Spontaneous breathing and respiratory support of preterm infants at birth

Pas, A.B. de

CHAPTER 9

Positive end expiratory pressure is required to develop a functional residual capacity in preterm rabbits ventilated from birth

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Submitted

Abstract

Objective. To determine the effect of PEEP on the spatial and temporal pattern of lung aeration in preterm rabbit pups mechanically ventilated from birth using simultaneous phase contrast X-ray imaging and plethysmography.

Methods. Preterm rabbit pups were delivered by caesarean section at 27d of gestational age, immediately placed within a water-filled plethysmograph (head out) and imaged as they were mechanically ventilated from birth with either a PEEP of 0 cmH_2O or 5 cmH_2O. The peak inflation pressure was held constant at 35 cmH_2O.

Results. Without PEEP, gas only entered into the distal airways during inflation. The distal airways collapsed during expiration and as a result, the functional residual capacity (FRC) did not increase above the lung's anatomical dead space volume (2.5±0.8 mL/kg). In contrast, ventilation with 5 cmH_2O PEEP gradually increased aeration of the distal airways with each inflation and they did not collapse at end expiration. The FRC achieved in pups ventilated with PEEP (19.9±3.2 mL/kg) was significantly greater than in pups ventilated without PEEP (-2.3±3.5 mL/kg).

Conclusion. PEEP is essential for facilitating aeration of the distal airways, accumulating FRC and preventing distal airway collapse at end-expiration in very preterm rabbit pups mechanically ventilated from birth.

Introduction

At birth, the airways must be cleared of liquid to allow air entry and the onset of pulmonary gas exchange (1-3), which precipitates major changes in cardio-pulmonary physiology essential for the transition to air-breathing (4, 5). Although airway liquid clearance is thought to result from an osmotic gradient induced by Na+ reabsorption (3, 6), hydrostatic pressures generated by inspiration may play a significant role (7, 8). As Na+ re-absorptive mechanisms are immature and potentially non-functional in very preterm infants (3, 9, 10), trans-pulmonary hydrostatic pressures may be critical for airway liquid clearance in preterm infants.

Infants born very preterm have immature lungs that are surfactant deficient, partially liquid-filled and prone to collapse at end-expiration, and commonly require assisted ventilation at birth (11). Little is known about procedures that facilitate uniform lung aeration at birth, particularly in very preterm infants, when the lung is partially liquid-filled and highly susceptible to injury (12-14). Although ventilating very preterm infants with a positive end expiratory pressure (PEEP) has many advantages (15-18), particularly in preventing airway collapse (19,20), its affects on lung aeration at birth are unknown. If airway liquid clearance is mainly regulated by hydrostatic pressures in the immature lung (7-8), the application of PEEP at birth may enhance airway liquid clearance and uniform lung aeration.

Phase contrast X-ray imaging (PCI) can measure the temporal and spatial pattern of lung aeration from birth in newborn rabbit pups (7, 21). PCI exploits the refractive index differences between air and water to produce contrast of air/liquid boundaries in the lung (21-27). As a result, the liquid-filled fetal lung only becomes visible following the entry of air at birth, providing high resolution images of all air-filled structures, including alveoli (7). This imaging technique is ideal for measuring the temporal and spatial pattern of lung aeration and has been used in spontaneously breathing rabbit pups (7, 8). In this study, we hypothesized that PEEP, applied from the first inflation after birth, enhances airway liquid clearance and uniform lung aeration in very preterm rabbit pups. To investigate this, we imaged lung aeration in ventilated very preterm rabbit pups from birth, in the presence and absence of PEEP, and simultaneously measured changes in lung gas volumes using plethysmography.

Methods

Experimental procedure

All experiments were approved by SPring-8 and Monash University's School of Biomedical Science's Animal Ethics Committees. Studies were conducted in experimental hutch 3 of beam line 20B2, in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan.

