mitigating RD severity and to date, there has been limited low-quality evidence from small studies on the effects of different management strategies for term RD (6). Imaging studies have now shown that after birth, airway liquid clearance predominantly results from pressure gradients generated by breathing, independent of Na^+ reabsorption (7, 8). These findings have led to a revised understanding of airway liquid clearance during and after birth.

The volume of airway liquid in a singleton fetus with a normal volume of amniotic fluid can be up to 35–45 mL/kg near term, before the onset of labor (9). During the transition from fetal to newborn life, airway liquid can either be *1*) lost from the lung via the nose and mouth during labor and vaginal delivery due to uterine contractions or *2*) cleared across the distal airway wall into the lung tissue. At birth, irrespective of whether the liquid is cleared into lung tissue via Na^+ reabsorption or the pressure gradients generated by inspiration, all liquid present in the airways must be reabsorbed into the lung tissue.

Term RD most often affects infants born by elective cesarean section without labor (10, 11). As these infants do not undergo labor and vaginal delivery, they are not exposed to the postural changes that drive liquid loss from the respiratory system via the nose and mouth. As a result, they are at risk of having larger volumes of liquid in their airways when they commence breathing after birth, all of which must be reabsorbed into the lung tissue. This is known to cause a form of pulmonary edema (12, 13), which in adults causes tachypnea and dyspnea (14, 15). Elevated airway liquid volumes at birth also reduce lung compliance and end-expiratory lung gas volumes (functional residual capacity; FRC), expand the chest wall, and flatten the diaphragm in newborn rabbits (16). These effects readily explain the symptoms of tachypnea, labored breathing, grunting, and expiratory braking in term infants with RD.

Our studies in near-term newborn rabbits suggest that the reduction in FRC caused by elevated airway liquid volumes in near-term newborn rabbits results from liquid re-entry into the airways between breaths (8, 16, 17). As the application of a positive end-expiratory pressure (PEEP) prevents airway liquid re-entry at FRC in preterm rabbits (18), we hypothesized that it would have similar benefits in near-term rabbits with elevated airway liquid volumes. In this study, we investigated whether applying an end-expiratory pressure (8 cmH_2O PEEP), compared with 0 cmH_2O PEEP, improves lung function, FRC, and the distribution of gas within the lung in the near-term newborn rabbits with elevated airway liquid volumes at birth.

MATERIALS AND METHODS

Ethical Approval

All animal procedures were approved by the SPring-8 Animal Care and Monash University Animal Ethics Committees. Experiments were conducted in accordance with the National Health and Medical Research Council (NHMRC) Australian code of practice for the care and use of animals for scientific purposes (19).

Experimental Procedure

Pregnant New Zealand White rabbits at 30 days gestational age (term ∼32 days; n = 8) were sedated using propofol (iv; 8 mg/kg bolus, followed by 40–100 mg/kg/h; Rapinovet, Merck Animal Health), intubated and then anesthetized using inhaled isoflurane (1.5%–4%; Isoflurane, DS Pharma Animal Health) as previously described (16). Fetal rabbits (kittens, n = 22) were exteriorized by cesarean section (the umbilical cord remained intact), anesthetized with sodium pentobarbitone (Somnopentyl; 0.1 mg ip, Abbot Laboratories) and an endotracheal tube (18G intracath; BD Australia) inserted via a tracheostomy. After placement of the endotracheal tube, as much airway liquid as possible was gently withdrawn using a 1 mL syringe attached to the endotracheal tube. The mean volume withdrawn was 0.15 ± 0.02 mL, but significant volumes of lung liquid were likely lost via the trachea due to loss of laryngeal reflexes in the kittens following induction of general anesthesia in the doe. Kittens were randomized into the four different experimental groups just before delivery, once it was established that the kitten was viable (not stillborn), according to a randomized sequence that was established before commencing the study. As multiple kittens were used from any one doe, the pre-established randomized sequence reduced the chance that multiple kittens from one doe were included in any one group to avoid the risk of litter bias. As previously described (16), the control group (n = 11) had no replacement liquid added to their airways (to mimic the volume of airway liquid at birth similar to natural clearance during labor and vaginal delivery), whereas the elevated liquid (EL; n = 11) group had 30 mL/kg of liquid (0.9% sodium chloride) added to their airways to mimic the volume expected at birth in newborns delivered by cesarean section near term without labor (mean volume = 1.12 ± 0.05 mL). As the volume of residual liquid remaining in the distal airways after drainage is ∼7 mL/kg (9), at ventilation onset, we estimate that kittens in the control group had ∼7 mL/kg and EL kittens had ∼37 mL/kg of airway liquid. This model allows us to investigate the effect of elevated airway liquid volumes on lung function between the groups. Following removal/addition of liquid, the endotracheal tube was blocked to prevent air from entering the lung during delivery and before ventilation and imaging commenced.

Ventilation Protocol

Following delivery, kittens were immediately placed, upright and head out, in a warmed (39°C) water-filled plethysmograph (custom-made) located within the experimental imaging hutch, as previously described (7, 16, 20). The endotracheal tube was connected to a purpose-built pressure-limited ventilator (21) that also triggered image acquisition to synchronize the imaging and pulmonary ventilation. In this study, kittens were mechanically ventilated, whereas near-term infants who subsequently develop RD usually breathe unassisted for the first few hours after birth. We chose to anesthetize and mechanically ventilate the kittens to control for differences in breathing rates and inspiratory effort between individuals as it is well established that inspiration drives lung liquid clearance (22). This ventilation approach allowed ventilation of the kittens with a continuous and uniform airway pressure wave to accurately assess the effect of end-expiratory pressures on lung aeration and function in kittens with EL. Investigating the effect of airway pressure is much more difficult and inconsistent when applying end-expiratory pressures noninvasively due to leak and the presence of the larynx, which can close the airways and prevent the transmission of applied pressures into the lung (23).

Following imaging onset, kittens within both the control and EL groups were randomly assigned to receive ventilation either commencing with a PEEP of 0 cmH_2O (0PEEP-I; control, n = 6; EL, n = 6) or 8 cmH_2O (8PEEP-I; control, n = 5; EL, n = 5). Mechanical ventilation commenced with intermittent positive pressure ventilation (iPPV), with a peak inflation pressure (PIP) of 25 cmH_2O, inspiratory and expiratory times of 0.5 s. The PIP was increased to achieve a tidal volume (Vt) of 8 mL/kg, which was measured directly from the plethysmograph as previously described (16, 24). Once Vt was reached and had stabilized, kittens in each group underwent a sequence of changing PEEP levels, either increasing from 0 cmH_2O to 8 cmH_2O (Fig. 1*A*) or decreasing from 8 cmH_2O to 0 cmH_2O (Fig. 1*B*), before returning back to the initial PEEP level in the final phase (0PEEP-F or 8 PEEP-F). Throughout the PEEP sequence ventilation protocol, the PIP was adjusted to maintain a constant Vt of 8 mL/kg. Each PEEP level was maintained until FRC values (measured from the plethysmograph) and all other ventilation parameters had stabilized. A PEEP of 8 cmH_2O was chosen as our previous studies using 3 cmH_2O and 5 cmH_2O PEEP were unable to overcome the detrimental effects of elevated airway liquid volume at birth on lung function (16, 25). During the ventilation period, airway pressures and lung gas volumes (measured from the plethysmograph) were digitally recorded (PowerLab, ADInstruments; Sydney, Australia) and phase-contrast X-ray images were acquired. All animals were euthanized at the conclusion of the experiment with an overdose of pentobarbitone (somnopentyl >100 mg/kg) administered intravenously (doe) or intraperitoneally (kittens).

