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IMAGING LUNG AERATION AND LUNG LIQUID CLEARANCE AT BIRTH USING PHASE CONTRAST X-RAY IMAGING

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SUMMARY

1. The transition to extra-uterine life at birth is critically dependent on airway liquid clearance to allow the entry of air and the onset of gaseous ventilation. We have used phase contrast X-ray imaging to identify factors that regulate lung aeration at birth in spontaneously breathing term and mechanically ventilated preterm rabbit pups.

2. Phase contrast X-ray imaging exploits the difference in refractive index between air and water to enhance image contrast, enabling the smallest air-filled structures of the lung (alveoli; < 100 μm) to be resolved. Using this technique, the lungs become visible as they aerate, allowing the air–liquid interface to be observed as it moves distally during lung aeration.

3. Spontaneously breathing term rabbit pups rapidly aerate their lungs, with most fully recruiting their functional residual capacity (FRC) within the first few breaths. The increase in FRC occurs mainly during individual breaths, demonstrating that airway liquid clearance and lung aeration is closely associated with inspiration. We suggest that transpulmonary pressures generated by inspiration provide a hydrostatic pressure gradient for the movement of water out of the airways and into the surrounding lung tissue after birth.

4. In mechanically ventilated preterm pups, lung aeration is closely associated with lung inflation and a positive end-expiratory pressure is required to generate and maintain FRC after birth.

5. In summary, phase contrast X-ray imaging can image the air-filled lung with high temporal and spatial resolution and is ideal for identifying factors that regulate lung aeration at birth in both spontaneously breathing term and mechanically ventilated preterm neonates.

Key words: birth, fetus, lung aeration, mechanical ventilation, preterm birth, spontaneous breathing at birth.

INTRODUCTION

The transition to air breathing at birth is a major physiological challenge that all humans must face to survive extra-uterine life. Thus, it is not surprising that respiratory failure after birth is the greatest cause of morbidity and mortality in newborn infants. Before birth, the fetal lungs take no part in gas exchange, which occurs across the placenta, and the future airways are liquid filled.[1,2]

"at birth the airways must be cleared of liquid"

Pulmonary blood flow (PBF) is low because pulmonary vascular resistance (PVR) is high and the majority (approximately 90%) of blood exiting the right ventricle bypasses the lungs and enters the systemic circulation directly (descending aorta) via a large vascular shunt (ductus arteriosus).[3,4] Thus, at birth the airways must be cleared of liquid to allow the entry of air and the onset of gaseous ventilation, PVR must decrease so that PBF can increase to accept the entire output of the right ventricle and the ductus arteriosus must close to separate the systemic and pulmonary circulations. Although these major physiological events are essential for survival at birth and are intrinsically linked to the clearance of lung liquid and the entry of air into the lungs, the nature of the association and the underlying mechanisms are unclear.[4]

It is widely acknowledged that airway liquid clearance and lung aeration are determined primarily by transepithelial osmotic gradients generated by Na^+ reabsorption.[5,6] However, this is unlikely to be the only mechanism,[7] particularly in very preterm infants, because this

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mechanism matures late in gestation[8–10] and is unlikely to be active in these infants.[11] Recent studies using phase contrast X-ray imaging of the lungs at birth have provided compelling evidence to indicate that pressure gradients generated by inspiration are also involved.[12] Using phase contrast X-ray imaging, we have investigated the factors responsible for, and the respiratory patterns that promote, lung aeration in both spontaneously breathing term and mechanically ventilated preterm, neonatal rabbits.

PHASE CONTRAST X-RAY IMAGING

Phase contrast X-ray imaging greatly enhances image contrast by using the phase change of X-rays as they propagate through objects with inhomogeneous refractive indices.[13–15] When X-rays pass through an object comprised of media with differing refractive indices, the X-ray wave fronts are refracted at each boundary between different media. If the incident X-ray beam is spatially coherent, the refracted wave fronts produce interference patterns that, with sufficient propagation distance beyond the object, provide strong contrast of boundaries between structures with differing refractive indices. The resulting contrast is much greater than that produced by X-ray absorption alone. The lung is ideally suited to phase contrast X-ray imaging[13–15] because it is comprised mainly of air (approximately 80% by volume at end expiration), divided by thin tissue structures (mainly water). The air–tissue interfaces yield significant phase shifts and, as a result, the air-filled structures that weakly absorb X-rays become highly visible.[12–16] Although phase contrast X-ray imaging can use a variety of X-ray sources, synchrotron radiation is ideal for this type of imaging because of its coherence and brightness.[15]

