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RESEARCH ARTICLE

Improving lung aeration in ventilated newborn preterm rabbits with a partially aerated lung

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Pryor EJ, Kitchen MJ, Croughan MK, Crossley KJ, Wallace MJ, Lee K, te Pas AB, McGillick EV, Hooper SB. Improving lung aeration in ventilated newborn preterm rabbits with a partially aerated lung. *J Appl Physiol* 129: 891–900, 2020. First published August 20, 2020; doi:10.1152/japplphysiol.00426.2020.—Preterm newborns commonly receive intermittent positive pressure ventilation (iPPV) at birth, but the optimal approach that facilitates uniform lung aeration is unknown, particularly in a partially aerated lung. As both inflation time and exogenous surfactant facilitate uniform lung aeration, we investigated whether they can improve lung aeration and lung mechanics in a partially aerated lung immediately after birth. Preterm rabbit kittens (29 days of gestation, term ~32 days) were delivered by caesarean section and partial lung aeration was created by intubating and mechanically ventilating the right lung. The tube was then withdrawn to ventilate both lungs using inflation times of 0.2 s or 1.0 s, with or without exogenous surfactant (200 mg/kg; Curosurf) and a tidal volume (Vt) of 8 mL/kg. Simultaneous phase contrast X-ray imaging and plethysmography were used to measure lung aeration and mechanics. Kittens ventilated with longer inflation times (1.0 s) reached their target Vt with fewer inflations, required lower inflation pressures (28.5 ± 1.1 vs. 33.5 ± 1.3 cmH_2O, $P = 0.01$) and had higher dynamic lung compliances (0.54 ± 0.3 vs. 0.40 ± 0.3 $cmH_2O{\cdot}mL^{-1}{\cdot}kg^{-1}$, $P = 0.003$). Surfactant increased functional residual capacity (FRC; 31.9 ± 3.2 vs. 18.0 ± 3.9 mL/kg, $P = 0.02$) and the proportion of the Vt entering the previously unaerated lung but had no effect on dynamic lung compliance. Combining early surfactant treatment with longer inflation times increases FRC levels, improves dynamic lung compliance, reduces inflation pressures and markedly increases the proportion of the lungs being ventilated during iPPV in preterm kittens with a partially aerated lung.

NEW & NOTEWORTHY Preterm newborns commonly receive intermittent positive pressure ventilation (iPPV) at birth, but the optimal approach that facilitates uniform lung aeration is unknown, particularly in a partially aerated lung. Using phase contrast X-ray imaging, we showed that combining a long inflation time (1.0 s) with surfactant improved lung mechanics and aeration in the immediate newborn period. The current clinical practice of using short inflation times during iPPV might be suboptimal and a different approach is needed.

airway liquid clearance; lung aeration; preterm birth; respiratory support; surfactant

INTRODUCTION

Nearly one-fifth of all babies born in Australia require some form of assistance at birth to transition to newborn life (3). Approximately one-third of these newborns receive intermittent positive pressure ventilation (iPPV) applied via a face mask, and a further 4% receive intubation and mechanical ventilation (3). The majority of newborns requiring respiratory support at birth are born preterm (<37 wk gestation), whereas the majority of newborns receiving more invasive interventions, such as intubation and mechanical ventilation, are born extremely preterm (<28 wk) (5). Overall, nearly 90% of babies born extremely preterm require some form of assisted ventilation in the delivery room (1).

The clearance of airway liquid is critical for the transition to newborn life at birth, as it triggers the cardiovascular transition and the onset of pulmonary gas exchange by allowing air to enter the distal regions of the lung (7, 12). As such, lung aeration and the onset of pulmonary ventilation is the cornerstone of neonatal resuscitation. Although term newborns can rapidly aerate their entire lung, aeration in extremely preterm newborns is often delayed and restricted to isolated lung regions (20). As a result, ventilation of the lung is nonuniform. This reduces the lung's gas exchange potential, necessitates the use of high inspired oxygen levels and greatly increases the risk of injury to the immature lung (26). However, it is currently unclear why the immature lung does not aerate uniformly or what approaches can be used to subsequently increase the amount of lung that has aerated in very preterm newborns.