Phase-Contrast X-Ray Imaging

All studies were conducted in experimental hutch 3 of beamline 20B2, in the Biomedical Imaging Centre at the SPring-8 Synchrotron in Japan. Partially coherent X-rays provided by the synchrotron radiation, tuned to an energy of 24 keV, enabled the entire thoracic cavity of the kittens to be imaged using propagation-based phase-contrast X-ray imaging, as previously described (16, 20, 25). The X-ray source-to-sample distance was ∼210 m and the sample-to-detector distance was 2 m. A Hamamatsu ORCA flash C11440-22C detector was coupled to a 25-μm thick gadolinium oxysulfide (Gd2O2S:Tb+) powdered phosphor and a tandem lens system that provided an effective pixel size of 15.2 μm and an active field of view of 31 (W) ×31 (H) mm^2. Flat-field and dark-field images were acquired after each kitten was imaged to correct for variations in the beam intensity and detector dark current signal.

Image Analysis

Lung gas volumes.

Regional lung gas volumes were measured using previously described methods (16, 26). Images of the chest obtained from kittens imaged upright in the plethysmograph were divided into quadrants; *upper left* (UL), *upper right* (UR), *lower left* (LL), and *lower right* (LR) lung regions using the 7th rib as the boundary between upper and lower lung regions. From these partitioned images, regional lung volumes at FRC and at peak inflation within each region were measured. The initial aeration period during the first PEEP level was used to determine the time taken to achieve a stable FRC and total FRC (normalized to kitten weight) for the whole lung.

Airway dimensions and numbers.

The speckle patterns produced by phase-contrast X-ray imaging of the lungs were used to measure regional airway

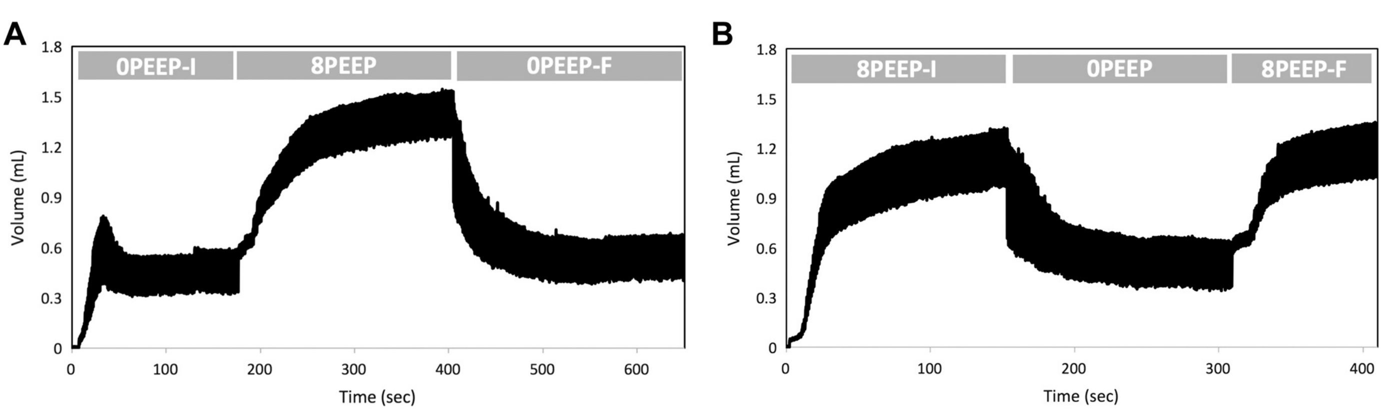

Figure 1. Typical lung gas volume recording of newborn kittens showing the changes in lung gas volumes during the positive end-expiratory pressure (PEEP) sequences. *A*: kittens began ventilation initially with 0 cmH_2O PEEP (0PEEP-I), before being increased to 8 cmH_2O (8PEEP) and then returning to the final 0 cmH_2O PEEP (0PEEP-F) phase. *B*: kittens underwent a PEEP sequence initiating ventilation with 8 cmH_2O (8PEEP-I), before being reduced to 0 cmH_2O (0PEEP) and then returning to the final 8 cmH_2O PEEP phase (8PEEP-F).

dimensions, as previously described (16, 27, 28). The airway dimension analysis provides a measure of the dominant visible airway size in two-dimensional (2-D) projection, as determined using power spectral analysis (27), and was measured for each region of the lung (UL, UR, LL, and LR) at FRC following each inflation during the experimental period. Using phase-contrast X-ray imaging, the airways remain invisible until they are aerated and as the larger airways aerate faster than the smaller airways, initially fewer smaller airways contribute to the dominant airway size measurement. However, as the lung aerates, more smaller airways become visible and contribute to dominant airway size measurement, which then decreases (27). For a visual description of airway size, a representative kitten from each group was selected and a power spectrum analysis was used to produce a heat map at FRC and peak inflation in each group by analyzing the 128 × 128 pixel region around each pixel contained in the lung (Fig. 4).

Physiological Analysis

LabChart 8 (ADInstruments) recordings of ventilation parameters were obtained during the initial recruitment phase (Vt, PIP, and number of breaths to reach Vt) and once Vt had stabilized at each PEEP level during the ventilation sequence (16). Dynamic lung compliance was derived from raw values obtained from the LabChart during each PEEP level (Vt/[PIP–PEEP]). Changes in Vt and airway pressure measure respiratory system compliance (including both lung and chest wall compliance), but as the chest wall is considerably more compliant than the lung we have referred to it as "lung compliance" for simplicity.

Statistical Analysis

Our study aimed to investigate the effect of EL compared with controls *1*) during the initial period of lung aeration when ventilated with two different levels of PEEP and *2*) changes in PEEP during the ventilation sequence. Volume measurements of the lung (including FRC, PIP, Vt, and compliance) and airway dimension analysis between control and EL groups were checked for normality and transformed if required. Data were analyzed using either the Student's unpaired *t* test (static measurements) or a two-way repeated-measures ANOVA for *1*) treatment (control vs. EL) and *2*) ventilation parameter (time from ventilation onset or %FRC or PEEP level depending on each outcome measure) with Sidak's multiple-comparisons post hoc test. When an interaction between the main effects of treatment and ventilation parameter was determined, the data were analyzed to determine the effect of treatment (control vs. EL) at each ventilation parameter. All data are presented as means ± SE. All statistical analyses were undertaken in Prism 7 and a significance level $P \leq 0.05$ was considered statistically significant.