"The lung is ideally suited to phase contrast X-ray imaging"

Because the fetal lungs are liquid filled, they are not visible using phase contrast X-ray imaging, but gradually become visible as the lungs fill with air after birth (Fig. 1), making this technique particularly useful for studying the factors regulating the entry of air into the lungs at birth. This technique can also image the air-filled structures of the lung with high spatial resolution (< 100 μm) and is capable of resolving even the smallest of airways (alveoli; Fig. 1). Image processing algorithms have also been developed that can derive quantitative information of lung gas volumes from single projection phase contrast X-ray image sequences.[17] This approach uses the Archimedean principle of volume measurement by fluid displacement, whereby the volume of air entering the lungs is determined by measuring the volume of water displaced from the imaging field-of-view. This is achieved by measuring the projected thickness of water in propagation-based phase contrast X-ray images acquired using monochromatic X-rays. The projected thickness of water is reconstructed per pixel by applying a single-image phase retrieval algorithm, which requires knowledge of the energy dependent refractive index decrement and attenuation coefficient of X-rays in water. The projected thickness is then summed across the image and multiplied by the known pixel size to yield the total volume of water. Because the thorax is comprised of multiple materials, the calculation is limited to measuring relative changes in water/air volume between successive image frames and cannot directly infer absolute lung air volume from a single image. These algorithms have been described in detail previously[16,17] and were validated by comparison with lung gas volumes measured by plethysmography.[18]

LUNG LIQUID CLEARANCE AND LUNG AERATION IN SPONTANEOUSLY BREATHING TERM NEONATES

The factors regulating lung liquid clearance at birth are a subject of considerable interest,[5,6] particularly because airway liquid retention is a significant cause of respiratory failure in newborn infants. The suggested mechanisms include mechanical forces imposed on the fetus during labour,[7,19] as well as transepithelial osmotic gradients generated by Na^+ reabsorption from the airways.[5,6] Although it is unlikely that 'vaginal squeeze' associated with delivery is a major contributing factor,[20] the postural changes imposed on the fetus during uterine contractions likely account for the loss of some lung liquid via the nose and mouth.[1,7] Increased spinal flexion of the fetus associated with uterine contractions increases fetal abdominal pressure, elevates the diaphragm and increases thoracic pressure, leading to lung liquid loss, particularly following the loss of amniotic fluid volume.[21] Because the fetal respiratory system is very compliant in late gestation,[22] large volumes of lung liquid can be lost shortly after the first signs of labour, before the onset of the second stage,[23] possibly due to small pressure gradients created by fetal postural changes.

Activation of epithelial Na^+ channels (ENaCs), particularly amiloride-sensitive ENaCs, are thought to play a major role in airway liquid clearance at birth, changing the epithelium from liquid secreting to liquid reabsorbing.[5,6] Specifically, fetal adrenaline (and vasopressin), released during the second stage of labour, are thought to activate apical-surface amiloride-sensitive Na^+ channels on distal airway epithelial cells.[5,8,24] This switches the epithelium from facilitated Cl^- secretion to active Na^+ reabsorption, which reverses the transepithelial osmotic gradient, causing liquid reabsorption.[5,8] Although considerable evidence supports a role for Na^+ uptake in alveolar fluid clearance at birth, particularly under stimulated conditions, it is likely that additional mechanisms, independent of Na^+ uptake, are also involved.[7,12] Indeed, although blockade of ENaCs with amiloride and inhibition of β-adrenoceptors can reduce or delay, it does not prevent lung liquid clearance at birth.[9,25] Similarly, although deletion of the gene encoding αENaC (but not βENaC or γENaC) impairs the normal reduction in lung water content at birth, $\alpha ENaC^{-/-}$ neonatal mice survive for up to 40 h after birth and so must establish some pulmonary gas exchange.[26]

