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CLINICAL RESEARCH ARTICLE

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Slowing lung deflation by increasing the expiratory resistance enhances FRC in preterm rabbits

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BACKGROUND: As very preterm infants have surfactant-deficient and highly incompliant lungs, slowing lung deflation during expiration might help preserve functional residual capacity(FRC) during lung aeration. In this study, we investigated the effect of expiratory resistance(Re) on lung aeration during positive pressure ventilation in preterm rabbits immediately after birth.
METHODS: Preterm rabbit pups were delivered at 29 days gestation, mechanically ventilated from birth and simultaneously imaged to measure lung aeration using phase-contrast X-ray. Re was varied by altering the length (0, 60 or 1000 mm) of the expiratory circuit.
RESULTS: Increasing Re led to a decrease in lung deflation rates and both peak expiratory flows and flow rates at mid-deflation. As a result, the rate of de-acceleration(slowing) in lung deflation when approaching FRC was markedly reduced with increasing resistance. During lung aeration, FRC was significantly different between resistance groups and was significantly higher over time in the high compared to the low resistance group. While FRC values tended to be higher with higher Re, they were not significantly different at end-ventilation ($t = 7$ min).
CONCLUSION: Increasing Re of the ventilation circuit during lung aeration in preterm rabbits immediately after birth decreased lung deflation rates and increased the accumulation of FRC over time.

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IMPACT:

- The expiratory phase of the ventilatory cycle has been largely overlooked as an opportunity to improve ventilation in preterm infants after birth.
- Increasing the expiratory resistance of the ventilator circuit during lung aeration in preterm rabbits immediately after birth markedly decreased lung deflation rates and increased FRC accumulation, compared to a low expiratory resistance.
- This indicates that ventilation devices that reduce the "work of breathing" by reducing the expiratory resistance, may have the unintended effect of reducing FRC, particularly in extremely preterm infants that have surfactant deficient highly incompliant lungs.

INTRODUCTION

Understanding how spontaneously breathing infants transition after birth has already provided several insights in how to improve ventilation for preterm infants after birth. During spontaneous breathing, sub-atmospheric pressures generated within lung tissue efficiently clears airway liquid into lung tissue, allowing the creation of an FRC and the onset of pulmonary gas exchange. As the transpulmonary pressure gradients generated by spontaneous breathing can be replicated by positive pressure ventilation (PPV), PPV can be used to aerate and ventilate the lungs when infants are apnoeic. However, the PPV characteristics required to aerate the liquid-filled lung are very different to those needed to ventilate the aerated lung, making extrapolation from one to the other problematic. For example, during the lung inflation phase, as liquid has a much higher viscosity than air, longer inspiratory times and higher pressure gradients are required. This is because the time constant of the liquid-filled lung is much longer than air-filled lung as the resistance to move liquids through the airways is much higher than air.[1,2]

During the expiratory phase, expiration is mostly passive and largely determined by lung recoil pressure, which is high in the stiff, surfactant deficient immature lung, making the time over which expiratory flow occurs very short. As a result, spontaneously breathing preterm infants make expiratory braking manoeuvres (EBMs) to regulate airflow during expiration and prevent the lung from collapsing at end-expiration.[3,4] During EBMs, the laryngeal resistance is increased which extends the expiratory flow time and increases the airway pressure during passive expiration to help

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retain gas within the lung at end-expiration.[1,4–6] However, this necessarily slows respiration rates and thereby reduces minute volumes.[7,8] Nevertheless, by opposing the lungs' elastic recoil during expiration, EBMs slow the rate of lung deflation and reduce the risk of small airway collapse when the lung reaches FRC.[1,2] While laryngeal resistance plays an important role in maintaining FRC in spontaneously breathing preterm infants, when infants are intubated the larynx is by-passed and laryngeal resistance is negated. Indeed, ventilation systems that have minimal expiratory resistance have higher peak expiratory flows and deflations rates,[5,9] which might lead to a loss of FRC due to the lung's higher momentum as it approaches FRC. While high positive end-expiratory pressures (PEEPs) help to reduce the risk of lung collapse at end-expiration in intubated infants, high PEEP levels impede venous return to the heart and reduce pulmonary blood flow.[10,11] As a result, the PEEP level used must balance the need to maintain FRC levels and to avoid compromising cardiovascular function in the immediate newborn period.