RESULTS

Changes in Respiratory Function during Lung Aeration

FRC recruitment.

FRC recruitment over time and the maximum FRC achieved were markedly lower in EL kittens ventilated initially with 0 cmH_2O compared with controls (Figs. 2, 3*A*, and 3*C*). In contrast, the rate and degree to which FRC increased were not different between control and EL kittens receiving 8 cmH_2O of PEEP (Figs. 2, 3*B*, and 3*D*).

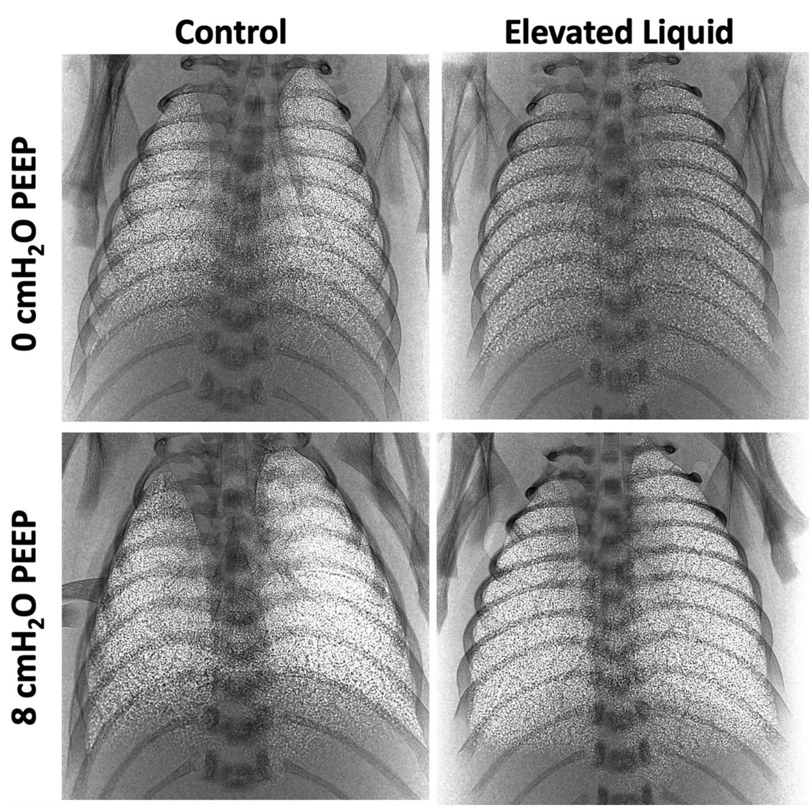

Figure 2. Phase-contrast X-ray images of lungs from control kittens (*left*) and kittens with elevated airway liquid volumes at birth (*right*) taken during the final stage of ventilation (see Fig. 1). Kittens received mechanical ventilation with a positive end-expiratory pressure (PEEP) of either 0 cmH_2O (*top*) or 8 cmH_2O (*bottom*). Visual differences in the speckle pattern highlight differences in the degree and uniformity of lung aeration between the groups at different PEEP levels.

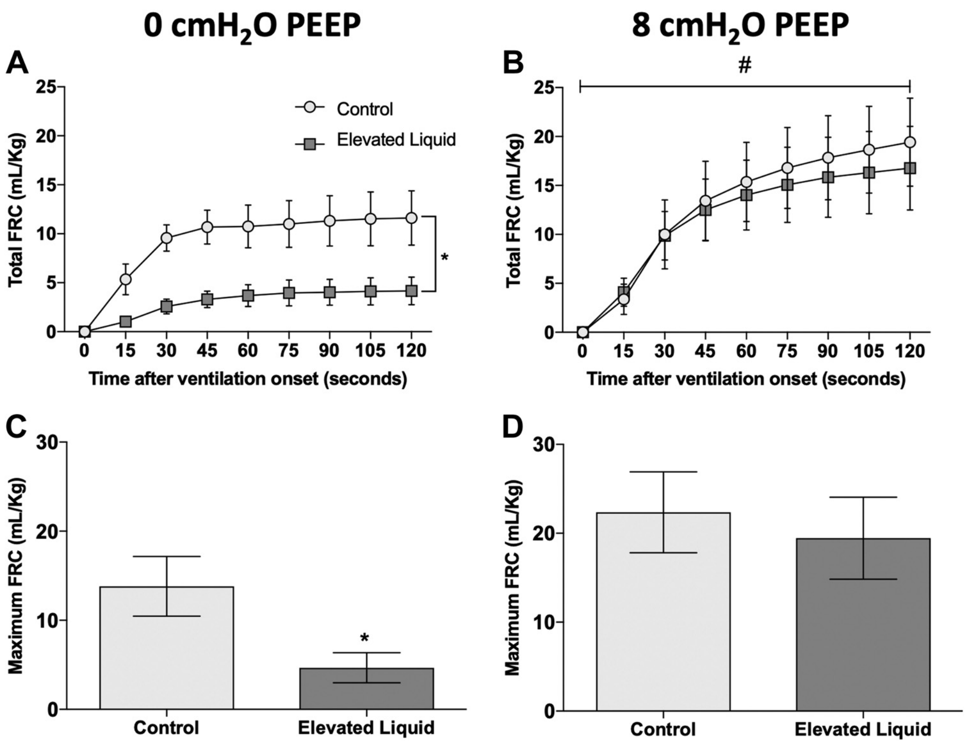

Figure 3. Total functional residual capacity (FRC; means ± SE) of the whole lung over time during initial lung aeration in control (open circles) and elevated liquid (gray squares) kittens receiving mechanical ventilation with a positive end-expiratory pressure (PEEP) of either 0 cmH_2O (*A*) or 8 cmH_2O (*B*). Difference in maximum FRC level achieved during the initial lung aeration phase between control (open bars) and elevated liquid kittens (gray bars) ventilated with 0 cmH_2O PEEP (*C*) and 8 cmH_2O PEEP (*D*). Two-way repeated-measures ANOVA with Sidak post hoc test (*A* and *B*); $*P \leq 0.05$ = effect of treatment (control vs. elevated liquid). $\#P \leq 0.05$ = effect over ventilation time in kittens. Student's *t* test (*C* and *D*); $*P \leq 0.05$ = effect of treatment (control vs. elevated liquid).

Tidal volume recruitment and lung compliance.
In kittens ventilated with 0 cmH_2O PEEP, the maximum PIP required to recruit a Vt of 8 mL/kg was greater in EL compared with control kittens (35.8 ± 1.2 cmH_2O vs. 30.8 ± 1.0 cmH_2O; $P = 0.008$) during initial lung aeration. Using the same respiratory rate, EL kittens also took significantly longer than controls to achieve a Vt of 8 mL/kg (132 ± 24 inflations vs. 71 ± 8 inflations; $P = 0.04$). In contrast, when kittens were ventilated with 8 cmH_2O PEEP, the maximum PIP required to recruit a Vt of 8 mL/kg was not different between EL and control kittens during initial lung aeration (36.3 ± 1.4 cmH_2O vs. 35.7 ± 0.6 cmH_2O; $P = 0.74$). The number of inflations required to reach Vt was also not different between EL and control kittens (88 ± 18 inflations vs. 71 ± 12 inflations; $P = 0.45$).