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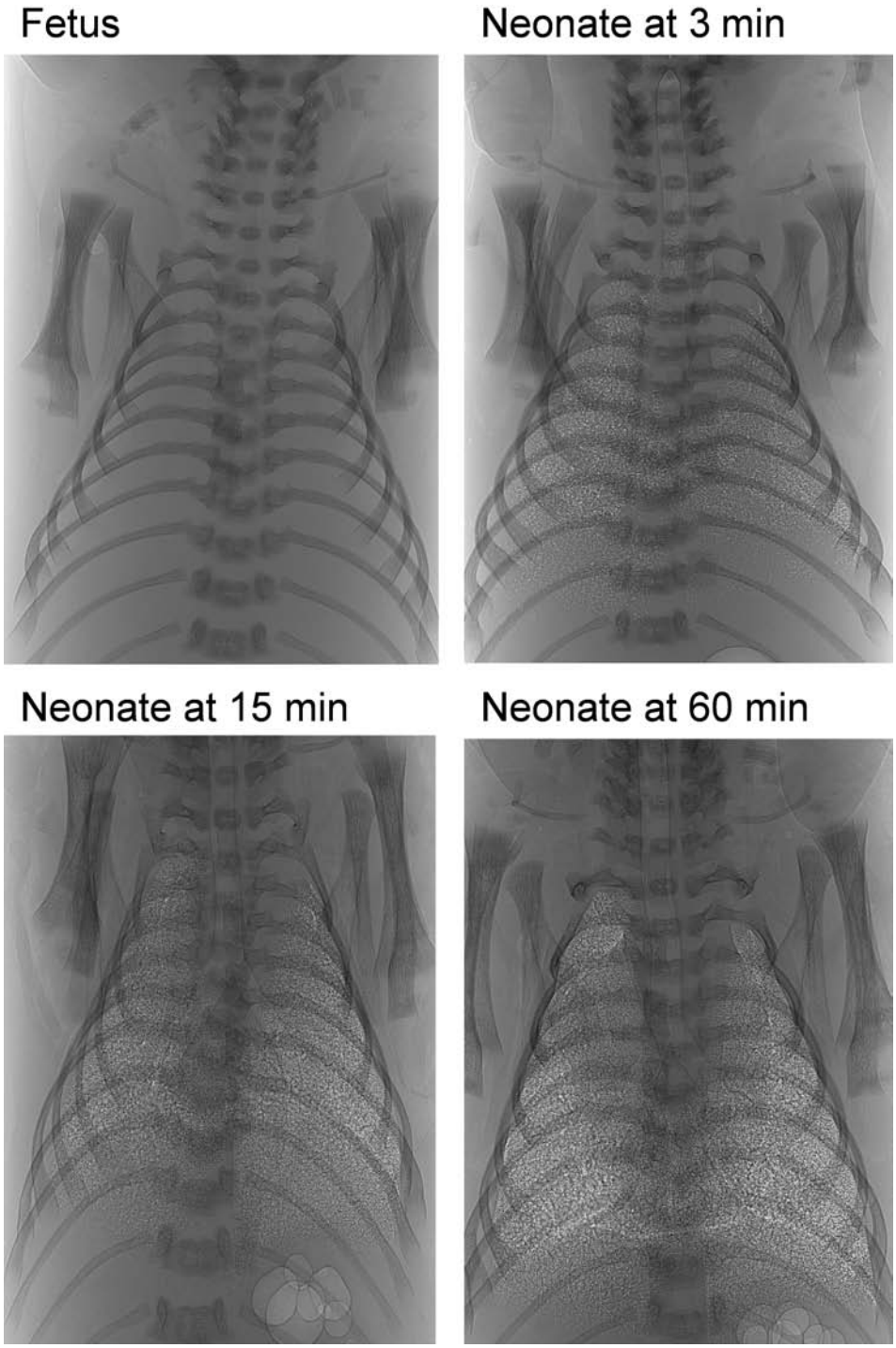

Fig. 1 Phase contrast X-ray image of spontaneously breathing newborn rabbit pups delivered by Caesarean section near term (31 days gestational age). Images were acquired before the onset of aeration (fetus) and then at 3, 15 and 60 min after birth. Following lung aeration, the difference in the refractive index between air and water produces strong phase contrast at all air–liquid boundaries, rendering the air-filled lung visible. A detailed examination of the neonatal lung at 60 min after birth demonstrates that this technique is able to resolve even the smallest of air-filled structures (alveoli, at approximately 100 μm in diameter).[12]