In order to maintain FRC levels after birth, a possible alternative to the use of high PEEP levels is to slow the rate of lung deflation by increasing expiratory resistance in the ventilatory circuit, just like EBM's. Our aim was to investigate the effect of expiratory resistance on lung aeration during PPV in preterm rabbits immediately after birth. We hypothesised that a higher expiratory resistance would result in lower deflation rates, lower peak expiratory flows and the need for lower de-acceleration rates at end-deflation, leading to higher FRC levels.

METHODS

Animal experiments

All experiments were conducted in accordance with the National Health and Medical Research Council (NHMRC) of Australia code of practice for the care and use of animals for scientific purposes.[12] All animal procedures were approved by the SPring-8/Japan Synchrotron Radiation Institute (JASRI) Animal Care (Proposal #2011B0022) and Monash Medical Centre Animal Ethics Committee (Monash University) (Proposal #MMCA2011/17). Methodological reporting is provided as per the relevant ARRIVE guidelines.[13] This study was conducted in experimental hutch 3 of beamline 20B2 in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan.

At 29 days gestation (~26–28 weeks of gestation in human infants), pregnant New Zealand white rabbits ($n = 5$) were initially anesthetised with propofol (12 mg/kg). Following intubation, anaesthesia was maintained with inhaled isoflurane (1–4%) during mechanical ventilation. Pups were delivered via caesarean section one at a time, leaving the foetal membranes over the nose and mouth to prevent air entering the lungs. The pups were weighed, sedated (0.375 mg pentobarbitone sodium) and intubated (18–21 G tube), via tracheostomy, using a blocked endotracheal tube to prevent lung aeration due to spontaneous breathing efforts. The foetal membranes were then removed and the umbilical cord ligated. Pups were placed in a water-filled plethysmograph (37 °C), with its head out, positioned within the expected path of the X-ray beam (see below). The endotracheal tube was unblocked and connected to a custom-designed small-animal ventilator[14] which triggered image acquisition to synchronise the imaging with each ventilation cycle; imaging commenced 1–2 min after delivery.

Experimental protocol

All pups were ventilated using positive pressure ventilation (PPV), with the peak inspiratory pressure (PIP) being adjusted to achieve a set tidal volume (Vt) of 10 mL/kg. Initially, the PIP was set at 35 cmH_2O, then adjusted as needed. Fixed inspiration (1 s) and expiration (1.5 s) times were used for a rate of 24 inflations/min. The positive end-expiratory pressure (PEEP) was set at 4 cmH_2O in the high expiratory resistance group to ensure expiration was completed within the pre-set expiratory time to avoid any potential for gas trapping at end-expiration; a PEEP of 5 cm H_2O was used in the low and medium expiratory resistance groups. Pups were randomly allocated to one of the following three expiratory resistance groups by extending the length of the expiratory tubing:

- Group 1 low resistance: Length of additional tubing = 0 mm
- Group 2 medium resistance: Length of additional tubing = 60 mm
- Group 3 high resistance: Length of additional tubing = 1000 mm

Ventilation of the pups continued for 7 min while they were being imaged. The doe and all pups were humanely euthanised at the end of the experiment using sodium pentobarbitone at a dose of >100 mg/kg for both doe and pups.

Physiological measures

Lung air volumes were measured throughout the respiratory cycle from birth using a water-filled plethysmograph and were digitally recorded using a computerised data acquisition system (Powerlab, ADInstruments Sydney, Australia). The plethysmograph was calibrated (two point calibration) before each experiment by measuring the pressure change caused by injecting a known volume of water (1 mL) into the sealed chamber.[15] We recorded the initial volume, tidal volume (Vt), airway pressure, and inspiratory and expiratory gas flows. From this, we were able to calculate peak inspiratory and expiratory flow rate (PIFR, PEFR), lung-deflation rate at mid-deflation (i.e. flow rate at mid-deflation) and the rate of de-acceleration at end-deflation (i.e. how quickly the lung must de-accelerate (slow) at the end of the deflation to maintain FRC). Phase contrast x-ray imaging (see below) was used to measure FRC, as increased floatation of the pup over time (due to lung aeration) caused drift in the plethysmograph signal.

