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EXPERIMENTAL

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Variable ventilation improves ventilation and lung compliance in preterm lambs

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Abstract *Purpose:* In adult animals, ventilation with variable tidal volume and rate improves lung mechanics, arterial oxygenation and ventilation compared to a monotonously controlled ventilation pattern. We assessed the physiological consequences of variable ventilation in the immature lung. *Methods:* Lambs delivered at 129 days (term = 150 days) were euthanised ($n = 9$) or anaesthetised, tracheostomised and suctioned prior to prophylactic intra-tracheal surfactant instillation (Curosurf®, 100 mg/kg) and commencement of controlled ventilation (50 breaths/min, tidal volume 7.7 ± 0.8 mL/kg). Volume history was standardised at 20 min with two sustained (3 s) inflations to 30 cmH_2O followed immediately by measurement of baseline dynamic lung mechanics (FlexiVent™, Scireq, Canada). Ventilation was continued according to prior randomisation (variable or conventional ventilation). For variable ventilation ($n = 9$), breath-to-breath tidal volume and respiratory rate varied but intra-breath minute volume (MV) and average tidal volume were equivalent to the conventional ventilation group with fixed tidal volume and rate ($n = 7$). Lung mechanics and gas exchange were measured at intervals. Lambs were euthanised at 2 h. Inflammatory cell counts and protein from bronchoalveolar lavage fluid and lung tissue cytokine mRNA were quantified. *Results:* At study completion, $PaCO_2$ ($p = 0.026$) and mean airway pressure ($p = 0.002$) were lower and pH ($p = 0.047$), ventilation efficiency index ($p = 0.021$) and dynamic compliance were higher ($p = 0.003$) in lambs on variable rather than conventional ventilation. However, oxygenation indices and post-mortem static compliances were not different between groups. *Conclusion:* Variable ventilation improves ventilation efficiency and in vivo lung compliance in the preterm lung, but unlike adult models, had no effect on arterial oxygenation.

Keywords Infant · Preterm · Artificial respiration · Homeokinesis · Respiratory distress syndrome · Stochastic resonance

Introduction

Preterm infant lungs are very different to adult lungs. At birth, the lungs are fluid filled and the fetal lung fluid needs to be absorbed to establish a functional residual capacity. The extremely preterm lung has only primitive saccules and a thickened alveolar-capillary barrier at the zone of gas exchange. The highly compliant chest wall

and reduced lung recoil leave the parenchyma susceptible to collapse and re-inflation/stretch injury. The ductus arteriosus may remain patent for some time after birth and high pulmonary vascular resistance results in right-to-left shunting across fetal vascular channels, impairing oxygenation [1]. High heart rates increase pulmonary flow transit time limiting the opportunity for oxygen diffusion into the capillary.

Spontaneously breathing infants utilise a range of pressures, tidal volumes (V_T) and breathing rates [2] and are highly dependent on sighs to maintain lung volume [3]. This breathing pattern contrasts markedly with traditional approaches to conventional ventilation (CV) of newborn infants that strictly regulate tidal volume (V_T), respiratory rate (RR) and peak inspiratory pressures (PIP). Variability is an important component of homeokinesis and is integral to biological well-being. Targeting a more physiological and variable ventilation strategy may be desirable.

Ventilatory patterns using variable inspiratory to expiratory ratios and positive end expiratory pressure (PEEP) were suggested in 1992 [4]. More recently, Mutch and colleagues proposed that variable V_T and respiratory rate ventilation (VV) [5] may promote lung volume recruitment. Compared to CV that uses a fixed tidal volume (V_T) and RR, VV delivers the same average V_T and RR, but inversely varies V_T and RR on a breath-to-breath basis. Thus VV maintains a constant breath-to-breath minute volume throughout the study but delivered V_Ts are distributed according to a power law such that the lung may receive tidal volumes less than or greater than the average V_T [6].

In theoretical [7] and adult animal models of healthy [8, 9] and injured or diseased lungs [5, 10–13], VV improves oxygenation and enhances gas exchange compared to CV. The potential applications and benefits of VV in the immature preterm lung are unstudied.

We hypothesised that in a preterm lamb model, VV would improve arterial oxygenation, ventilation efficiency and lung compliance.