At 28 days gestational age (GA; term = 32 days), pregnant New Zealand white rabbits (n=8) were anaesthetised using propofol (Rapinovet; i.v.; 12mg/kg bolus), intubated and anaesthesia maintained by isoflourane inhalation (1.5-4%). Pups were exteriorised by caesarean section, sedated with sodium pentobarbitone (Nembutal; 0.1mg, i.p.), intubated (via a tracheotomy) with an 18G tube and delivered. Spontaneous breathing was prevented before imaging started by occluding the endotracheal tube. Immediately after delivery, the pup was placed vertically in a water-filled (40°C) plethysmograph with the head out and positioned so the chest was in the path of the X-ray beam. The endotracheal tube was connected to a small animal ventilator (SAR-830/P; CWE) and ventilation commenced with the onset of imaging (~1-2 min after delivery). Pups were ventilated with air using a peak inflation pressure (PIP) of 35 cmH_2O and 16 inflations per minute with a 1 sec inflation time. They were randomly allocated to either no PEEP (0PEEP) or a PEEP of 5 cmH_2O (5PEEP). It was not possible to alter the PEEP or PIP settings during imaging as the ventilator could not be controlled remotely. All pups were ventilated and imaged for 7-10 mins after birth and then killed with sodium pentobarbitone (Nembutal; 100mg/kg; i.p.).

Plethysmography

Lung air volume changes were measured from birth using a custom made water-filled plethysmograph (8, 28), comprised of an upright (height 12cm), cylindrical (diameter 32mm), water-filled chamber, sealed at the top by a rubber diaphragm and open to atmosphere via a vertical water column. The pup's head was exteriorised from the chamber via a hole in the diaphragm which formed a water-tight seal around the pup's neck. Changes in thoracic volume due to air inhalation caused water to move between the chamber and water column. The resulting pressure changes were measured using a pressure transducer (DTX™ Plus TNF-R; Becton Dickinson) and recorded digitally (Powerlab, ADInstruments; Sydney, Australia). The plethysmograph was calibrated by injecting 1mL of water before each experiment.

Phase contrast X-ray imaging

Phase contrast X-ray imaging was used to image air entry into the lungs from birth to investigate the rate and spatial pattern of lung aeration. Simultaneous plethysmography measured changes in gas volumes, although this could also be obtained from the images (28). Details describing the imaging procedures have been reported previously (7). Briefly, the X-ray energy was 24 keV, the pups were 2.0 m upstream of the detector (EMCCD, C9100-02); a 50msec exposure time and a 1 sec inflation time were used to minimise motion blur. Image acquisition and ventilation were synchronised with inflation onset triggering a sequence of 6 images (300 msec intervals). A pre-object shutter prevented radiation exposure between images and the timing of image acquisition was recorded digitally simultaneously with the plethysmograph recording.

Data and statistical analysis

All lung gas volumes were adjusted for body weight. FRC was determined as the lung gas volume at end-expiration and tidal volume (Vt) as the volume at end-inflation minus the post-inflation FRC (expired gas volume). FRC and Vt values were an average of 3 inflations and measured at inflation 5±1 (i.e. average of inflations 4-6), inflation 20±1 and at every 20±1 inflations for 120 inflations.

The anatomical dead space (ADS) volume was determined from the images as the volume of air in the lung at the first appearance of a fine speckle within the image. Speckling is caused by the focusing effects of multiple over-lying air/liquid boundaries in projection which act as a series of aberrant compound refractive lenses (7,21-23). Speckle appears in the image when air reaches the small terminal airways and exactly corresponds in timing with a marked inflection in the lung pressure-volume curve (Fig 1). As the timing of image acquisition occurred in unison with plethysmograph volume measurements, the lung gas volume when speckle first appeared in the image could be determined. The total lung gas