After birth, lung aeration results from pressure gradients generated by inspiration or artificial inflations, which drive movement of the air-liquid interface through the airways into the distal gas exchange regions of the lung (6, 22). Currently, it is unclear why the air-liquid interface preferentially enters some airways during lung aeration but not others, although surface tension is thought to be a major contributing factor (20). Nevertheless, in a partially aerated lung, the very large (100-fold) difference in resistance between liquid- and air-filled airways restricts air entry into air-filled lung regions during tidal ventilation. As a result, ongoing tidal ventilation is unlikely to further aerate unaerated lung regions. Imaging studies have shown that the uniformity of lung aeration is greatly improved by pro-

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longed (sustained) inflations or surfactant, when given with the first inflation in preterm newborns (20, 25). However, it is unclear whether longer inflation times and/or surfactant can improve lung aeration during ongoing iPPV in a lung that is already partially aerated.

In this study, we aimed to determine the effect of inflation time, with and without surfactant, on lung mechanics and the uniformity of lung aeration in a partially aerated lung during iPPV in preterm newborn rabbit kittens. We hypothesized that following partial lung aeration, lung mechanics and the proportion of the lung being ventilated will increase with longer inflation times, leading to an increase in functional residual capacity (FRC), and that surfactant will enhance this effect. We also hypothesized that, as surfactant acts to increase lung compliance, it may reduce expiratory gas flows and increase expiratory times.

METHODS

Animal Ethics

All experimental procedures were approved by both the Monash University's Animal Ethics Committee and the Australian Synchrotron Animal Ethics Committee and conducted in accordance with the National Health and Medical Research Council (NHMRC) Australian code of practice for the care and use of animals for scientific purposes (15). All studies were conducted in experimental hutch 3 of the Imaging and Medical Beamline at the Australian Synchrotron.

Experimental Procedure

Pregnant preterm New Zealand white rabbit kittens (29 days of gestational age, term ~32 days) were anaesthetized using propofol (8 mg/kg iv bolus, followed by 40–100 $mg \cdot kg^{-1} \cdot h^{-1}$, Rapinovet, Merck Animal Health) and intubated, and then anesthesia was maintained using inhaled isoflurane (1.5–4%; Isoflurane, DS Pharma Animal Health) as previously described (14). Kittens were exteriorized by caesarean section and anaesthetized (sodium pentobarbitone, Jurox New South Wales, Australia; 3 mg/kg via intraperitoneal injection), and an endotracheal tube (18 G intracath; BD Australia) was inserted into the right main bronchus via a tracheostomy immediately before the kittens were delivered.

Preexperimental period. Following delivery, kittens ($n = 28$) were placed, head-out, in a warm (39°C), water-filled plethysmograph located within the synchrotron's experimental hutch; the volume of water displaced from the chamber equals the volume of air entering the lung (8). The endotracheal tube was connected to a purpose-built pressure-limited ventilator, which triggered the onset of imaging (14). The correct positioning of the endotracheal tube (within the right main bronchus) was verified by imaging before the preexperimental ventilation period commenced. Kittens were imaged and mechanically ventilated throughout both the preexperimental and experimental periods. During the preexperimental ventilation period, ventilation commenced with a peak inflation pressure (PIP) of 30 cmH_2O, a positive end-expiratory pressure (PEEP) of 5 cmH_2O, and inflation (Ti) and expiratory (Te) times of 0.5 s. The PIP was adjusted to facilitate partial lung aeration and to achieve a stable tidal volume (Vt) of 5 mL/kg for ~30 s. This preexperimental, unilateral ventilation protocol resulted in partial aeration of the right lung, usually the basal right lobe, thereby creating a model of nonuniform lung aeration that was relatively consistent between animals (Fig. 1). At the end of the preexperimental ventilation period, imaging was paused and the endotracheal tube was retracted until the tip was proximal to the bifurcation of the trachea. The position was verified by imaging and allowed both lungs to be ventilated during the subsequent experimental period.