Following initial lung aeration, when FRC recruitment and Vt had stabilized, the PIP required to maintain a Vt of 8 mL/kg was significantly higher in EL kittens compared with controls when ventilated with 0 cmH_2O PEEP (31.8 ± 1.2 cmH_2O vs. 20.4 ± 1.2 cmH_2O; $P < 0.001$). As a result, lung compliance was 37.5% lower in EL compared with control kittens (0.010 ± 0.001 mL/kg/cmH_2O vs. 0.016 ± 0.001 mL/kg/cmH_2O; $P < 0.001$). However, when kittens were ventilated with 8 cmH_2O PEEP, the PIP required to maintain Vt of 8 mL/kg was not different between EL and control kittens (29.8 ± 0.6 cmH_2O vs. 29.8 ± 0.3 cmH_2O; $P = 0.96$). Similarly, after initial lung aeration, lung compliance was not different in EL compared with control kittens (0.015 ± 0.001 mL/kg/cmH_2O vs. 0.015 ± 0.001 mL/kg/cmH_2O; $P = 0.61$).

Airway dimensions during lung aeration.
Color maps of airway dimensions demonstrate the spatial distribution of the dominant airway size in 2-D projection at both FRC and peak inflation in a representative control and EL kitten ventilated with either 0 cmH_2O or 8 cmH_2O (Fig. 4). In kittens ventilated with 0 cmH_2O PEEP, the dominant airway size was significantly larger in all lung quadrants in EL kittens compared with controls at FRC (Fig. 5, *A*, *C*, *E*, and *G*). During initial aeration, a larger dominant airway size means that fewer smaller airways are aerated, but as more alveoli are recruited the dominant airway size trends downward over time as the smaller airways aerate and contribute more to the dominant airway size calculation. When kittens were ventilated with 8 cmH_2O PEEP, the dominant airway size in EL kittens was significantly larger in all lung quadrants (Fig. 5, *B*, *D*, and *F*), except for the lower right lobe (Fig. 5*H*), compared with control kittens.

Changes in Respiratory Function following Lung Aeration

Effect of changing PEEP on FRC and lung compliance.
Prior to changing PEEP, EL kittens that commenced ventilation with 0 cmH_2O had lower lung compliances and required higher PIPs to maintain Vt compared with controls (Fig. 6, *A* and *C*). However, when the PEEP was increased to 8 cmH_2O, both the lung compliance and PIP required to maintain Vt were similar in both EL kittens and control kittens (Fig. 6, *A* and *C*). In contrast, when kittens commenced ventilation with 8 cmH_2O PEEP, reducing the PEEP to 0 cmH_2O significantly affected lung compliance and the required PIP during the PEEP sequence (Fig. 6, *B* and *D*). Although there was no significant difference between control and EL kittens, this was likely due to insufficient power as the study was not powered to detect a difference in these specific measures. There were clear visual differences in the degree of lung aeration and FRC

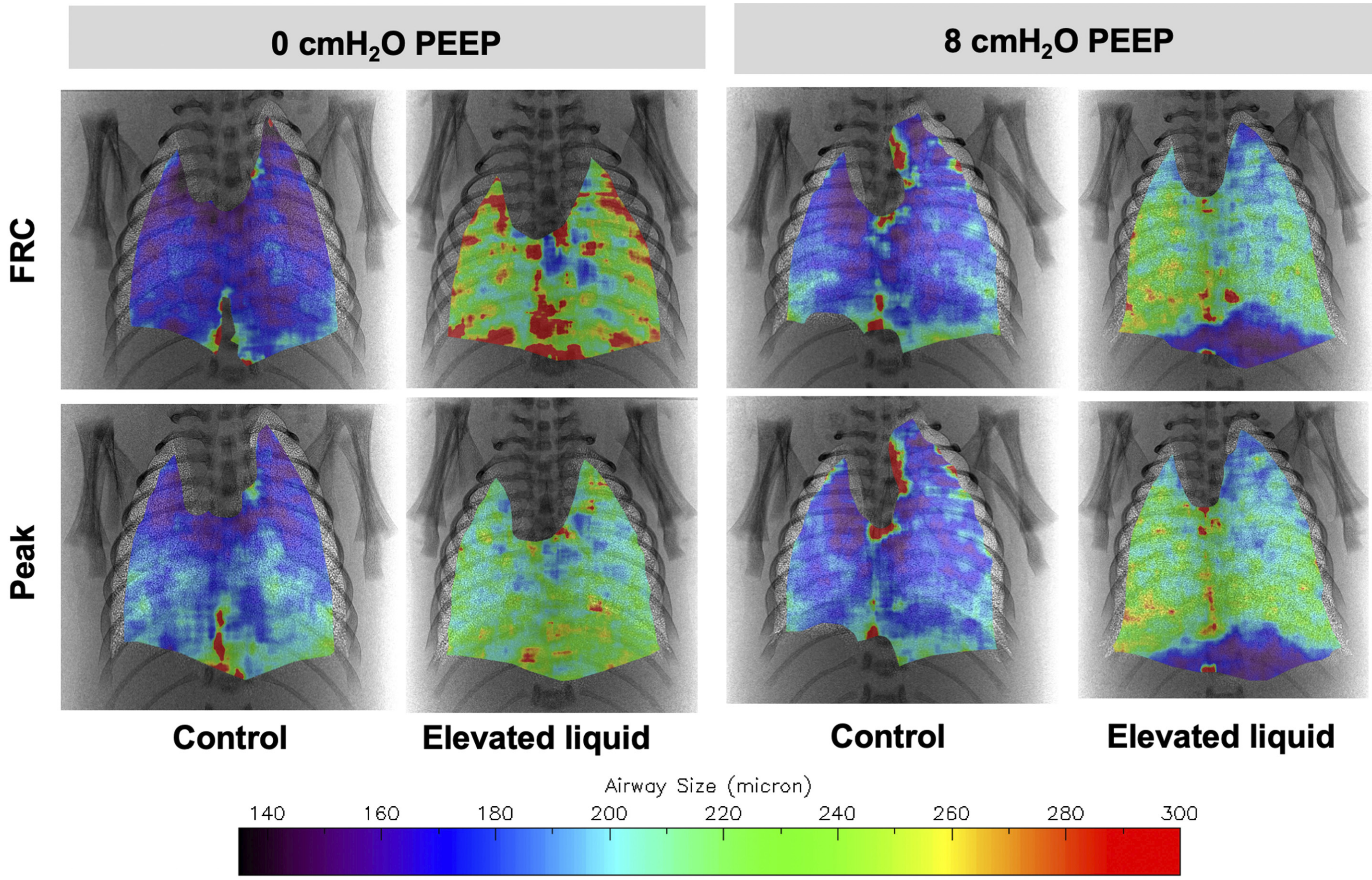

Figure 4. Color maps of a representative kitten from each group showing the regional distribution of the dominant airway dimension across the entire lung of control (left image within each panel) and elevated liquid kittens (right image within each panel) at functional residual capacity (FRC; *top*) and peak inflation (peak; *bottom*) when receiving respiratory support with 0 cmH_2O positive end-expiratory pressure (PEEP; *left*) or 8 cmH_2O PEEP (*right*).

between *1*) control and EL kittens and *2*) during the 0–8–0 cmH_2O PEEP sequence (Supplemental Movie S1; see https://doi.org/10.6084/m9.figshare.12838130).