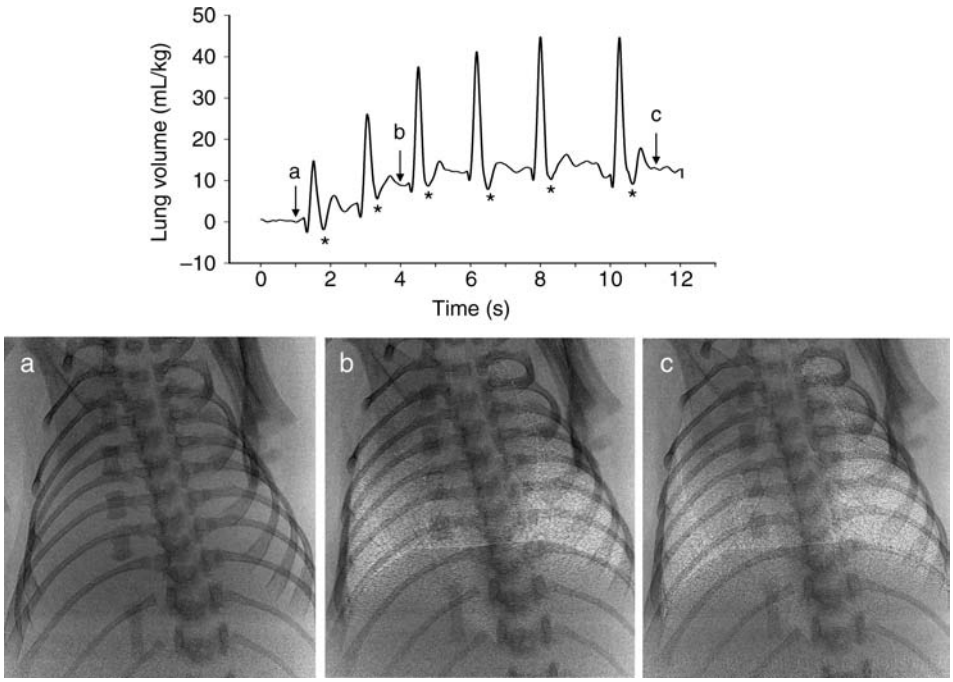

Fig. 2 Plethysmograph recording of breathing activity and the increase in end-expiratory lung gas volumes from birth in a spontaneously breathing newborn rabbit pup delivered near term. The recording demonstrates that pups can rapidly generate an end-expiratory lung air volume of approximately 16 mL/kg within 10–12 s of the onset of breathing. Note that the end-expiratory lung air volume increases with each breath. (a–c) Phase contrast X-ray images were acquired at the times indicated by the arrows in the plethysomgraph recording and demonstrate the increase in lung aeration associated with each breath. Reductions in gas volume immediately following inspiration (asterisk) are recording artefacts.

Investigations of the temporal and spatial pattern of lung aeration in spontaneously breathing newborn rabbit pups using phase contrast X-ray imaging[12] clearly demonstrate that lung aeration is closely associated with inspiration. To quantify the relative contribution of inspiration to lung aeration and the creation of an end-expiratory gas volume from birth, phase contrast X-ray imaging has been used simultaneously with plethysmography (MLL Siew *et al*., unpubl. obs., 2008). Individual breaths accounted for 94.8 ± 1.4% of the increase in functional residual capacity (FRC) after birth (Fig. 2) and only small increases in FRC (5.2 ± 1.6%) could be detected between breaths (in 15 of 26 pups). Images acquired immediately before and after a single breath (Fig. 2) clearly demonstrate the increase in lung aeration associated with a single inspiration, which occurs, on average, at a rate of 9.7 ± 0.8 mL/kg per s (or approximately 35 L/kg per h) over a single breath; breath duration is, on average, approximately 0.3 s.

"lung aeration associated with a single inspiration"

In these experiments, measures of lung aeration must equate to airway liquid clearance, because the only other possible explanation is that liquid remains within the distal airways and coexists with air following lung aeration. For that to occur, the distal airways must expand considerably to accommodate both the pre-existing liquid volume (at least 20 mL/kg[1,2]) and the increase in air volume (approximately 16 mL/kg) acquired during aeration (MLL Siew *et al*., unpubl. obs., 2008). Consequently, with tidal volumes of up to 15 mL/kg immediately after birth, lung volumes at end-inspiration would increase above 50 mL/kg. This would be injurious and force neonates to breathe at the top of their pressure–volume curve, thereby decreasing lung compliance with increasing lung aeration. Clearly this does not normally occur and would cause a thick liquid layer to line the distal airways, greatly increasing the barrier for gas diffusion. The potential thickness of this layer can be calculated knowing the radii of the air-filled components of the terminal sacs (approximately 70 μm[12]) and assuming that they are spherical. If no liquid leaves the sacs, to accommodate the addition of an equal volume of air, the terminal sac radius must increase 1.26-fold (to approximately 88 μm), making the liquid layer approximately 18 μm thick. Normally, the intersaccular wall thickness is 4–6 μm and the air–blood gas barrier is < 1 μm for efficient gas exchange. A liquid layer this thick would markedly reduce postnatal respiratory function and clearly does not normally occur at birth. Furthermore, if liquid was retained within the distal airways, recoil of the expanded airways should force liquid to refill the airways and push the air–liquid interface proximally during expiration. The image sequences clearly show that this does not happen.