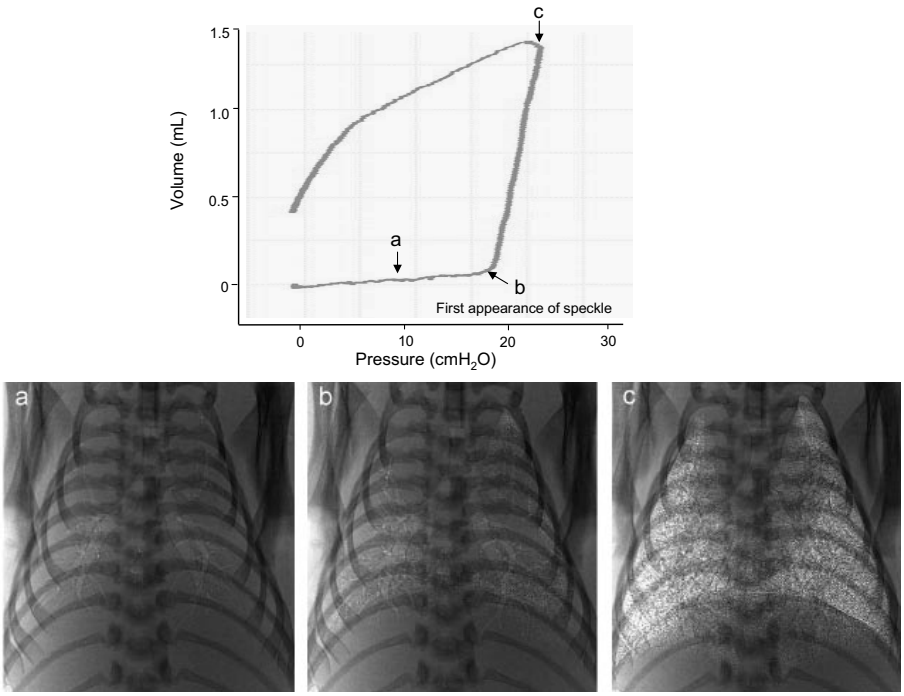

Figure 1. A pressure-volume (PV) curve of a newborn rabbit pup during its first inflation with air and phase contrast X-ray images acquired at different points on the curve as indicated by the arrows. Image (b) was acquired immediately after the point of inflection on the inflation limb of the PV curve and shows aeration just beginning in the distal airways. The lung air volume at this point was taken to be the anatomical dead space volume.

volume minus the ADS volume provided an estimate of gas volume within the terminal air sacs. The ADS volume was subtracted from the lung gas volume and the area under the curve (above the ADS volume) was calculated over 3 inflations (from inflation onset to inflation onset) at inflation 5, 20 and every 20th inflation for 120 inflations. The time the lung gas volume exceeded the ADS volume was calculated over 3 inflations and expressed as a percentage of the total duration of the 3 inflations.

Results are presented as means ± SEM. A p value of <0.05 was considered statistically significant. Student unpaired t-tests were used to compare differences between groups. Changes in FRC, Vt, alveolar gas volume and duration above ADS volume per inflation over time were analyzed using a 2-way repeated measures analysis of variance (ANOVA) with a Fishers Least Significant Difference (LSD) post-hoc test.

Results

Animal data: Sixteen preterm pups (from 8 rabbits) were ventilated from birth; 8 with no PEEP (0PEEP) and 8 with 5cmH_2O of PEEP (5PEEP). The GA (28.1 ± 0.1 vs 28.3 ± 0.2 days) and mean pup weight (35.3 ± 1.1 and 33.8 ± 2.6 g) were similar in both groups. Four pups were excluded (2 per group) from ADS volume analyses due to synchronization problems between image acquisition and plethysmograph recording. The calculated ADS volume was similar in 0PEEP (2.5 ± 0.8 mL/kg) and 5PEEP (3.0 ± 0.3 mL/kg) pups.

Phase contrast X-ray imaging of lung aeration: Movies of lung aeration were constructed by compiling the PC X-ray images sequentially (at ~1.5x normal speed) and demonstrate the beneficial effect of PEEP on lung aeration from birth. In the absence of PEEP (see Movie PEEP-1 on disc), although air eventually entered and aerated the distal gas exchange regions of the lung, the lungs did not retain gas at end-expiration for at least the first 120 inflations. Numerous inflations were required before air reached the terminal airways, which remained briefly only during inflation (Fig 2). During expiration, most airways either collapsed or re-filled with liquid in 0PEEP pups, so that much of the respiratory tree was un-aerated at end-expiration (Fig 2); some air could be seen trapped in small conducting airways in some pups. In contrast, the lungs of pups ventilated with 5 cmH_2O PEEP (see Movie PEEP-2 on disc) aerated rapidly, beginning in the large proximal airways and extending to the distal regions. Lung aeration increased with each inflation and air was retained in both the distal and larger conducting airways at end-expiration, with no evidence of lung collapse or liquid re-filling the airways (Movie 2; Fig 2). Lung aeration in 5PEEP pups was not uniform, beginning in the apical lobes, particularly the right apical lobe, and followed by aeration of the medial and then basal lobes.