Methods

Studies were performed at the University of Western Australia and were approved by the institution's Animal Ethics Committee. Detailed methods are provided in Online Resource 1.

Animal care and monitoring

Anaesthesia was induced in date-mated pregnant ewes at 129 days gestation (term $\approx$ 150 days) prior to spinal regional anaesthetic block and caesarean section. Nonventilated control lambs (NVC, $n = 9$) were euthanised at delivery and a post-mortem performed immediately.

Fetuses assigned to ventilation were tracheostomised (4.5-mm-ID tube) and fetal lung fluid was suctioned prior to intra-tracheal surfactant instillation (100 mg/kg, Curosurf®, Chiesi Pharma, Italy) and delivery. The lamb was weighed and commenced volume-controlled pressure-limited ventilation (FlexiVent, Scireq, Canada) using standardised initial settings (11 mL/kg cylinder V_T to achieve delivered $V_T = 7.7 \pm 0.8$ mL/kg, 40 cmH_2O maximum PIP, 50 breaths/min). Anaesthesia (propofol 0.1 mg/kg/min, Norbrook Laboratories, Australia) and analgesia (remifentanil 0.05 μg/kg/min, GlaxoSmithKline, Australia) were administered by continuous umbilical venous catheter infusion, ablating spontaneous respiratory activity. Blood gases were sampled at intervals from an umbilical arterial catheter, and postductal oxyhaemoglobin saturation (SpO_2) was monitored continuously (N95, Covidien-Nellcor, CO, USA).

Baseline respiratory mechanics (see below) were obtained with the FlexiVent using a standardised volume-controlled waveform at 20 min, immediately after lung volume was normalised with two successive 3-s inflations to 30 cmH_2O and prior to continuing lambs on CV ($n = 7$) or commencing VV ($n = 9$).

For VV lambs, the FlexiVent was pre-programmed to deliver a range of V_Ts, with an average (SD) delivered (tracheal) tidal V_T of 7.7 (2.1) mL/kg (CV% −27.1%) and a median (range) of 7.1 (5.5, 17.5) mL/kg [6]. Respiratory rate was varied inversely with V_T (average 50 breaths/min), to match the breath-to-breath and average minute ventilation in the CV group.

Respiratory mechanics measurements were repeated at 25-min intervals throughout the study, paired with arterial blood gas measurements and recording of ventilatory pressures. Oxygenation index (OI) was calculated as

$$\mathrm{OI} = P_{tr} \times \mathrm{FiO_2} \times 100 / P\mathrm{aO_2} \quad (1)$$

where P_{tr} is the mean pressure in the trachea and PaO_2 is the partial pressure of oxygen in arterial blood.

FiO_2 was adjusted to target a peripheral oxyhaemoglobin saturation (SpO_2) of 88–95%. The alveolar–arterial (aA) ratio was calculated as

$$\mathrm{aA\ Ratio} = P\mathrm{aO_2} / (713 \times \mathrm{FiO_2} - P\mathrm{aO_2})$$

where $PaCO_2$ is the partial pressure of carbon dioxide in arterial blood. Ventilation efficiency index (VEI) was calculated as

$$\mathrm{VEI} = 3800 / (\Delta P \times \mathrm{RR} \times P\mathrm{aCO_2})$$

where 3,800 is a constant for production of carbon dioxide (mL mmHg kg^{-1} min^{-1}) and ΔP is the difference between PIP and PEEP (in mmHg).

Cylinder volume remained constant for the duration of the study. Lambs were euthanised with intravenous sodium pentobarbitone (100 mg/kg) at 2 h.

Respiratory mechanics analysis

Cylinder displacement volume, cylinder pressure and airway opening pressure were recorded and used to calculate P_{tr} and volume delivered to the trachea ($V_{T,tr}$) after correction for the physical properties of the circuit and tracheal tube [14]. Respiratory mechanics were determined from standardised volume-controlled breaths (cylinder volume 11 mL/kg, inspiratory time 0.6 s, RR 60 breaths/min) programmed and delivered by the FlexiVent at intervals to coincide with blood gas measurements. Calculated P_{tr} and $V_{T,tr}$ waveforms were analysed using the single-compartment model via multi-linear regression to provide measures of respiratory system resistance (R_{rs}) and elastance (E_{rs}). Dynamic compliance was calculated as $C_{rs} = 1/E_{rs}$.