The observation that lung aeration occurs during inspiration in spontaneously breathing rabbit pups is contrary to most previous commentaries on this topic,[5,6] including our own.[1,2,4] It is widely acknowledged that lung liquid reabsorption at birth results from the

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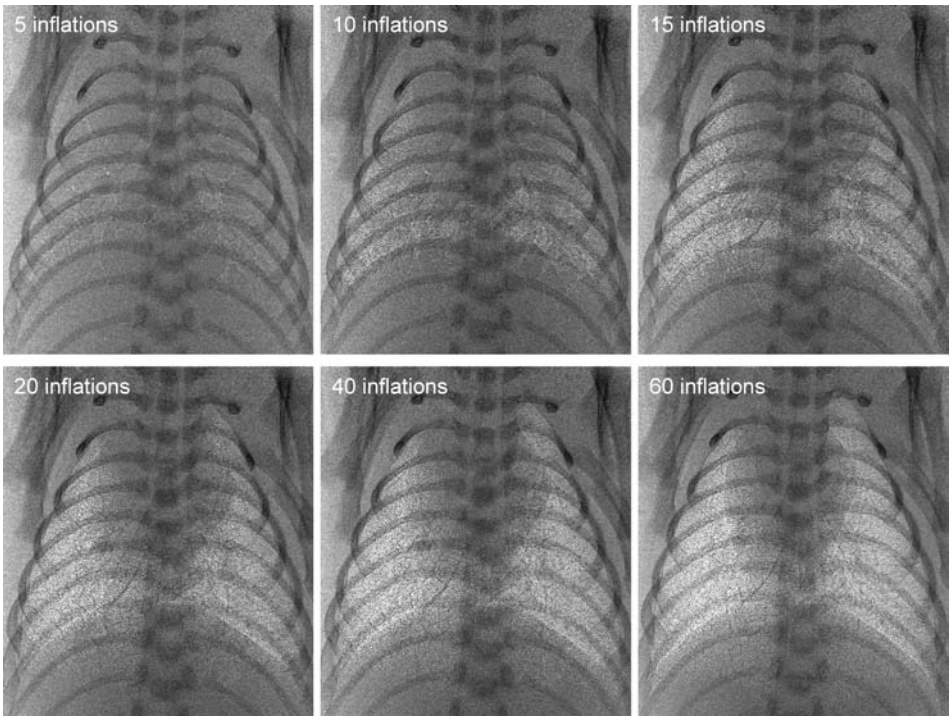

Fig. 3 Phase contrast X-ray images acquired at functional residual capacity (FRC) in a dead rabbit fetus that was ventilated, starting approximately 2 h after death, using a peak inflating pressure of 35 cmH_2O and a positive end-expiratory pressure of 5 cmH_2O at 24 inflations/min. The phase contrast X-ray images were acquired after 5, 10, 15, 20, 40 and 60 inflations and demonstrate that ventilation alone, in the absence of any active endogenous mechanisms, can cause lung aeration.

activation of ENaCs, causing Na^+ reabsorption and reversal of the transepithelial osmotic gradient, leading to water uptake from the airways.[5,6] However, lung aeration is clearly associated with inspiration and occurs much more rapidly than could be achieved by liquid reabsorption alone (see Fig. 2). Thus, we suggest that transepithelial hydrostatic pressures generated by inspiration are the primary driving force for airway liquid clearance after birth.[12] This suggestion is consistent with the finding that lung aeration occurs rapidly in ventilated dead fetal rabbits, in which labour-induced liquid clearance mechanisms, such as adrenaline-induced Na^+ reabsorption, could not be activated (Fig. 3). Because FRC increased and lung compliance decreased with each breath in these dead pups, it appears that hydrostatic pressures can clear airway liquid in the absence of endogenous mechanisms (MLL Siew *et al*., unpubl. obs., 2008).

"hydrostatic pressures can clear airway liquid"

Upon clearance from the airways, the liquid must enter the interstitial tissue compartment before it is eventually cleared from the lung via the lymphatics and vascular system, which can take a number of hours.[27] Because the liquid rapidly leaves the airways (in minutes) and is cleared from the tissue much more slowly (over hours), the temporary accumulation of water within the interstitial tissue compartment most probably explains the transient (2–4 h) increase in interstitial tissue pressure that occurs immediately after birth.[28] Furthermore, the combination of liquid retention within the tissue and the increase in airway gas volume most probably explains the increase in chest wall expansion that occurs shortly after birth.[12] Then, as the water is cleared from lung tissue, the interstitial tissue pressure gradually declines to eventually become subatmospheric (within 6 h), as measured *in situ* in the adult.[28]