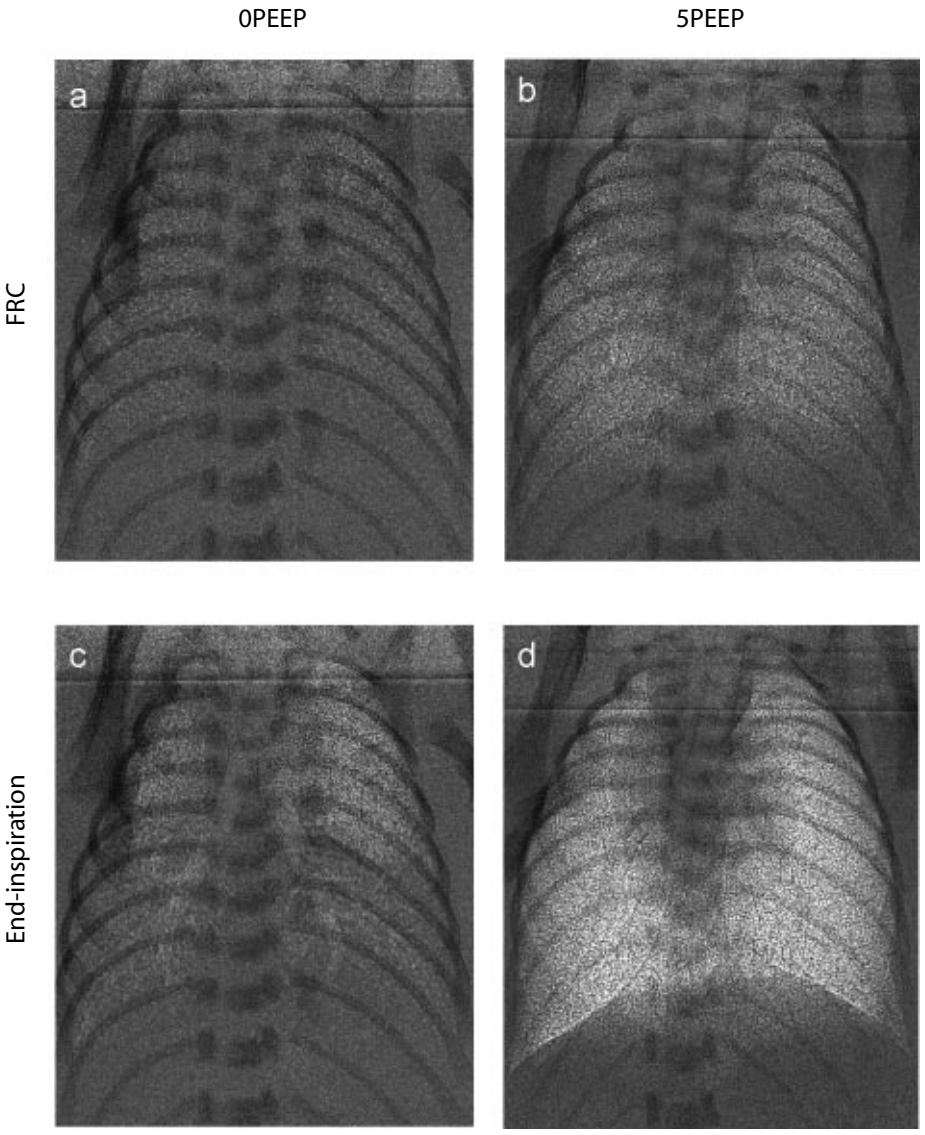

Figure 2. Phase contrast X-ray images of preterm rabbit pups ventilated with 0 cmH_2O (0PEEP; a & c) and 5 cmH_2O (5PEEP; b & d) of positive end-expiratory pressure (PEEP). Images were acquired at either functional residual capacity (FRC; a & b) or near end-inspiration (c & d) at approximately the 20th breath after the onset of ventilation.