Phase contrast x-ray imaging

All pups were imaged at the SPring-8 Biomedical Imaging Centre, Japan, in Hutch 3 of beamline 20B2. The partially-coherent synchrotron-based x-rays were tuned to 24 keV, with a source-to-sample distance of approximately 210 metres and a sample-to-detector distance of three metres to allow for adequate phase contrast effects. A Hamamatsu C9300-124A phosphor charge-coupled device (CCD) with tapered fibre optics bonded between the CCD chip and the 20-µm thick gadolinium oxysulfide (Gd_2O_2S; P43) phosphor. The taper ratio was 1.8:1, converting the native pixel size of 9 µm to an effective pixel size of 16.2 µm. Four images were taken during each phase of the respiratory cycle (inspiratory time 1 s and expiratory time 1.5 s) at a frame rate of 3.3 Hz. Direct beam and detector dark current images were used to correct for detector artefacts, beam inhomogeneities and variations in detector dark current signal.

Lung gas volumes at FRC were measured from the high-resolution phase-contrast X-ray image sequences using the power spectrum analysis technique developed by Leong et al.[16] This technique measures the Fourier spectrum associated with the speckle patterns produced by phase contrast imaging of overlapping alveoli, whose spectral power increases with increasing lung air volume. The integral of the azimuthally-averaged power spectrum of the lung region of each frame was calculated and correlated to the total lung gas volume via the calibration coefficients reported by Leong et al.[16] We were able to measure absolute lung gas volumes since the lungs were unaerated in the initial frame of each sequence. The data points corresponding to FRC were then extracted for analysis via the application of a gradient filter over the absolute lung gas volume data to find the local minima between breaths, which have steep gradients during inspiration and expiration.

Statistical analysis

To minimise animal use,[17] the sample size was calculated utilising a large effect size (~80%) and a variance in FRC levels measured in similar previous study.[18] This gave a sample size of 5–6 rabbits per group to detect a difference between groups at an alpha value of 0.05. All statistical analyses were performed with IBM SPSS Statistics version 25 (IBM Software, Chicago, IL, United States, 2016). A p-value < 0.05 was considered statistically significant. Quantitative continuous data were judged for normality based on the inspection of histograms. Numerical skewed outcomes were compared using independent-samples Kruskal–Wallis test or Mann Whitney U test and presented as median (IQR). To compare the effect of expiratory resistance on FRC over time, a mean was calculated in 15 s intervals every minute during positive pressure ventilation. Linear mixed-effect regression models accounted for the relation between multiple measurements of the same kitten with an uncorrected covariance structure. Group, time and group × time interactions were included in the model as fixed factors. Group p values were used to compare means between groups. Data are presented as median (IQR).

Table 1. Baseline characteristics.

	Low resistance $n = 7$	Medium resistance $n = 5$	High resistance $n = 5$	p-value[a]
Birthweight (g)	33 (24–34)	36 (29–37)	31 (26–33)	0.295

[a]Kruskal–Wallis test.

Table 2. Physiological and respiratory parameters during positive pressure ventilation.

	Low resistance $n = 7$	Medium resistance $n = 5$	High resistance $n = 5$	p-value[a]
Resistance				
Ri (cmH_2O/mL/s)	11.6 (5.6–15.4)	9.6 (3.9–15.2)	6.8 (5.2–11.9)	0.062
Re (cmH_2O/mL/s)	5.8 (4.8–7.4)[e]	7.0 (4.5–8.7)	9.1 (8.0–11.0)[c]	0.027
Pressure				
Set PIP level (cmH_2O)	28.3 (25.2–29.2)	25.8 (24.1–29.9)	26.2 (24.1–28.4)	0.816
Set PEEP level (cmH_2O)	5.5 (5.3–5.7)[e]	5.3 (4.7–5.5)[e]	4.1 (3.8–4.6)[c,d]	0.011
Mean airway pressure (cmH_2O)	15.1 (13.6–15.3)	13.9 (13.1–16.0)	15.2 (14.3–16.4)	0.625
Duration from PIP to PEEP (sec)	0.1 (0.1–0.2)[d,e]	0.3 (0.2–03)[c,e]	0.9 (0.8–0.9)[c,d]	<0.001
Pressure time integral (cmH_2O.s)	23.6 (20.6–24.3)	21.7 (20.0–26.5)	29.1 (25.3–31.1)	0.053
Flow rates				
PIFR (mL/kg/s)	78.8 (59.1–107.1)	70.2 (57.8–144.7)	115.5 (80.3–129.9)	0.376
PEFR (mL/kg/s)	125.0 (120.3–158.1)[d,e]	105.6 (89.4–120.8)[c,e]	81.9 (72.3–89.0)[c,d]	0.004
Lung Deflation				
Deflation rate at mid-deflation (mL/kg/s)	84.6 (75.2–112.1)[d,e]	51.4 (46.6–63.5)[c,e]	9.7 (9.4–13.2)[c,d]	<0.001
De-acceleration rate at end-deflation (mL/kg/s^2)	1265.2 (1014.4–1464.8)[d,e]	615.8 (458.7–713.1)[c,e]	47.0 (41.5–58.4)[c,d]	<0.001
Tidal volume				
Vt (mL/kg)	11.9 (10.7–14.06)	10.5 (10.0–11.6)	10.7 (10.4–11.6)	0.111
FRC				
Average FRC over time (t0– t405) (mL/kg)	9.9 (5.7–14.3)[e]	10.3 (7.2–21.5)	17.0 (9.3–19.5)[c]	0.012[b]
FRC at end-ventilation t405 sec (mL/kg)	12.4 (9.9–22.5)	12.9 (8.6–25.7)	18.8 (13.4–23.1)	0.719