EL kittens receiving initial ventilation with 0 cmH_2O PEEP had significantly lower FRC across the entire lung, compared with controls and this persisted despite increasing the PEEP to 8 cmH_2O (Fig. 7*A*). The FRC was significantly lower in the EL compared with control kittens in the upper but did not reach statistical significance in the lower lung quadrants during the PEEP sequence in kittens commencing ventilation with 0 cmH_2O (see Supplemental Fig. S1; https://doi.org/10.6084/m9.figshare.12991598). In contrast, in kittens initially ventilated with 8 cmH_2O PEEP, FRC levels for the entire lung (and for all quadrants) remained similar between control and EL kittens during the changes in PEEP (Fig. 7*B* and Supplemental Fig. S1). Overall, FRC levels were significantly lower during ventilation with 0 cmH_2O PEEP compared with 8 cmH_2O PEEP in both control and EL kittens, irrespective of which initial PEEP sequence the kittens received (Fig. 7, *A* and *B*).

Effect of changing PEEP on airway dimensions and distal airway numbers.

Following lung aeration, EL kittens maintained a larger dominant airway size compared with control kittens across all PEEP levels (Fig. 8), irrespective of whether they were initially ventilated with a PEEP of 0 cmH_2O (average dominant airway size in the entire lung; 221 ± 7 µm vs. 165 ± 2 µm; $P < 0.0001$) or 8 cmH_2O (average dominant airway size in the entire lung; 191 ± 3 µm vs. 175 ± 3 µm; $P < 0.001$). The dominant airway size was significantly larger at all PEEP levels and in all lung quadrants in EL kittens, compared with controls when ventilation commenced with 0 cmH_2O PEEP (Fig. 8, *A*, *C*, *E*, and *G*). Similarly, the dominant airway size measured at FRC was significantly larger at all PEEP levels in all but the lower right quadrant, in EL kittens compared with control kittens when ventilation commenced with 8 cmH_2O PEEP (Fig. 8, *B*, *D*, *F*, and *H*). Furthermore, the magnitude of difference in dominant airway size between EL and control kittens was larger when ventilation commenced with 0 cmH_2O compared with 8 cmH_2O PEEP (Fig. 8).

DISCUSSION

Although interventions aimed at preventing or treating term RD have been relatively ineffective (6), this may be due to a misunderstanding of the potential underlying mechanisms. Animal studies have provided compelling evidence that term RD may primarily result from elevated airway liquid volumes at birth, rather than an inability to clear the liquid (16, 25). In this study, we have shown that

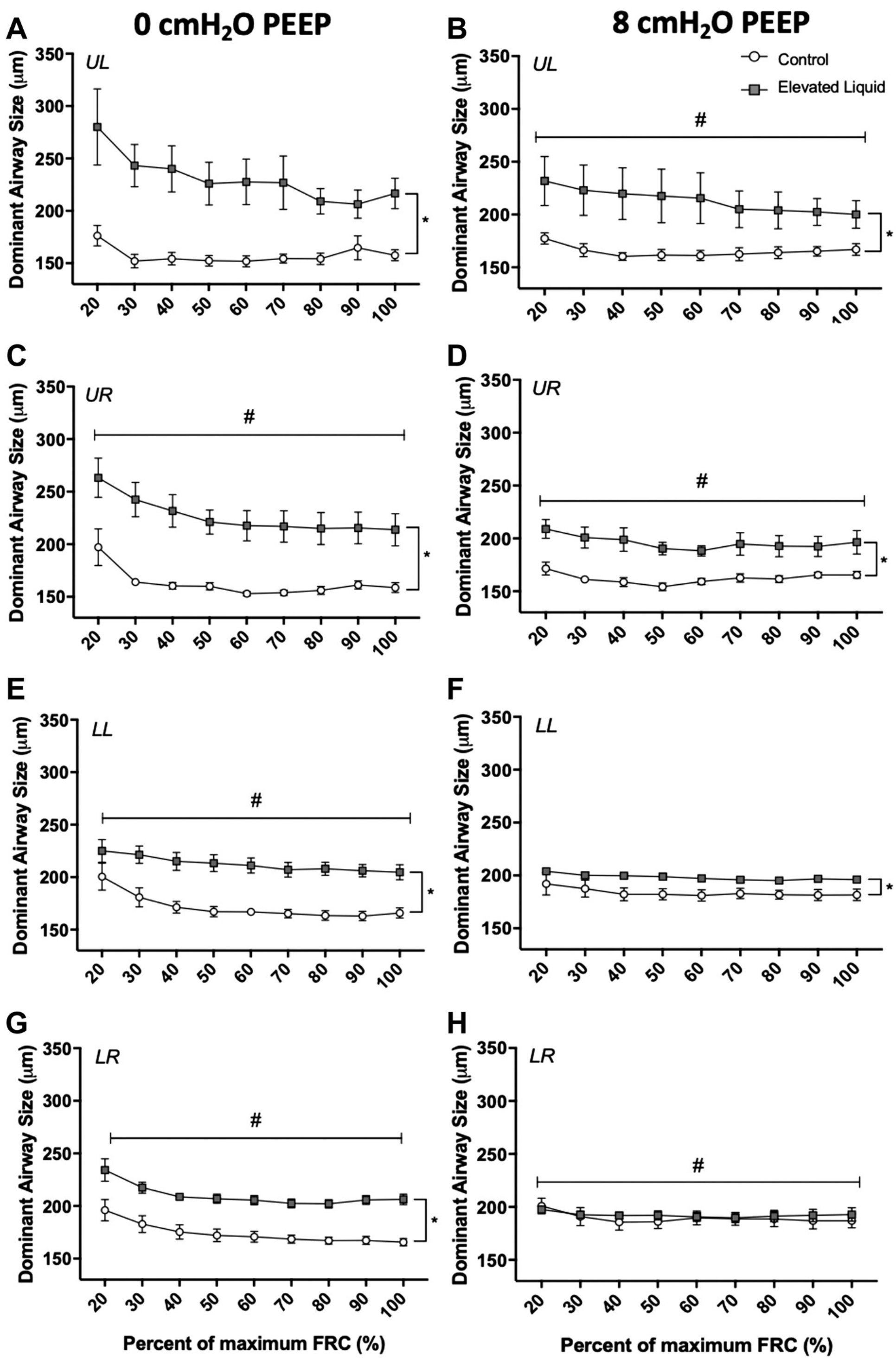

Figure 5. Dominant airway size (means ± SE) measured at functional residual capacity (FRC) during the initial lung aeration phase for each level of FRC in control (open circles) and elevated liquid kittens (gray squares) receiving mechanical ventilation with a positive end-expiratory pressure (PEEP) of either 0 cmH_2O (*A*, *C*, *E*, *G*) or 8 cmH_2O (*B*, *D*, *F*, *H*). Airway size is presented in lung quadrants; upper left (UL; *A*, *B*), upper right (UR; *C*, *D*), lower left (LL; *E*, *F*), and lower right (LR; *G*, *H*). A smaller dominant airway size indicates more air in distal airspaces (e.g., alveoli). Two-way repeated-measures ANOVA with Sidak post hoc test; $*P \leq 0.05$ = effect of treatment (control vs. elevated liquid). $\#P \leq 0.05$ = effect over time to achieve FRC in kittens.