A volumetric analysis of the lungs based on partitioning of the image into quadrants (Fig. 4a) demonstrates that lung aeration in spontaneously breathing pups is not uniform. Aeration begins in the basal lobes and the rate of increase in air volume is much greater in the basal compared with the apical lobes. As a result, the rate of increase in end-expiratory lung volume is much greater in the basal lobes than the apical lobes, which reflects the size difference, and therefore the volume difference, across these regions. Indeed, when the volumes are corrected for the average end-expiratory gas volume achieved in each region, the relative volume change was similar. However, the relative volume change associated with tidal breathing appeared much greater in the apical than basal lobes, suggesting that ventilation predominantly occurred in these lobes, but further investigation is required because image acquisition did not always coincide with end-inspiration (Fig. 4b). Nevertheless, this technique is ideal for investigating regional ventilation within the lung, providing information that cannot be obtained by plethysmography.

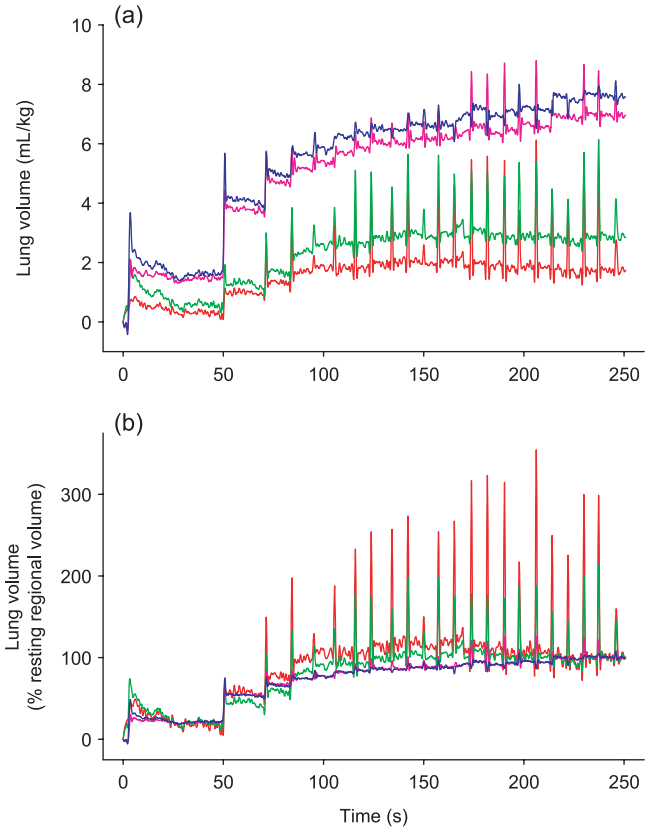

Fig. 4 Regional changes in (a) absolute and (b) relative lung gas volumes from birth in a spontaneously breathing newborn rabbit pup. The images acquired during the lung aeration process were partitioned (based on changing rib positions) and the changes in lung gas volumes were measured using phase retrieval algorithms in each partitioned image. This analytical technique is able to measure the breath-by-breath changes in lung gas volumes within selected regions of the lung. Red, upper left lobe; green, upper right lobe; pink, lower left lobe; blue, lower right lobe. (b) The relative changes in lung gas volume are expressed in relation to the resting gas volume achieved in each region 250 s after birth. This figure indicates that, relative to the resting gas volume, ventilation predominantly occurs in the upper (caudal) lobes of the lung after birth. These figures have been redrawn using data published in Kitchen *et al*.[18]