Post-mortem

The chest was opened, the lung was inflated to 40 cmH_2O and an in situ deflation pressure–volume curve was recorded. Static compliance was calculated from the slope of the deflation pressure–volume curve. Lungs were excised and weighed. A bronchoalveolar lavage (BAL) was performed on the left lung [15] for measurement of protein [16], and quantification of inflammatory and differential cell counts [17]. Right lower lobe lung pieces were immediately snap frozen in liquid nitrogen for later analysis of lung tissue IL-1β mRNA levels by quantitative real-time polymerase chain reaction (qRT-PCR).

Statistics

Outcome variables are expressed as mean ± (SD) unless specified. Comparisons between intervention groups used Student's t test or analysis of variance (ANOVA, parametric) or Mann–Whitney rank sum test (non-parametric) as appropriate, whereas multiple comparisons were performed using two-way ANOVA (Sigmastat^{TM} v3.5, Systat Software, USA). Statistical significance was accepted at $p < 0.05$.

Results

There were no differences between the study groups in the gestation, birth weight, or baseline acid–base status at delivery (Table 1).

The variability of breath-to-breath V_T and RR for the VV group contrasted against the monotonously fixed delivered V_T of 7.7 mL at 50 breaths/min in the CV group (Online Resource 2). Breath-to-breath V_Ts and RR were distributed around the average value in the VV group according to a power law (Online Resource 2). Both groups had a breath-to-breath delivered minute volume of 385 mL/kg/min.

Arterial pH increased throughout the study in the VV group and was higher compared to the CV group at study completion (see Online Resource 2, mean difference 0.11; $p = 0.047$). For the same average delivered $V_{T,tr}$, RR and minute volume, $PaCO_2$ was lower in VV lambs compared to the CV group from 70 min until 2 h (Fig. 1a; median difference at 2 h −18.3 mmHg; $p = 0.026$). VV did not influence PaO_2, ($p = 0.95$; Fig. 1b), FiO_2, OI or aA Ratio (Online Resource 2).

Mean P_{tr} decreased in the VV group between 20 and 120 min (mean difference (95% CI) −2.7 (−3.5, −2.0) cmH_2O; $p < 0.0001$) but remained unchanged in the CV group (see Online Resource 2). Mean pressure was lower in the VV group compared to CV lambs within 25 min of commencing VV and remained significantly lower until study completion ($p = 0.04$, Online Resource 2).

R_{rs} was unchanged between 20 and 120 min in CV and VV groups and did not differ between the groups (Online Resource 2). There was no difference in mean (SD) specific dynamic compliance at baseline (20 min) between VV and CV (Fig. 2a). C_{rs} increased steadily in the VV group (mean difference (95% CI) 0.38 (0.19, 0.56) mL/kg/cmH_2O; $p = 0.001$) whereas C_{rs} remained unchanged in the CV group (mean difference (95% CI) −0.04 (−0.21, 0.13) mL/kg/cmH_2O). Dynamic C_{rs} was significantly higher in the VV group from 70 min until the end of the study (Fig. 2a). Differences in VEI between the two groups were clearly evident from 70 min (higher in VV group) and increased throughout the study (Online Resource 2). Post-mortem static compliance in the collapsed lung was not different between the CV (1.12 (0.29) mL/kg/cmH_2O) and VV (1.23 (0.27) mL/kg/cmH_2O) groups (mean difference (95% CI) 0.04 (−0.30, 0.39) mL/cmH_2O; $p = 0.23$; Fig. 2b).