the application of an end-expiratory pressure, both before and after lung aeration, improves lung mechanics and FRC in the presence of elevated airway liquid at birth. Following lung aeration, the application of PEEP was able to mitigate many of the deficits associated with elevated airway liquid at birth. However, surprisingly, it only had a limited effect on reducing the dominant airway size at FRC, which suggests that even a high level of PEEP (8 cmH_2O) is unable to prevent liquid re-entry into the distal airways.

Effect of PEEP on Lung Aeration in Kittens with Elevated Liquid

End-expiratory positive airway pressures are widely regarded as essential for supporting very preterm newborns at birth (18, 20, 29–31), but considerably less is known about the role of

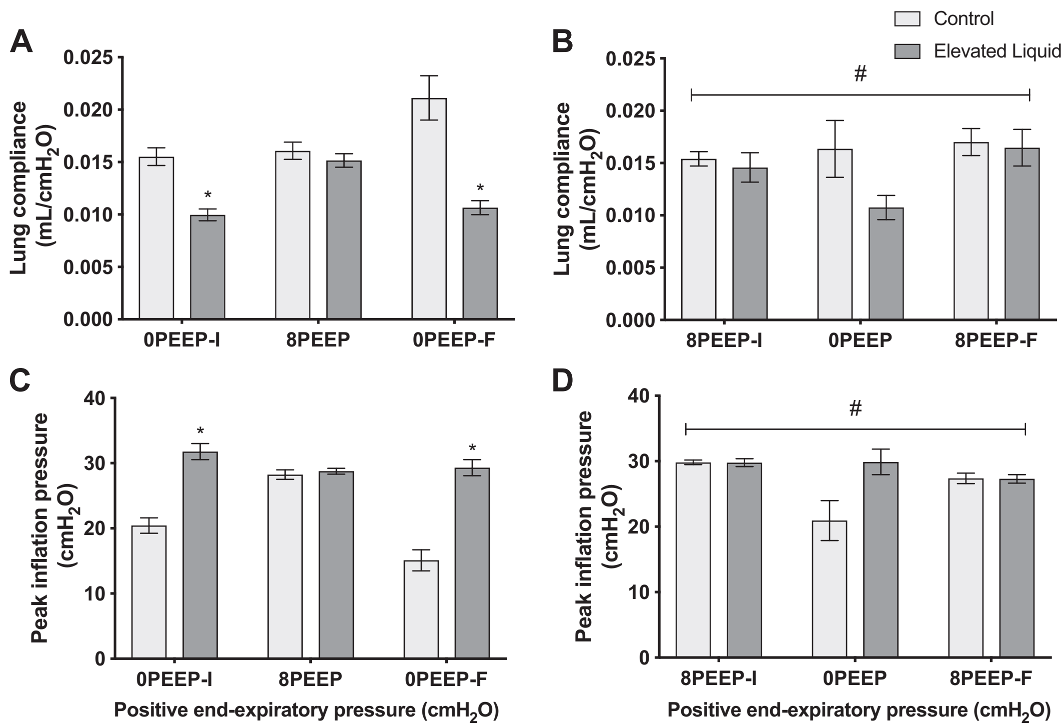

Figure 6. The effect of changes in positive end-expiratory pressure (PEEP) from 0–8–0 or 8–0–8 cmH_2O on lung compliance (*A*, *B*) and peak inflation pressure (*C*, *D*) required to maintain tidal volume (means ± SE) in control (open bars) and elevated liquid (gray bars) kittens. I = PEEP level during initial ventilation phase. F = PEEP level during final ventilation phase. Two-way repeated-measures ANOVA with Sidak post hoc test; *$P \leq 0.05$ = effect of treatment (control vs. elevated liquid). #$P \leq 0.05$ = effect of PEEP sequence in kittens.

PEEP in supporting near-term newborns with RD. We found that during lung aeration, EL kittens initially ventilated with 8 cmH_2O PEEP were able to aerate their lungs with fewer inflations, required lower inflation pressures, and achieved higher FRCs than EL kittens ventilated with 0 cmH_2O PEEP. However, the dominant airway size measured at both FRC and peak inflation remained significantly greater in EL kittens compared with control kittens during lung aeration, irrespective of the PEEP level (Fig. 4 and 8).

We have previously shown that a shift in the dominant airway size to a larger size is associated with a reduction in the number of aerated distal airways that can be visualized using phase-contrast X-ray imaging (16). As the presence of liquid in the airways renders them invisible, an increase in the dominant airway size indicates that more of the smaller airways are liquid-filled and so the analysis is skewed by limiting the measurement to larger air-filled alveoli and airways. This explains the counter-intuitive finding that the dominant airway size is larger at FRC than at peak inflation in EL kittens ventilated with 0 cmH_2O PEEP (Fig. 4). That is, at FRC the smaller alveoli refill with liquid, which is then recleared during the next inflation (8). As a result, at peak inflation, a larger number of smaller alveoli are aerated, which reduces the dominant airway size measurement. This is consistent with the finding that FRC levels were lower in EL kittens ventilated with 0 cmH_2O PEEP and that 8 cmH_2O of PEEP was sufficient to prevent alveolar reflooding in EL kittens, as indicated by similar FRC levels in control and EL kittens. The contribution of increasing aeration of the distal airways to the reduction in dominant airway size is demonstrated in the phase-contrast X-ray video (see Supplemental Movie S1 and Figs. 5 and 8) of *1*) control and EL kittens over time and *2*) between inflations at different PEEP levels.