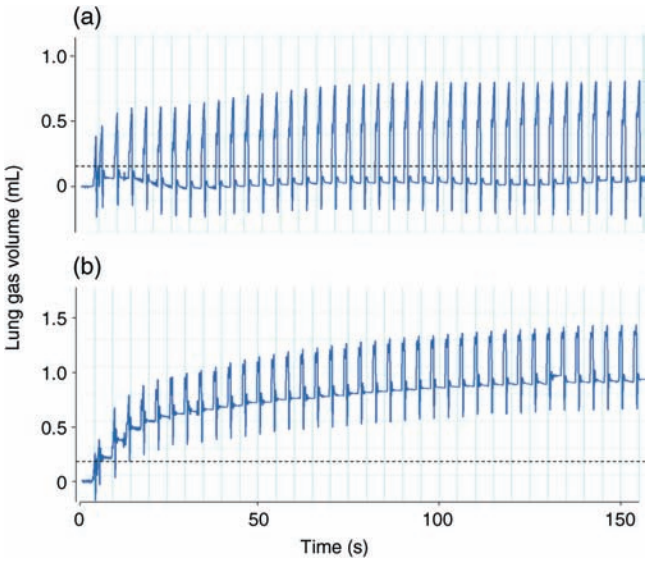

Fig. 5 Plethysmograph recordings of changes in lung gas volumes in intubated and mechanically ventilated preterm rabbit pups delivered by Caesarean section at 27 days of gestation. Pups were ventilated using a peak inspiratory pressure (PIP) of 35 cmH_2O at a positive end-expiratory pressure (PEEP) of (a) 0 or (b) 5 cmH_2O. All preterm pups that were ventilated in the absence of PEEP (0 cmH_2O) failed to develop an end-expiratory lung gas volume, resulting in lung collapse at end-expiration. Transient reductions in lung gas volumes immediately following a mechanical inflation are recording artefacts. The dashed line indicates dead space volume.

LUNG LIQUID CLEARANCE AND LUNG AERATION IN MECHANICALLY VENTILATED PRETERM NEONATES

Preterm birth is the greatest cause of neonatal morbidity and mortality, occurring in approximately 7.5% of all births in Australia. Infants at greatest risk[29] are born very preterm (< 28 weeks gestational age), which occurs in approximately 1.5% of births (approximately 3600 babies/year in Australia). These infants usually need resuscitation at birth and many require weeks of assisted ventilation because their lungs are too immature to support their respiratory needs. However, ventilating these infants can injure their lungs, leading to the development of bronchopulmonary dysplasia (BPD),[30,31] which can be lethal and has major long-term health implications.[32–34] As a result, very preterm infants require prolonged intensive care, usually spending months in hospital following birth, and survivors commonly require rehospitalization in their first year of life for serious respiratory diseases.

"preterm infants require prolonged intensive care"

Because more very premature infants are surviving the immediate postnatal period, the incidence of BPD is increasing,[29] making it the most common, difficult and expensive problem in neonatal medicine. The increasing incidence of BPD, which is indicative of ventilation-induced lung injury, has occurred despite major advances in neonatal ventilation and therapeutic treatments.[30] Clearly, critical factors have been missed and current practices need re-evaluation.[35] In particular, although considerable attention has focused on best practises for ventilating very preterm infants following stabilization, little attention has focused on strategies for ventilating these infants at birth, when

the lungs are partially liquid filled. For instance, it is still common to manually resuscitate very preterm babies at birth with self-inflating bags that allow little control or knowledge of the tidal volumes and pressures administered. The potential for lung injury to occur during this period, when the lung is largely liquid filled, is thought to be high, prompting the comment that lung injury 'can begin right from the first breath, in the delivery room where we often ignore the tidal volume and the end-expiratory pressure we use to support gas exchange'.[31] However, little attention has focused on this critical period, mainly because it has not been possible (until recently) to examine how ventilation procedures influence lung aeration and regional ventilation on a breath-by-breath basis.

Phase contrast X-ray imaging is an ideal technique for identifying ventilation strategies that promote lung aeration in mechanically ventilated rabbit pups delivered very preterm. In particular, it can identify how different ventilation procedures influence the uniformity of lung aeration at birth and counteract the propensity for the immature lung to collapse at end-expiration. Using this technique, the effects of a positive end-expiratory pressure (PEEP), a sustained (5, 10 and 20 s) first inflation, exogenous surfactant, body position and antenatal corticosteroids on the temporal and spatial patterns of lung aeration have been investigated. However, for the purposes of the present review, we will focus on the effect of PEEP.

Positive end-expiratory pressure is known to have major benefits for ventilating very preterm infants once they have been stabilized that include improving lung gas volumes,[36–38] blood oxygenation[36,37,39] and reducing alveolar collapse at end-expiration.[40,41] Because airway collapse is a major cause of lung injury, PEEP plays an important role in protecting against lung injury, particularly in intubated very preterm infants, by maintaining an end-expiratory distending pressure on the airways. However, PEEP is not commonly used during the immediate newborn period, particularly in infants requiring resuscitation at birth. Indeed, the guidelines provided by the International Liaison Committee on Resuscitation[42] for the ventilation of preterm infants at birth fail to recommend the use of PEEP.