There were no differences between nonventilated controls and either ventilated group in the protein levels in the BAL fluid (Fig. 3a, $p = 0.10$). The percentage of inflammatory cells (monocytes and neutrophils) compared to total cells in the BAL fluid, and lung tissue IL-1β mRNA expression were increased in both ventilated

Table 1 Baseline characteristics

	NVC	CV	VV
N (male)	9 (5)	7 (4)	9 (5)
Gestation (days)	129 (0.9)	129 (0.9)	129 (0.7)
Birth weight (kg)	3.1 (0.2)	3.1 (0.4)	3.0 (0.3)
Cord pH	7.1 (0.2)	7.1 (0.1)	7.1 (0.1)
Cord $PaCO_2$ (mmHg)	77 (20)	86 (22)	75 (11)
Cord PaO_2 (mmHg)	7 (2)	8 (2)	12 (4)

Values displayed as mean (SD)
NVC nonventilated controls, *CV* conventional ventilation, *VV* variable ventilation

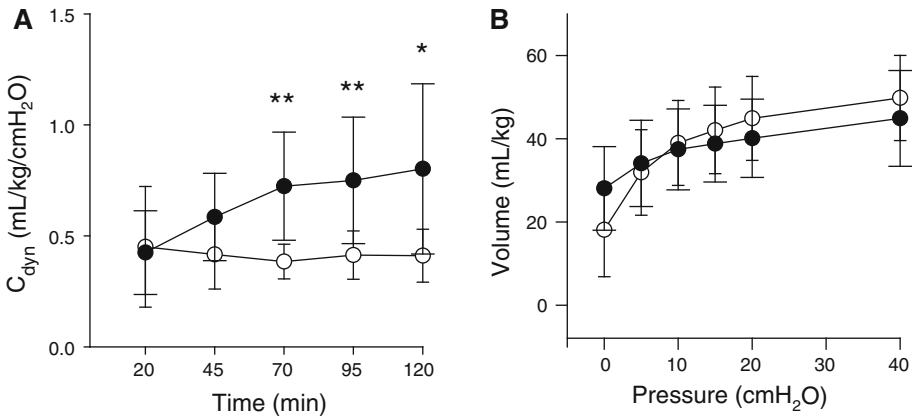

Fig. 1 Gas exchange and arterial oxygenation: plots depict **a** partial pressure of arterial carbon dioxide ($PaCO_2$) and **b** partial pressure of arterial oxygen (PaO_2) in conventional (*open circles*) and variable ventilation (*closed circles*) groups. OI data are displayed as median (25–75th centile). $PaCO_2$ decreased over the duration of the study in the variable ventilation group compared to the conventional ventilation group, despite no difference in average delivered tidal volume, respiratory rate or breath-to-breath minute volume. *$p < 0.05$

groups compared to nonventilated controls ($p < 0.001$ and $p = 0.026$ respectively) but did not differ between VV and CV groups (Online Resource 2 and Fig. 3b respectively).

Discussion

We investigated the physiological and mechanical responses of the immature preterm lung to VV. Compared to ventilation of the preterm lung with fixed V_T and RR, VV with equivalent breath–breath minute volume enhances ventilation efficiency (via more efficient CO_2 removal and reduced ventilator pressures) and improves in vivo lung compliance, while having no effect on arterial oxygenation.

Despite initial transition and stabilisation on controlled ventilation, all lambs had comparable gas exchange and mechanical properties of the lung at 20 min of life when baseline measurements were obtained. Physiological differences occurring subsequently were due to the allocated ventilation modality (CV or VV) commenced at 20 min.

The steadily improving arterial pH and CO_2 levels in the VV group compared to the CV group achieved at lower P_{tr} are an important outcome in this preterm model. Lower arterial CO_2 values were shown in some adult animal model VV studies [5, 9, 13] but not others [10, 18]. Enhanced efficiency of CO_2 clearance with VV was linked previously to reduced dead space ventilation [9]. Variable ventilation may enhance ventilation/perfusion matching with a redistribution of pulmonary blood flow to the caudal zones compared to ventilation using the Acute Respiratory Distress Syndrome (ARDS) Network protocol, to peripheral zones compared with the open lung approach [18]. Redistribution of pulmonary blood flow was also observed with noisy pressure support ventilation compared to pressure control ventilation, and routine pressure support ventilation [19]. Likewise, we showed previously that a noisy pressure waveform arising from bubble continuous positive airway pressure increases peripheral airway patency and decreases the lung clearance index in preterm lambs [20], suggesting that variability reduces ventilation inhomogeneity and increases gas mixing efficiency in the preterm model.