Functional Residual Capacity (FRC)

All pups ventilated without PEEP (0PEEP) failed to accumulate a FRC that exceeded the ADS volume (Fig 3). FRC in 0PEEP pups increased transiently to 0.9±0.7mL/kg after the first 5 inflations, but was significantly reduced by inflations 20 and 40 ($p<0.05$) and did not significantly differ from zero throughout the remainder of the experiment. As a result, the percentage of time that lung gas volumes were greater than the ADS volume in 0PEEP pups was <20% for the first 40 inflations, remaining below 50% for the first 120 inflations (Figs 3 & 4b). Thus, without PEEP, lung gas volumes only increased above the ADS volume briefly during, and shortly, after inflation.

All 5PEEP pups rapidly accumulated a FRC and, at all time points, had a significantly higher FRC than pups ventilated with no PEEP ($p<0.001$) (Fig 4a). The FRC increased rapidly to 8.4 ± 2.4 mL/kg within the first 5 inflations, to 18.4 ± 1.8mL/kg by inflation 40 and then remained at this level (Fig 4a). Thus, in 5PEEP pups, the percentage of time that lung gas volumes were greater than the ADS volumes increased to 100% within the first 5 inflations and remained above the ADS volume throughout the respiratory cycle (Fig 4b).

Tidal volume (Vt) and lung compliance

The expired Vt in 0PEEP and 5PEEP pups significantly increased from the onset of ventilation until the 120th inflation ($p<0.05$; Figs 3 and 5a). The increase in expired Vt in 5PEEP pups

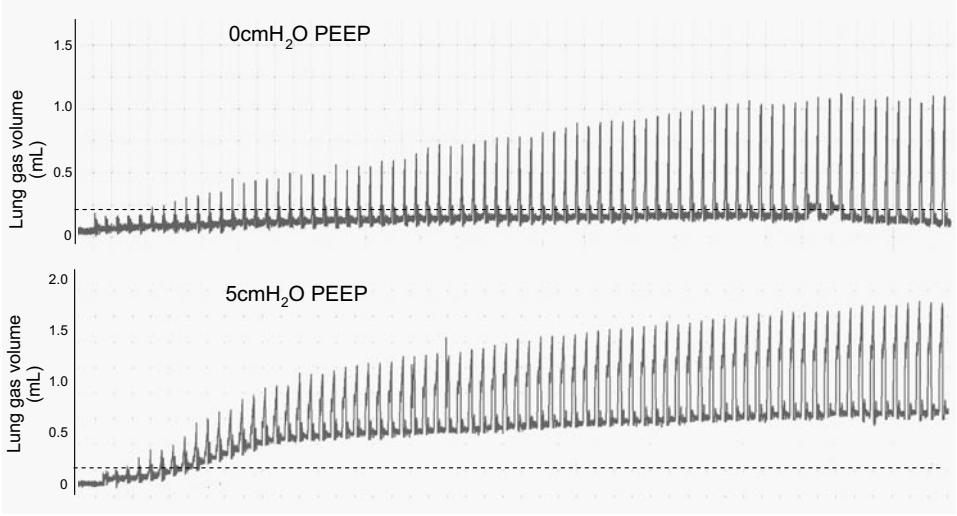

Figure 3. Lung gas volumes, measured using plethysmography, in preterm rabbit pups ventilated from birth with either 0 cmH_2O (top panel) or 5 cmH_2O (bottom panel) of positive end-expiratory pressure (PEEP). The dotted line represents the calculated anatomical dead space volume. Note that pups ventilated with 5 cmH_2O PEEP rapidly established a functional residual capacity (FRC) whereas pups ventilated in the absence of PEEP (0 cmH_2O PEEP) did not develop a FRC.