Ri inspiratory resistance, *Re* expiratory resistance, *PIP* peak inspiratory pressure, *PEEP* positive end-expiratory pressure, *PIFR* peak inspiratory flow rate, *PEFR* peak expiratory flow rate, *Vt* tidal volume, *FRC* functional residual capacity.
[a]Kruskal–Wallis test.
[b]Linear mixed model.
[c]Significantly different from the low resistance group
[d]Significantly different from the medium resistance group.
[e]Significantly different from the high resistance group.

RESULTS

In total, 17 newborn rabbit pups from 5 adult rabbits were included in this study: 7 in the low resistance group, 5 in the medium resistance group and 5 in the high resistance group. A similar birthweight was observed between the pups in all three expiratory resistance groups (Table 1). Set PIP levels were similar between all three groups, while set PEEP levels were significantly different ($p = 0.011$). The PEEP level in the low resistance group (5.5 (5.3–5.7 cmH_2O) was higher than the high (4.1 (3.8–4.6) cmH_2O) and medium (5.3 (4.7–5.5 cmH_2O) resistance groups (Table 2).

During PPV, inspiratory resistance was similar, while expiratory resistance was significantly different between the three resistance groups ($p = 0.027$). The lung deflation time, from maximal to minimal volume, was significantly longer with increasing expiratory resistance ($p = 0.001$) (Table 2, Fig. 1). In addition, lung deflation rates at mid-deflation were markedly reduced by increasing resistance (84.6 (75.2–112.1) vs 51.4 (46.6–63.5) vs 9.7 (9.4–13.2) mL/kg/s, $p < 0.001$). As a result, lung aeration was markedly greater at 300 and 600 msec after peak inflation with high resistance (Fig. 2). Similarly, peak expiratory flow rates (PEFR) (125.0 (120.3–158.1) vs 105.6 (89.4–120.8) vs 81.9 (72.3–89.0) mL/kg/s, $p = 0.004$), and lung deflation de-acceleration rates at end-deflation (1265.2 (1014.4–1464.8) vs 615.8 (458.7–713.1) vs 47.0 (41.5–58.4) mL/kg/s^2, $p < 0.001$) were significantly reduced by increasing resistance. Peak inspiratory flow rates (PIFR) remained similar between groups (Table 2). As a result of the high deflation rates in the low expiratory resistance group, the lung volume briefly decreased below FRC, as lung volumes oscillation around FRC, which can be observed in the pressure-volume curves (Fig. 1).

The increase in FRC during lung aeration was significantly different between the three resistance groups ($p = 0.0012$) with the low resistance group having a significantly lower FRC over time compared to the high resistance group ($p = 0.004$; Table 2, Fig. 3). While FRC values were not significantly different at the end of the ventilation period (Table 2), the difference in FRC appeared greatest at the onset of lung aeration, which is when compliance is at its lowest (Fig. 3). The cumulative pressure-time integral of inflation breaths during the entire ventilation period tended to be increased in the high resistance, but this just failed to reach statistical significance ($p = 0.053$); Table 2).