Our observation of steady improvement in dynamic lung compliance and associated reduced ventilatory pressures is also a feature of VV in adult lung models. Given the ventilation efficiency index increased in the VV group before statistical differences in CO_2 clearance were evident, improved lung compliance (and accompanying reduced ventilator pressures) contributed to the improved ventilator efficiency and a physiological benefit of VV in this preterm lamb model. The comparable total static compliance and end expiratory lung volume between groups in the totally collapsed post-mortem in situ lung make differences in physical deformation, alveolar edema, or proteinaceous exudates unlikely to explain the improved in vivo dynamic compliance. Enhanced lung compliance with VV may also result from increased surfactant secretion and ventilation homogeneity [8, 21]. Surfactant secretion was not compared between the two lamb groups as all ventilated lambs received prophylactic surfactant at delivery to ensure survival over the 2-h study period. Furthermore, the endogenous surfactant pool in naïve lambs at this gestation is very low in the hours immediately after delivery [15]. These factors, plus the lack of evidence of difference in pressure–volume curves obtained from collapsed lungs post-mortem make it

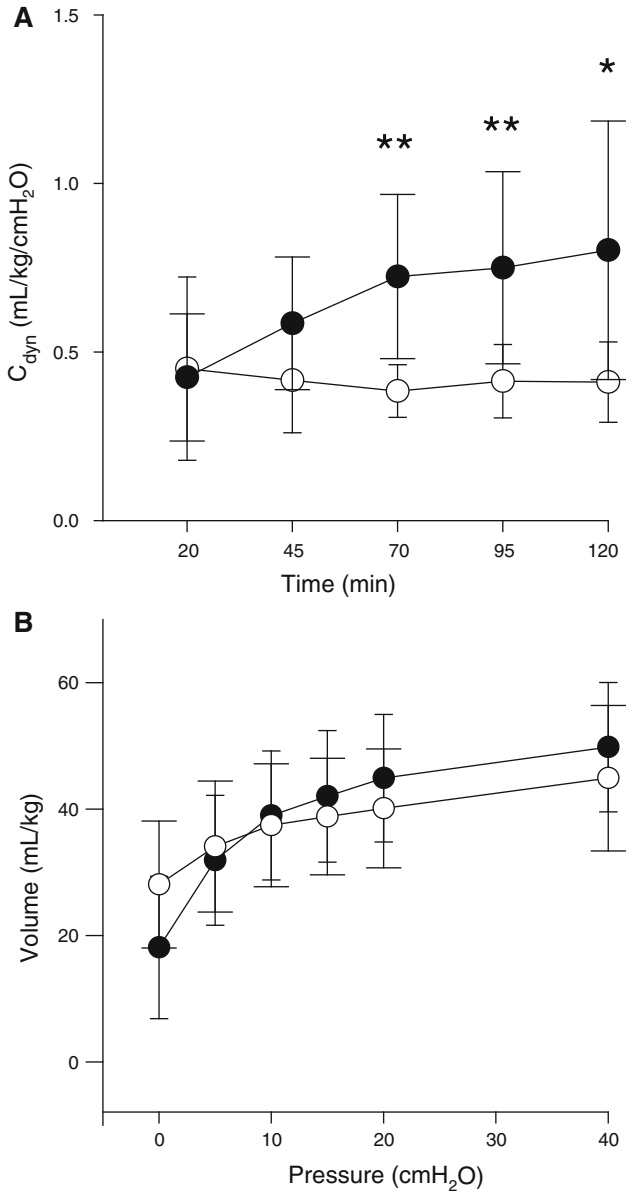

Fig. 2 Lung mechanics. Plots depict **a** dynamic compliance (C_{dyn}) and **b** in situ deflation pressure–volume curve obtained at post-mortem after inflating collapsed lungs. Symbols represent control (*open circles*) and variable ventilation (*closed circles*) groups. C_{dyn} over the duration of the study in the variable ventilation group compared to the conventional ventilation group. There were no statistically significant differences between groups in the static specific compliance, or the stability of the lung at atmospheric pressure as determined from the deflation pressure–volume curve. *$p < 0.05$; **$p < 0.01$