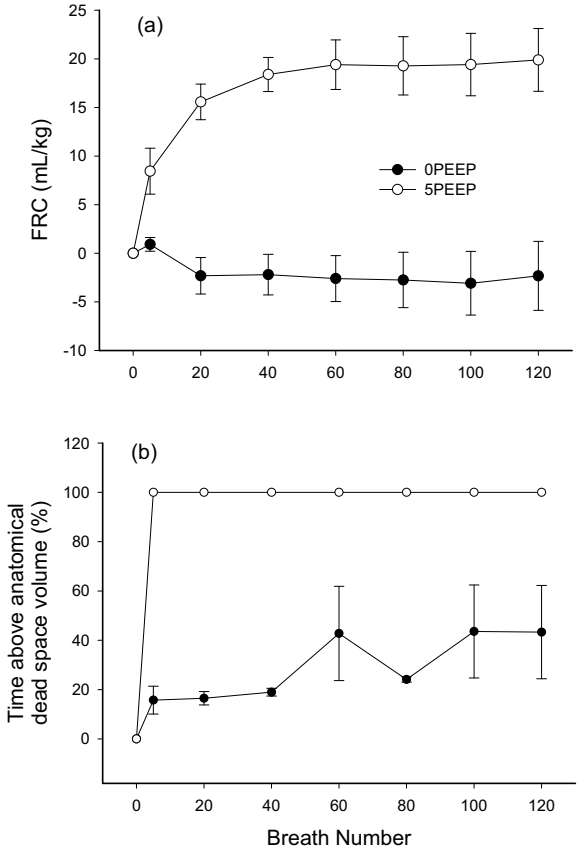

Figure 4. (a) The change in functional residual capacity (FRC) with time measured from birth in preterm rabbit pups ventilated from birth with either 0 cmH_2O (0PEEP, filled circles) or 5 cmH_2O (5PEEP, open circles) of positive end-expiratory pressure (PEEP). **(b)** The percentage of time that the lung gas volume remained above the anatomical dead space volume in 0PEEP (filled circles) and 5PEEP (open circles) pups. For each pup, values were averaged over a 3 breath cycle at breath 5 ± 1, 20 ± 1 and every 20th breath thereafter. Values are mean ± SEM.

was less than in 0PEEP pups with increasing inflation number. As a result, the mean Vt of 0PEEP pups was significantly greater than that of 5PEEP pups after inflation 60 ($p<0.05$) (Fig 5a). At this time, the difference in Vt between 0PEEP and 5PEEP pups was equal to the FRC in 5PEEP pups, as pups in both groups achieved the same lung gas volume at end-inflation (45.7±5.6 vs 48.2±3.2 mL/kg respectively). However, at end-expiration the lung gas volume was 19.9±3.2mL/kg in 5PEEP pups and 0.9 ± 0.7mL/kg in 0PEEP pups. Respiratory system compliance increased during lung aeration, but no difference could be detected between the groups (Fig 5b).

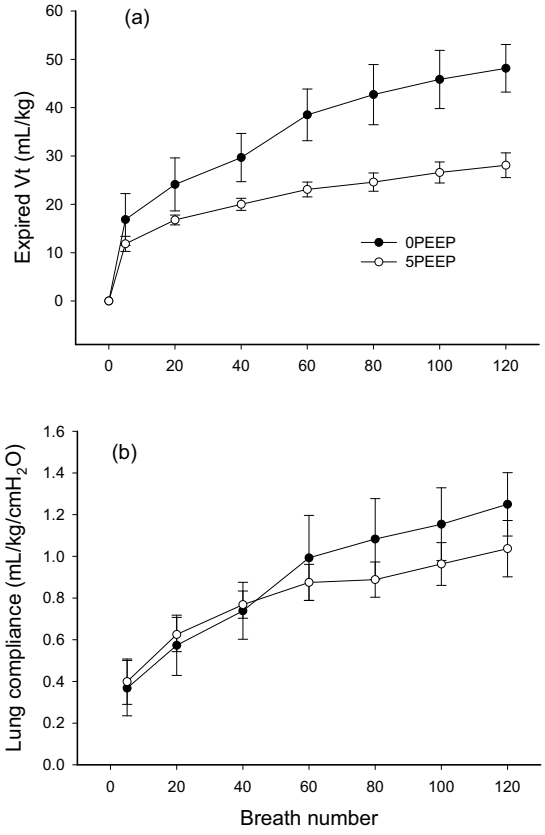

Figure 5. Increase in tidal volume (Vt; a) and lung compliance (b) in preterm rabbit pups ventilated from birth with either 0 cmH_2O (0PEEP, filled circles) or 5 cmH_2O (5PEEP, open circles) of positive end-expiratory pressure (PEEP). Values are mean ± SEM.