DISCUSSION

Previous studies investigating approaches for optimising ventilation of extremely preterm infants in the delivery room have almost

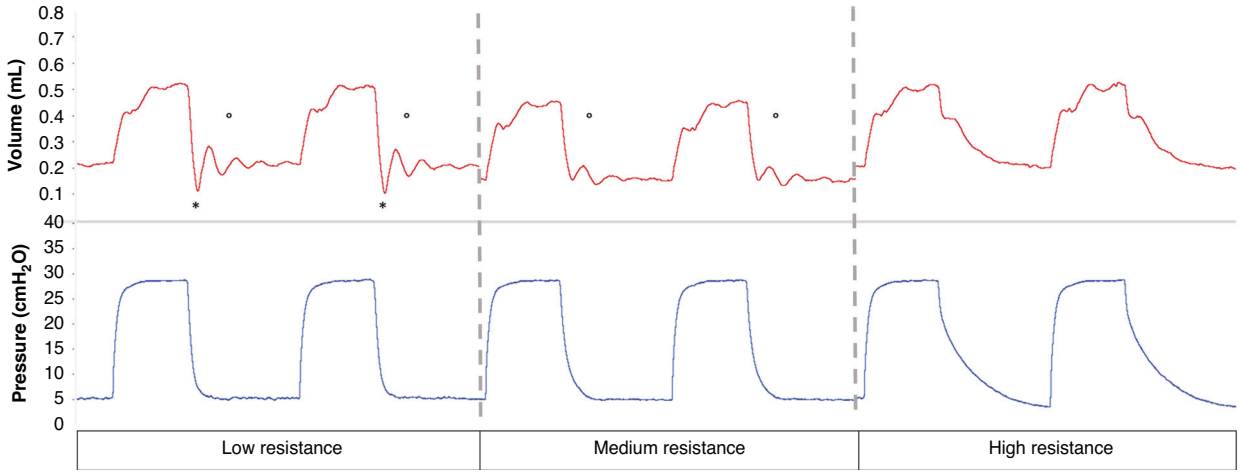

Fig. 1 The effect of expiratory resistance on pressure curves (blue; bottom panel) and tidal volumes (red; top panel) during positive pressure ventilation. Pressure volume curves are displayed of the low, medium and high expiratory resistance groups. The * indicates that lung volume goes briefly below FRC and the ° indicates an oscillation in lung volume at FRC.

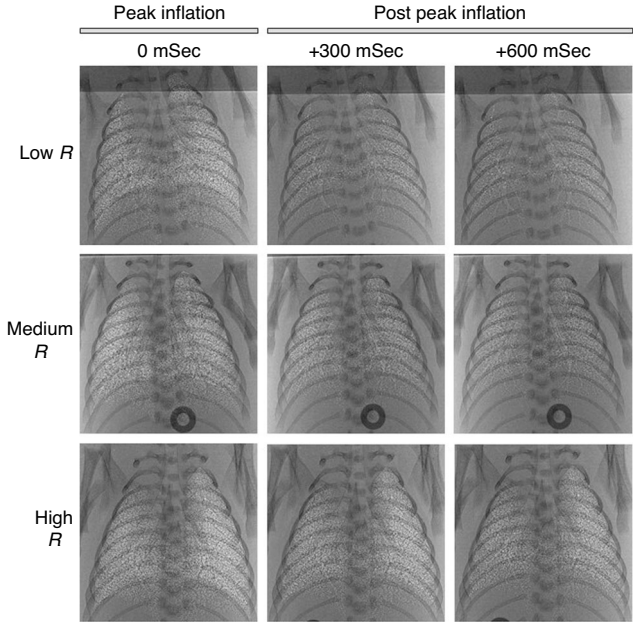

Fig. 2 Phase contrast X-ray imaging. Phase contrast X-ray imaging showing lung aeration 90 s after ventilation onset at peak inflation (0 msec) and post inflation (300 and 600 msec after peak inflation) for a rabbit pup in the low, medium and high expiratory resistance (R) group.