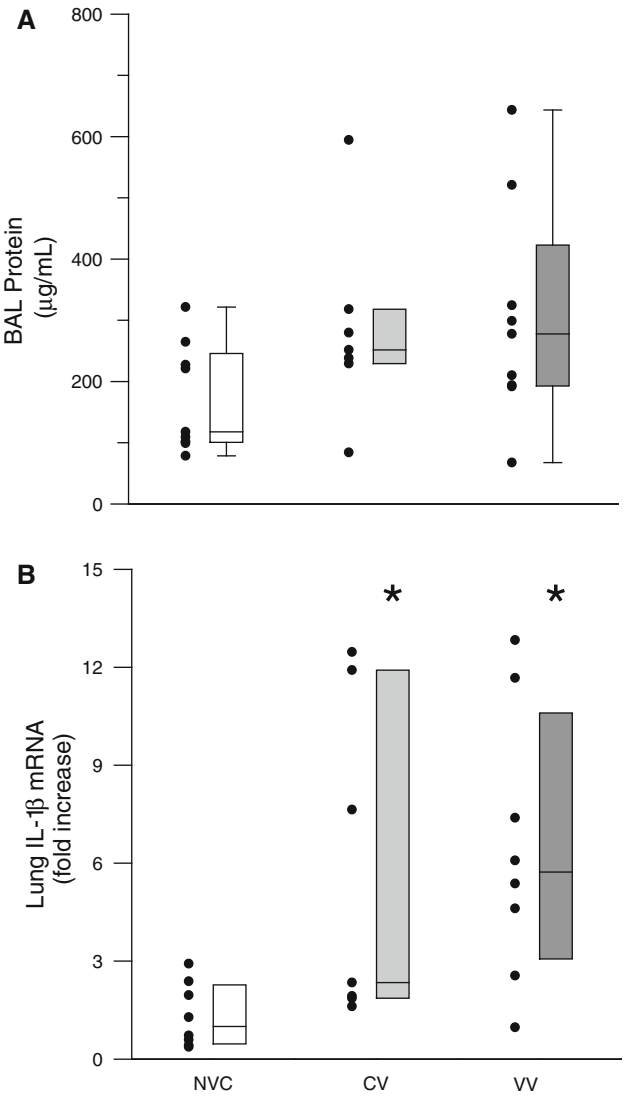

Fig. 3 Markers of inflammation. Figure shows individual data points and box plots for **a** bronchoalveolar lavage (BAL) protein and **b** lung tissue IL-1β mRNA (fold increase) in nonventilated (NVC—*white bar*), control ventilation (CV—*light grey bar*) and variable ventilation (VV—*dark grey bar*) lamb groups. The lung tissue IL-1β mRNA expression was higher in ventilated compared to nonventilated groups, but no differences were apparent between control and variable ventilation groups. *$p < 0.05$

unlikely that differences in surfactant secretion played more than a minor role in volume recruitment. Likewise, whilst VV has been associated with reduced pulmonary artery pressures, this would normally be associated with increased pulmonary blood flows and reduced compliance. Thus, we believe that enhanced lung volume recruitment is the most probable explanation for increased dynamic lung compliance [22].

Lack of a parallel improvement in arterial oxygenation in preterm lambs contrasts starkly with findings from VV in adult animals with either saline lavage [18, 23] or oleic acid injury models [5, 12]. Whereas atelectasis is a prominent feature of both adult and preterm models of RDS, arterial oxygenation in the transitional premature lung is influenced by factors other than lung volume. At 129 days gestation, the ovine lung is in the late saccular/early alveolar phase of development [24] with an

increased alveolar-capillary membrane thickness. The resultant impaired diffusion capacity and limited oxygen transfer may be compounded by a reduced time for equilibration because of a high heart rate and rapid transit of blood through the lung. Such diffusion impairment would affect CO_2 removal to a much lesser extent than O_2 as a result of the high solubility of CO_2 in blood and tissue, and rapid equilibration of CO_2 between pulmonary capillaries and the alveolus. Furthermore, the poor response to increased FiO_2 in our lambs (data not shown) suggests that intrapulmonary and/or right to left extrapulmonary shunts contributed to the arterial hypoxemia. We did not measure extrapulmonary shunting but it is common in the newborn as a result of elevated pulmonary artery pressures and persistence of right to left shunting across the fetal vascular channels. Our measurements of PaO_2 are postductal (via catheters inserted through the umbilical artery) and may be influenced by right to left shunting through the ductus arteriosus or foramen ovale. The gradual decline in aA ratio highlights the moderately severe respiratory disease evident in both ventilated groups whereas the elevated markers of injury highlight the susceptibility of the fragile preterm lung to mechanical ventilation.