Time-related analysis of gas in the distal airways

The time–dependent restraint on gas exchange was assessed by measuring the percentage of time spent at a gas volume greater than the ADS volume (Fig 4b; see above) and the time-related integral of the lung gas volume over a 3 inflation cycle (Fig. 6). The lung gas volume time integral increased rapidly in 5PEEP pups, reaching 36.0 ± 2.7 mL.sec/kg at inflation 20, which was significantly greater than in 0PEEP pups (6.4 ± 2.0 mL.sec/kg). By inflation 40, the lung gas volume integral had increased further to 44.6 ± 2.6 mL.sec/kg in 5PEEP pups and then remained at this level. In contrast, in 0PEEP pups the lung gas volume integral increased from 9.5 ± 2.1mL.sec/kg at inflation 40 to 23.9 ± 4.0 mL.sec/kg at inflation 120, which was significantly lower than in 5PEEP pups (Fig 6).

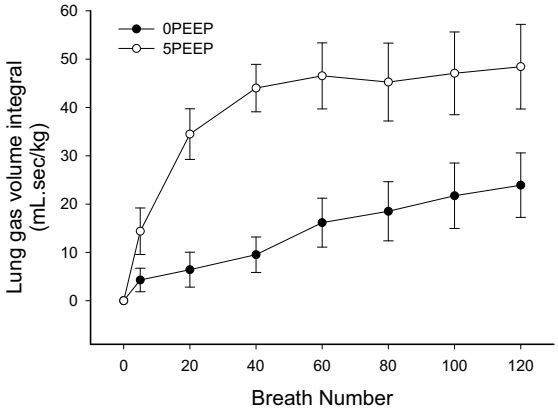

Figure 6. The lung gas volume time integral (area under the lung gas volume curve) in preterm rabbit pups ventilated from birth with either 0 cmH_2O (0PEEP, filled circles) or 5 cmH_2O (5PEEP, open circles) of positive end-expiratory pressure (PEEP). Values are mean ± SEM.

Discussion

Our study demonstrates that ventilation of preterm rabbit pups from birth with 5 cmH_2O of PEEP greatly improves aeration of the distal airways and prevents airway collapse at end-expiration (see Movie PEEP-2 on disc). As a result, pups ventilated with 5 cmH_2O of PEEP rapidly accumulated an FRC with each inflation (increasing to 19.9 ± 3.2mL/kg) and the distal gas exchange regions remained gas-filled at end-expiration. Without PEEP (see Movie PEEP-1 on disc), air only penetrated the distal gas exchange regions briefly during inflation and the airways either partially collapsed or re-filled with liquid at end-expiration. As these pups were intubated and sedated, these results are most applicable to intubated apneic newborn very preterm infants. However, our results may also provide an additional explanation for the beneficial effects of continuous positive airway pressure (CPAP) in a very preterm infant.

Airway liquid removal at birth is thought to result from adrenaline-induced activation of amiloride sensitive sodium channels which increases sodium reabsorption thereby creating an osmotic gradient for water uptake across the pulmonary epithelium (3, 6). As this process matures in late gestation (9, 29), partly due to the action of cortisol (30, 31), this mechanism is unlikely to be active in the immature lung of very preterm infants. Thus, the mechanisms driving airway liquid clearance and lung aeration in these infants are unclear. We have recently suggested that trans-epithelial hydrostatic pressures generated during inspiration provides a hydrostatic pressure gradient for liquid to leave the airways (7, 8). The finding that PEEP facilitates airway liquid clearance and lung aeration in mechanically ventilated

pups is consistent with this suggestion and provides further evidence that trans-epithelial hydrostatic pressures play an important role in this process, particularly in the immature lung.

The phase contrast X-ray movie sequences demonstrate the positive effect of PEEP on lung aeration in very preterm rabbit pups mechanically ventilated from birth. Although the spatial pattern of lung aeration was not uniform, the extent of lung aeration increased with each inflation and once aerated, regions did not collapse or re-fill with liquid during expiration. These observations were supported by plethysmography measurements, demonstrating that FRC rapidly increased to 19.9 ± 3.2mL/kg, values similar to that observed in spontaneously breathing term pups (8). As a result, after the first 5 inflations, the lung gas volume remained permanently above the ADS volume, allowing gas exchange to occur throughout the respiratory cycle. Without PEEP, although gas eventually penetrated into the lung periphery, the phase contrast X-ray images show that air was not retained in the distal airways and some of their conducting airways, at end-expiration (Movie PEEP-1, Fig 2). These observations are supported by plethysmography measurements, demonstrating that without PEEP, a FRC greater than the ADS volume (3.0±0.3mL/kg) could not be formed. As a result, the mean gas volume was greater than the ADS volume for less than 50% of the time in 0PEEP pups.