"PEEP plays an important role in protecting against lung injury"

Phase contrast X-ray imaging clearly demonstrates that, in the absence of PEEP, preterm rabbit pups mechanically ventilated from birth gradually accumulate a tidal volume but fail to accumulate an FRC (Figs 5, 6). Indeed, the images show that despite developing a large tidal volume, alveolar ventilation was minimal and the lungs collapsed at end-expiration, including both small and large airway collapse (Fig. 6). In contrast, the application of 5 cmH_2O PEEP facilitated the accumulation of an FRC that was similar in temporal

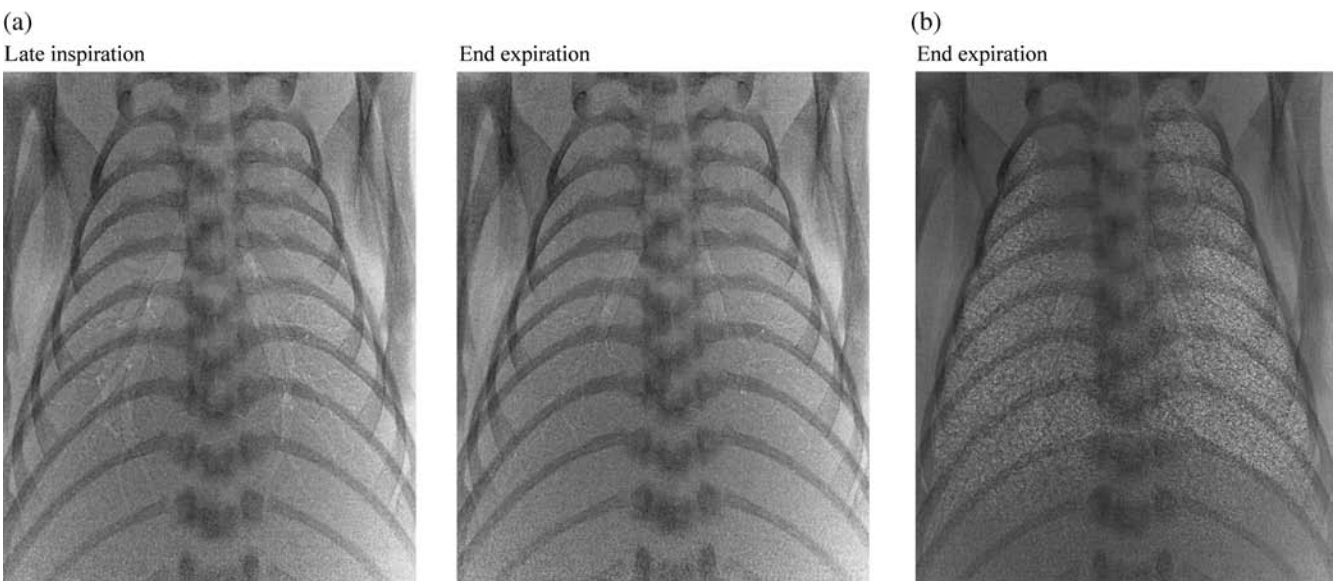

Fig. 6 Phase contrast X-ray images acquired 5 min after the onset of ventilation in preterm rabbit pups delivered by caesarean section at 27 days of gestation. Pups were ventilated mechanically from birth with a peak inspiratory pressure of 35 cmH_2O and either in (a) the absence of a positive end-expiratory pressure (PEEP; 0 cmH_2O) or (b) the presence of 5 cmH_2O PEEP. Two images are displayed from a pup being ventilated with no PEEP; one acquired late in inspiration and the other at end-expiration. The other image was acquired at end-expiration in a pup ventilated with 5 cmH_2O PEEP. Note the airway collapse that occurs at end-expiration in the absence of PEEP.

pattern to that observed in spontaneously breathing term pups[12] (Fig. 5). The phase contrast X-ray images clearly demonstrate that the lungs gradually aerate and the airways remain open at end-expiration following ventilation with PEEP (Fig. 6). Because repeated opening and collapse of respiratory units is believed to be a major cause of lung injury, ventilation of neonates from birth in the absence of PEEP is likely to be injurious in very preterm infants with immature lungs.

"airways remain open at end-expiration following ventilation with PEEP"

CONCLUSIONS

Phase contrast X-ray imaging can image the lung with high spatial and temporal resolution. In particular, its unique ability to resolve boundaries between media of differing refractive indices makes it an ideal technique for identifying the factors regulating lung aeration at birth. Before birth, the lungs are not visible using this technique and only become visible as they aerate, allowing the progression of the air–liquid interfaces to be visualized and tracked as they move from the proximal and into the distal airways after birth. Using this technique, we have demonstrated that inspiration is a primary factor regulating lung aeration in spontaneously breathing rabbit pups at term and that, in the absence of PEEP, mechanically ventilated preterm rabbit pups do not accumulate an end-expiratory lung gas volume.

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