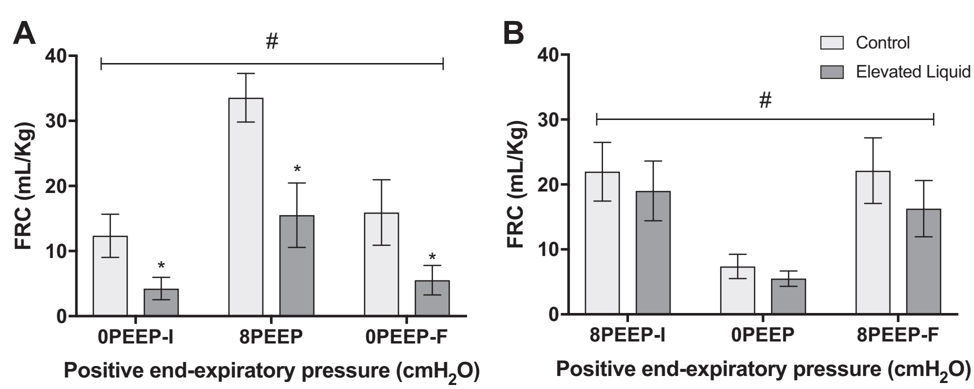

Figure 7. Effect of changes in positive end-expiratory pressure (PEEP) on functional residual capacity (FRC; means ± SE; normalized to kitten weight) in control (open bars) and elevated liquid (gray bars) receiving mechanical ventilation commencing with a PEEP of either 0 cmH_2O (*A*) or 8 cmH_2O (*B*). I = PEEP level during initial ventilation phase. F = PEEP level during final ventilation phase. Two-way repeated-measures ANOVA with Sidak post hoc test; *$P \leq 0.05$ = effect of treatment (Control vs. Elevated Liquid). #$P \leq 0.05$ = effect of PEEP sequence in kittens.

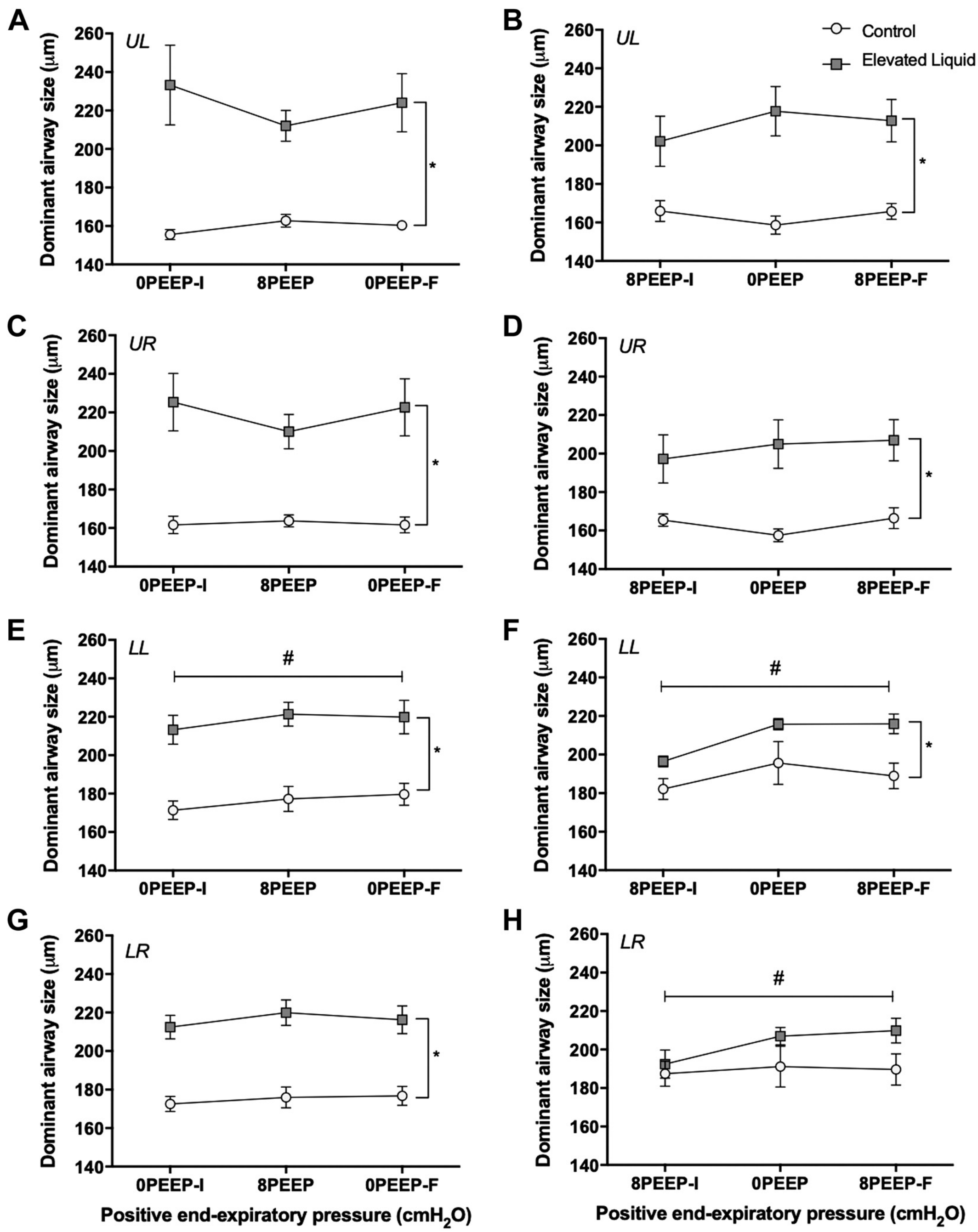

Figure 8. Dominant airway size (means ± SE) at functional residual capacity measured at each level of positive end-expiratory pressure (PEEP) in control (open circles) and elevated liquid (gray squares) kittens in upper left (UL; *A*, *B*), upper right (UR; *C*, *D*), lower left (LL; *E*, *F*), and lower right (LR; *G*, *H*) lung quadrants. A smaller dominant airway size indicates more air in distal airspaces (e.g., alveoli). I = PEEP level during initial ventilation phase. F = PEEP level during final ventilation phase. Two-way repeated-measures ANOVA with Sidak post hoc test; *$P \leq 0.05$ = effect of treatment (control vs. elevated liquid). #$P \leq 0.05$ = effect of PEEP sequence in kittens.

EL kittens ventilated with 0 cmH_2O PEEP had a heterogeneous distribution of airway size with a significant skew toward larger airway size in all lung quadrants at FRC compared with control kittens (Figs. 4 and 5). The heterogeneity in airway sizes (between control and EL kittens) at FRC tended to normalize at peak inflation (Fig. 4), which is consistent with the concept that the smaller airways are aerating during each inflation and then reflooding during expiration at 0 cmH_2O PEEP. These findings are more pronounced than our previous findings in EL kittens ventilated with 5 cmH_2O PEEP (16), whereas the dominant airway size distribution appeared more homogenous at both peak inflation and at FRC in EL kittens ventilated with 8 cmH_2O PEEP. Our finding is consistent with the concept that PEEP has a pressure-dependent effect on preventing liquid re-entry into the smaller airways between inflations. While these near-term newborns are not surfactant deficient, it is possible that the excess liquid "dilutes" the endogenous surfactant present in the airways, although we would expect this effect to disappear as the liquid is cleared from the airways. In either case, the application of PEEP can support FRC and oppose liquid re-entry in the presence of elevated airway liquid volumes and/or surfactant deficiency.