The improved arterial oxygenation and/or lung compliance [5, 8–13], and delivery of required V_Ts at lower mean airway pressure associated with use of VV compared to controlled mechanical ventilation [10] and the ARDS Network protocol [18] are partly due to stochastic processes that enhance airway opening and alveolar recruitment [7]. Given that airway branching is complete at 129 days gestation, recruitment in the preterm lamb lungs would most likely occur in a similar manner to that shown in adult models. However, incomplete alveolar development results in a lower total lung capacity and a higher V_T to functional residual capacity ratio. Therefore, the amount of variability in the ventilation pattern, or the magnitude and nature of the benefit observed may be different in the preterm to the mature lung.

Whereas some studies show decreased lung injury associated with VV [6, 8, 18, 25] or variable pressure support ventilation [26], others have demonstrated no benefit [27, 28]. In our limited assessments of lung injury markers, we found that any ventilation generated injury compared to the NVC group but saw no evidence of increased protein or inflammatory cells in the BAL, or in expression of the cytokine IL-1β mRNA between the ventilated groups. Absence of differences in lung injury may be explained by study design: we targeted physiological outcomes for gas exchange and lung mechanics between VV and fixed rate and volume CV by maintaining the same breath-to-breath minute volume for each group. Ventilation was not adjusted to achieve target $PaCO_2$ values, and hence lung injury was not a primary study outcome. Our CV group had moderately high $PaCO_2$ levels indicating hypoventilation. Targeting of ventilation to achieve normocapnea or permissive hypercapnea would have required higher V_T and potentially more evidence of injury in the CV group. Further studies targeting the effect of VV on injury will require an experimental protocol that targets comparable and clinically acceptable $PaCO_2$ range rather than comparable minute volume.

Volutrauma is a major contributor to ventilation-induced injury in the preterm lung [29]. We used a V_T distribution similar to that used successfully in injured mice [6] with a maximum delivered V_T of 17.3 mL/kg (2.25 times the average delivered V_T of 7.7 mL/kg). Whilst we did not record actual breath-to-breath delivered tidal volumes, there was a constant relationship between programmed and delivered tidal volume throughout the study; hence, we do not expect that our tidal volume magnitude or distribution changed from the programmed tidal volume ventilation. The most advantageous level of variability in V_T and RR in the preterm lung remains unknown. Preterm lungs have a substantially lower total lung capacity compared to adult lungs as a result of incomplete alveolar development and are susceptible to injury arising from large fluctuations in volume delivery. As few as five consecutive large consecutive tidal volume breaths immediately after birth causes injury in the newborn lung [30]. We have shown previously that IL-1β is markedly increased after as little as 15 min exposure to delivered tidal volumes of up to 15 mL/kg (i.e. twice the average V_T used) [30]. The absence of differences in injury markers between VV and CV suggests that the pattern and frequency of large tidal volume delivery (consecutive or distributed) may be an important feature of volutrauma in the preterm lung. Further studies comparing VV to CV need to assess different levels of V_T variability.

An important consideration in interpretation of these results is the relatively brief (2 h) ventilation period. With current clinical trends towards early extubation to non-invasive ventilation strategies, brief studies retain clinical relevance to study physiological consequences of different strategies during initiation of ventilation. Nonetheless, the potential long-term benefits of including variability in the ventilatory pattern used for the preterm infant remain unknown.

Conclusions

In a ventilated preterm lamb, VV improves lung compliance and ventilation efficiency compared to monotonous fixed volume-frequency ventilation. Contrary to previous studies in adult animal models, we observed no beneficial effects of VV on arterial oxygenation. A comparison of CV and VV with ventilation adjusted to target a clinical $PaCO_2$ range is needed to

ascertain if VV protects the preterm lung from ventilation-induced injury.

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