In the healthy mature lung, gas exchange is not limited by gas diffusion rates and are determined by the thickness and surface area of the air/blood gas barrier (32) as well as partial pressure gradients for O_2 and CO_2 (33). However, in a partially liquid-filled immature lung of a preterm infant, gas diffusion rates are likely to be limited, particularly for O_2 which is much less diffusible than CO_2 (32). This is because the lung gas barrier is structurally immature and distal airway collapse at end-expiration limits the volume of gas and the time available for alveolar gas exchange. A quantitative analysis of this relationship was assessed by measuring the lung gas volume time integral above the ADS volume. This integral rapidly increased in 5PEEP pups and was much greater than in 0PEEP pups (Fig 6), because the lung gas volume remained significantly above the ADS volume throughout expiration. Thus, the gas exchange potential of the lung, particularly for oxygen, was greater in 5PEEP pups compared to 0PEEP pups for the first 120 inflations after birth. It is possible that the affect of PEEP on increasing blood oxygenation during the newborn period (15, 16, 18) is due to an increase in the gas volume time integral.

After the first 120 inflations, the Vt was greater in 0PEEP pups compared with 5PEEP pups, despite all pups being ventilated with the same PIP. As the volume at peak inflation was similar in both groups, the difference in Vt was due to the higher FRC in 5PEEP pups as lung gas volumes decreased to near zero at end-expiration in 0PEEP pups. Apart from atelectrauma, this could cause greater shear stress and lung injury in 0PEEP pups compared

to 5PEEP pups. Other studies have shown that PEEP increases Vt (19, 34) by shifting lung volume into a more compliant region of the pressure-volume curve. Although this effect of PEEP is not immediate in the partially liquid-filled lung (18), it is possible that the maximum volumes achieved at end-inflation in our study were close to the top of the pup's pressure-volume curve. Thus, as 5PEEP pups had a higher FRC, the Vt may have been limited in these pups. It was necessary to ventilate pups with a PIP of 35 cmH_2O as preliminary experiments demonstrated that lower PIPs were unable to initiate lung aeration and it was not possible to alter the ventilator settings during imaging.

Phase contrast X-ray imaging provides a unique macroscopic view of the lung's transition into a gas-filled organ, demonstrating the importance of PEEP (see Movie PEEP-1 & Movie PEEP-2 on disc). The spatial pattern of lung aeration in preterm pups was different to that of spontaneously breathing term pups (8). In ventilated preterm pups, the apical lobes aerated first whereas the medial/basal lobes aerated first in spontaneously breathing term pups (7, 28). As diaphragmatic contraction drives lung expansion and reductions in airway pressure during spontaneous breathing, it is not surprising that lung aeration begins in the medial/basal regions, particularly as parts of the chest wall deform during spontaneous breathing (7). However, during mechanical ventilation, the application of positive pressure to the airways should provide relatively similar pressure gradients to apical and basal lobes, leading to uniform rates of aeration. As preterm pups were ventilated in an upright position, it is possible that gravity influenced the spatial pattern of lung aeration (7), however, spontaneously breathing pups were also studied in an upright position (8). It is pertinent to note that although very preterm human infants are usually ventilated in a horizontal position, and not upright as in this experiment, the effect of PEEP on FRC is independent of body position (unpublished observations).

This study has shown that mechanical ventilation from birth with PEEP greatly improves lung aeration and gas exchange potential of the lung immediately after birth. The phase contrast X-ray images, clearly demonstrate that PEEP prevents distal airway collapse at end-expiration allowing FRC to accumulate and lung aeration to increase with each inflation. These data also provide further evidence to indicate that trans-epithelial hydrostatic pressure gradients contribute to airway liquid clearance and lung aeration after birth.

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