Effect of Changes in PEEP in Kittens with Elevated Liquid following Lung Aeration

EL kittens initially ventilated with 0 cmH_2O PEEP, continued to have lower FRC levels than controls following the increase to 8 cmH_2O PEEP (Fig. 7*A*), although lung compliance was similar in control and EL kittens at this PEEP level. In contrast, EL kittens initially ventilated with 8 cmH_2O PEEP had similar FRC levels as control kittens before, during, and after PEEP was reduced to 0 cmH_2O (Fig. 7*B*). However, reducing PEEP to 0 cmH_2O, reduced lung compliance in EL but not in control kittens, although compliance was not significantly different between the two groups (Fig. 6*B*). These responses are surprising and indicate that, in EL kittens, there is a beneficial effect of commencing ventilation with PEEP on FRC, but not lung compliance that persists after the PEEP level has changed. The mechanisms for this are unclear, are likely to be complex, and require further physiological investigation. Nevertheless, these findings may underlie the observation that prophylactic continuous positive airway pressure (CPAP) for 20 min after birth reduced the incidence of RD symptoms in a small study of high-risk infants (32). It is possible that commencing ventilation with 0 cmH_2O PEEP results in a continuous cycle of alveolar liquid clearance and reflooding during each inflation/deflation, which damages the alveolar epithelium. As a result, increasing PEEP to 8 cmH_2O was unable to prevent alveolar reflooding during expiration, particularly in both upper lobes. This is indicated by the dominant airway size measurement, which tended to decrease in the upper lobes (Fig. 8, *A* and *C*) but remained unaltered in the lower lobes when PEEP was increased to 8 cmH_2O (Fig. 8, *E* and *G*).

The effect of changing PEEP level on lung aeration is clearly evident in the phase-contrast X-ray video (see Supplemental Movie S1) and in Fig. 2. When EL kittens were ventilated with 0 cmH_2O PEEP, it took more inflations to aerate the lung, and the degree of aeration in the distal airways was markedly reduced between inflations compared with control kittens. Then, increasing PEEP to 8 cmH_2O greatly increased the retention of air in the lungs between inflations, whereas reducing the PEEP from 8 to 0 cmH_2O caused an immediate loss of FRC. As there is a very clear reduction in the number of visible airways at FRC in EL kittens, this indicates that at FRC, liquid is reflooding the airways between breaths in the absence of an end-expiratory pressure. In control kittens, changes in FRC are visible during changes in PEEP but to a lesser extent as these kittens have less liquid and less reflooding of airways at FRC. Previous studies have shown that the clearance of liquid out of the airways and into lung tissue after birth is associated with an increase (to ~6 cmH_2O) in lung interstitial tissue pressure (33). Logically, therefore, the clearance of larger volumes will further increase interstitial tissue pressures, leading to a greater pressure gradient for liquid to re-enter the airways at FRC. As such, the application of an end-expiratory pressure will oppose this pressure gradient and prevent liquid from reflooding the airways.

Non-invasive Respiratory Support in Near-Term Newborns with Respiratory Distress

Our studies in mechanically ventilated newborn rabbits have now shown that the adverse effects of elevated liquid on lung mechanics are evident at both 0 and 5 cmH_2O of PEEP (16), but can be mitigated using a higher pressure of 8 cmH_2O PEEP. Hence, infants at risk for having elevated airway liquid volumes at birth likely require higher PEEP levels to support lung function and reduce airway liquid reflooding during the respiratory cycle. It is also important to consider when and how end-expiratory pressures can be weaned in newborns to prevent the adverse effects of EL on lung function. Our findings highlight the effects of end-expiratory pressure on lung function in ventilated newborn rabbits. Further studies are warranted to determine the optimal level of support in spontaneously breathing newborns with elevated airway liquid volumes at birth to more closely simulate the clinical scenario.

Small clinical studies examining the benefits of non-invasive respiratory support, including CPAP levels of 4–6 cmH_2O (32, 34–37), have shown positive effects on the duration of RD in near-term infants (6). However, there is considerable heterogeneity in the studies concerning the definition of RD, timing of intervention, the pressure level, devices used to apply CPAP, and the combination with oxygen supplementation. Although it is reported to be well tolerated in term/near-term newborns with RD, greater understanding is needed to assess the effects of pressure on the infant's respiratory physiology as higher airway pressures are known to induce complications such as air leak and reduced pulmonary blood flow (38). Furthermore, questions regarding prophylactic support compared with an interventional response once symptoms arise as well as the timing of treatment application following symptom onset are also important considerations. Indeed, the degree to which airway liquid volumes are elevated at birth is likely to determine both the time of onset and severity of RD symptoms. Nevertheless, our finding that the benefits of applying PEEP during lung aeration (8–0–8 cmH_2O sequence) are not entirely replicated by applying PEEP after lung aeration (0–8–0 cmH_2O sequence), supports the prophylactic approach of briefly applying end-expiratory pressures in at-risk infants in the delivery room immediately after birth (32). However, much more information is required in both preclinical and clinical studies to fully understand the impact of elevated airway liquid volume at birth and the type and level of respiratory support required to assist newborns born with elevated lung liquid volumes during and after transition.

Conclusions

We have shown that the use of PEEP (8 cmH_2O compared with 0 cmH_2O) can improve FRC and lung mechanics in near-term newborn rabbits with elevated airway liquid volumes at birth. In particular, PEEP: *1*) facilitates lung aeration and maintains FRC between inflations, *2*) prevents liquid from reflooding the airways between breaths before being recleared into lung tissue during the next inflation, which increases gas exchange potential, reduces the work of breathing, and likely reduces lung injury, and *3*) provides a pressure gradient that assists with the clearance of liquid from the airways. The effectiveness of interventions and level of PEEP/CPAP required, should take into consideration *1*) timing after birth, *2*) volume of airway liquid at birth, and *3*) timing relative to the initiation of respiratory support. Our study provides an increased understanding of the fundamental physiology and has identified an end-expiratory pressure level that can support lung function in near-term newborn rabbits with elevated airway liquid volumes after birth.

SUPPLEMENTAL DATA

Supplemental Movie S1: https://doi.org/10.6084/m9.figshare.12838130.

Supplemental Fig. S1: https://doi.org/10.6084/m9.figshare.12991598.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

E.V.M., A.B.T.P., K.J.C., M.J.W., S.B.H., and M.J.K. conceived and designed research; E.V.M., A.B.T.P., M.K.C., K.J.C., M.J.W., K.L., M.T., P.L.J.D., J.D., A.W.F., S.J.E.C., S.B.H., and M.J.K. performed experiments; E.V.M., M.K.C., and K.L. analyzed data; E.V.M., S.B.H., and M.J.K. interpreted results of experiments; E.V.M. and M.K.C. prepared figures; E.V.M. and S.B.H. drafted manuscript; E.V.M., A.B.T.P., M.K.C., K.J.C., M.J.W., K.L., P.L.J.D., J.D., A.W.F., S.B.H., and M.J.K. edited and revised manuscript; E.V.M., A.B.T.P., M.K.C., K.J.C., M.J.W., K.L., M.T., P.L.J.D., J.D., A.W.F., S.J.E.C., S.B.H., and M.J.K. approved final version of manuscript.

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