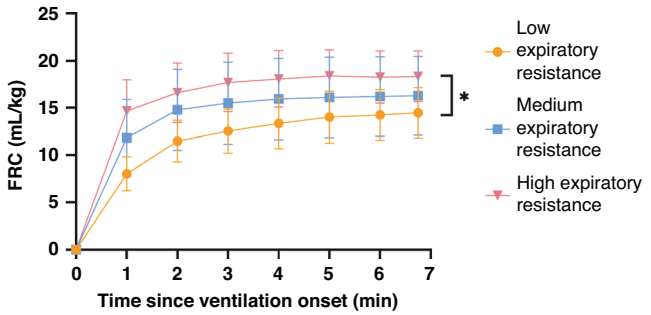

Fig. 3 Effect of expiratory resistance on functional residual capacity (FRC) during positive pressure ventilation. Median (Q1–Q3) FRC over time (t0 = start ventilation, t405 sec = end ventilation) within each group. An asterisk (*) indicates significant differences between groups.

exclusively focussed on the inspiratory phase of the ventilation cycle, except for PEEP. As expiration is a passive process, even during mechanical ventilation, the opportunity to alter expiratory parameters is more limited than during inflation. In this study, we investigated the effect of expiratory resistance on lung aeration during PPV in preterm rabbits immediately after birth. We observed that the accumulation of FRC over time was significantly higher with a high expiratory resistance compared to a low expiratory resistance. This finding indicates that ventilation devices that aim to reduce the "work of breathing", by reducing the expiratory resistance, may have the unintended effect of reducing FRC accumulation in extremely preterm infants that have surfactant deficient, highly incompliant lungs.

Our results are consistent with previous studies[19,20] investigating the effect of external expiratory resistance on lung volume in intubated and extubated neonates recovering from respiratory disease. In intubated infants, an increase in FRC of at least 50% was observed when an external expiratory resistance of 40–100 cm H_2O/L/s was applied.[20] In extubated infants, an increase in FRC of 40% was observed after application of an external expiratory resistance of 30 cm H_2O/L/s.[19] In both studies, the FRC increased in several seconds and was maintained in the breaths thereafter.[19,20]

Although a difference in FRC over time was observed between the high and low expiratory resistance group, the difference in FRC gradually decreased and by the end of the experimental period was no longer significant (Fig. 2). This finding was likely due to the difference in PEEP that was used between the different groups. Indeed, it is well established that PEEP assists in the creation and maintenance of FRC as it prevents repeated collapse and refilling of the distal airways at end-expiration; the higher the PEEP, the higher the FRC.[1,21,22] As we used a lower PEEP in the high resistance group it is likely that we have underestimated the influence of a high expiratory resistance on lung aeration. A lower PEEP level in the high expiratory resistance group was used to ensure that expiration was completed before the next inflation commenced so we could avoid air trapping, as this would artificially increase FRC (see Fig. 1). Nevertheless, while the lower PEEP likely resulted in lower than expected FRC levels in the high resistance group, particularly at the end of the ventilation period, the higher FRC we did measure over time in this group can only be attributed to the lower deflation rate. However, Fig. 1 clearly shows that caution is needed when using devices with a higher

expiratory resistance as air trapping can occur during PPV when the expiratory time is insufficient to reach the set PEEP. Furthermore, as lung compliance increases as the lung aerates, recoil pressure will decrease and so the expiratory resistance needed to slow lung deflation and optimise FRC recruitment/maintenance will likely decrease as the lung aerates.

Although we used a lower PEEP level with similar PIP levels during ventilation in these experiments, the mean airway pressure was similar. Nevertheless, the pressure-time integral tended to be higher in the high expiratory resistance group mainly because the time to deflate from PIP to PEEP was significantly longer in this group. It is possible that this could lead to a more uniform aeration of the lungs with less repeated collapse and refilling of the distal airways, allowing for more effective gas exchange and thus ventilation. Furthermore, as higher levels of lung aeration are persisting for longer during expiration within each respiratory cycle (Fig. 2), efficient gas exchange will likely increase and thereby reduce the newborn's ventilatory requirements.

Lung deflation rates at mid-deflation were ~9 fold higher in the low resistance group compared to the high resistance group (Fig. 1) indicating that the velocity of lung during deflation is markedly greater in the low resistance group. As a direct result of this higher deflation velocity, the lung must de-accelerate or slow at a markedly higher rate (~27 fold higher) as it approaches FRC. This is evident in Fig. 1, which clearly shows that the high rate of lung deflation causes an undershoot (ie lung volume briefly goes below FRC) and an oscillation in lung volume at FRC.[5,9] In addition, higher deflation and de-acceleration rates likely expose the lungs to higher levels of shear stress and subsequent airway epithelial cell damage, particularly in the fragile lungs of preterm infants at birth. Previous studies have shown that increased shear stress during the inspiratory phase of the ventilatory cycle is injurious to the lung, but the higher bias gas flows used to increase inflation rates in that study may also have increased deflation rates.[23] However, further studies are necessary to investigate the effects of deflation rates on FRC and lung injury. Nevertheless, as we found that PEFR was markedly higher than PIFR in the low resistance group, at the very least this indicates that expiratory flow rates should not be ignored when attempting to optimise the parameters used to ventilate extremely preterm infants at birth.

It is interesting that while PEFR was significantly increased by reducing the expiratory resistance, the percentage increase in PEFR (~50%) was markedly smaller than the percentage increase in lung deflation rate (~900%). This is likely because PEFR is a measure of peak flow, not average flow, that can be very short in duration and so does not accurately reflect bulk gas flow rates out of the lung during deflation. This is also evident in Fig. 1, which shows that in the high expiratory resistance group lung deflation is initially not too dissimilar to the lower resistance groups, but then rapidly slows and by mid deflation is markedly slower. As such, we consider that measures of PEFR markedly under-estimate the differences in lung deflation rates. Nevertheless, our finding of higher PEFRs are consistent with the finding of higher peak flows observed when the imposed resistance was reduced during passive expiration while using PPV in a bench test.[9]

While in this study, we were unable to measure physiological parameters such as end-tidal CO2 and oxygenation, we have previously shown that expired CO2 levels (averaged not end-tidal) are closely associated with tidal volumes and with the degree of lung aeration at peak inflation.[24] Thus, as tidal volumes were the same in each group and FRC levels were higher in the high resistance group, we would expect CO2 clearance to be similar or marginally higher in the high resistance group due to a higher end inspired gas volume. While the pups in this study were intubated, which bypasses the vocal cords, our findings indicate that the ability of vocal cords to increasing expiratory resistance during non-invasive ventilation is important for maintaining FRC in extremely preterm infants. While it is often assumed that expiratory braking manoeuvres help to defend FRC by pressurising the airways, it is also possible that their primary mode of action is to simply regulateresistance and slow the rate of lung deflation.

Except for regulating PEEP, the expiratory phase of the ventilatory cycle has been largely overlooked as an opportunity to improve the approaches used to ventilate preterm infants in the delivery room. While increasing PEEP levels necessarily reduces the pressure gradient driving expiration (i.e. PIP – PEEP) and, therefore, will reduce expiration rates, increasing PEEP levels adversely affects pulmonary blood flow[10] and cardiac output.[11] Alternatively, our findings indicate that regulating and perhaps slowing the rate of lung deflation may reduce shear stress and the PEEP required to maintain an appropriate FRC in preterm infants in the immediate newborn period. This suggestion is contrary to previous studies, advocating the use of respiratory devices that have a low expiratory resistance in order to "reduce the work of breathing". However, we consider that the logic of using this approach is questionable, particularly in the delivery room for infants with immature, liquid-filled and highly incompliant lungs. Indeed, as expiration is mostly passive, the only work associated with expiration is diaphragmatic- and expiratory braking, which acts to slow expiration and extend the expiratory phase.

CONCLUSION

Increasing the expiratory resistance of the ventilator circuit during lung aeration in preterm rabbits immediately after birth markedly decreased lung deflation rates and increased FRC accumulation, compared to a low expiratory resistance. This indicates that ventilation devices that aim to reduce the "work of breathing" by reducing the expiratory resistance, may have the unintended effect of reducing FRC, particularly in extremely preterm infants that have surfactant deficient highly incompliant lungs. Clinicians should be aware of this when choosing a ventilation device to resuscitate extremely preterm infants.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

K.K.: Conceptualisation, Methodology, Formal analysis and interpretation of data, Writing – original draft, Writing – review & editing. J.D.: Conceptualisation, Methodology, Supervision, Interpretation of data, Writing – review & editing. K.C., M.W.: Investigation, Interpretation of data, Writing – review & editing. S.C., I.D.: Interpretation of data, Writing – review & editing. D.J.: formal analysis and interpretation of data, Writing – review & editing. M.K.: Investigation, formal analysis and interpretation of data, Writing – review & editing. A.tP.: Conceptualisation, Investigation, Interpretation of data, Writing – review & editing. S.H.: Conceptualisation, Methodology, Funding acquisition, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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COMPETING INTERESTS

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ETHICS APPROVAL

All animal procedures were approved by the SPring-8 Animal Care and Monash University's Animal Ethics Committees.

ADDITIONAL INFORMATION

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