Experimental period. During the experimental period, we examined the effect of different inflation times and surfactant administration on lung mechanics and aeration in a partially aerated lung. Kittens were randomly assigned to receive ventilation with a Ti of 0.2 s ($n = 7$) or 1.0 s ($n = 8$) without surfactant, or with a short Ti of 0.2 s ($n = 6$) or a long Ti of 1.0 s ($n = 7$) with exogenous surfactant (200 mg/kg Curosurf, Chiesi Farmaceutici). In kittens receiving surfactant, the surfactant was administered via the endotracheal tube following its withdrawal at the end of the preexperimental ventilation period but before ventilation had recommenced. At the start of the experimental period, imaging recommenced before the onset of ventilation to ensure correct placement of the tube within the trachea. Ventilation commenced using the same PIP and PEEP that was used at the end of the preexperimental period for each kitten. The Te was set at 0.5 s for all groups. During the experimental period, the PIP was manually adjusted for each kitten until a stable Vt of 8 mL/kg was attained for at least 2 min.

Throughout both the preexperimental and experimental ventilation periods, kittens were imaged and physiological measurements of airway pressure and lung gas volumes were recorded digitally using PowerLab (ADInstruments, Sydney, Australia). At the conclusion of the experiment, all rabbits were killed with an overdose of Sodium thiopentobarbitone (Lethobarb >100 mg/kg, Virbac Australia, administered via intraperitoneal injection).

Phase Contrast X-ray Imaging

Phase contrast X-ray (PCX) images of the entire chest of preterm kittens were acquired as previously described, using a synchrotron source tuned to 24 keV (11, 24). PCX imaging provides clear and detailed images of the kitten lungs, including individual alveoli, which allow for accurate calculation of regional lung volumes (10). Experiments were performed at the Imaging and Medical Beamline (IMBL) of the Australian Synchrotron. The X-ray source-to-sample distance was ~135 m and the sample-to-detector distance was 1.5 m. Two detectors were used, one for each of the two beamtimes required to complete the experiment. The first was the IMBL's "Ruby" detector, and the second was our custom-designed detector. Both use a PCO.edge scientific CMOS CIS2051 camera coupled to 25-μm thick gadolinium oxysulfide (Gd2O2S:Tb+) powdered phosphor. The effective pixel sizes employed were 15.2 μm (Ruby) and 15.8 μm (custom detector), respectively, with a field-of-view of 39 (width) × 33 (height) mm^2 and 40.5 (width) × 34 (height) mm^2, respectively. Images were acquired using an exposure time of 30 ms and a frame rate of 10 Hz. At the conclusion of each imaging sequence, flat-field and dark-field images were acquired to correct for variations in beam intensity and detector dark current signal.

Image Analysis

Lung gas volumes were measured using previously described methods (10, 11, 21). Images of the chest were divided in half, using the center of the vertebral column as the boundary between the left and right lungs as previously described (10). Left and right lung volumes at FRC and peak inflation were calculated, and regional tidal volumes were calculated by subtracting the FRC from the peak inflation volume within each lung (Fig. 1).

Analysis of Physiological Parameters

The preexperimental and experimental ventilation periods were analyzed separately using LabChart (ADInstruments, Sydney, Australia). Measurements collected during the preexperimental period were used to demonstrate that physiological parameters were similar between groups before commencing the experimental period. For both preexperimental and experimental periods, each individual inflation was analyzed to measure PIP, PEEP, mean airway pressure, FRC, Vt, and dynamic lung compliance [Vt/(PIP − PEEP)]. The inflation-by-

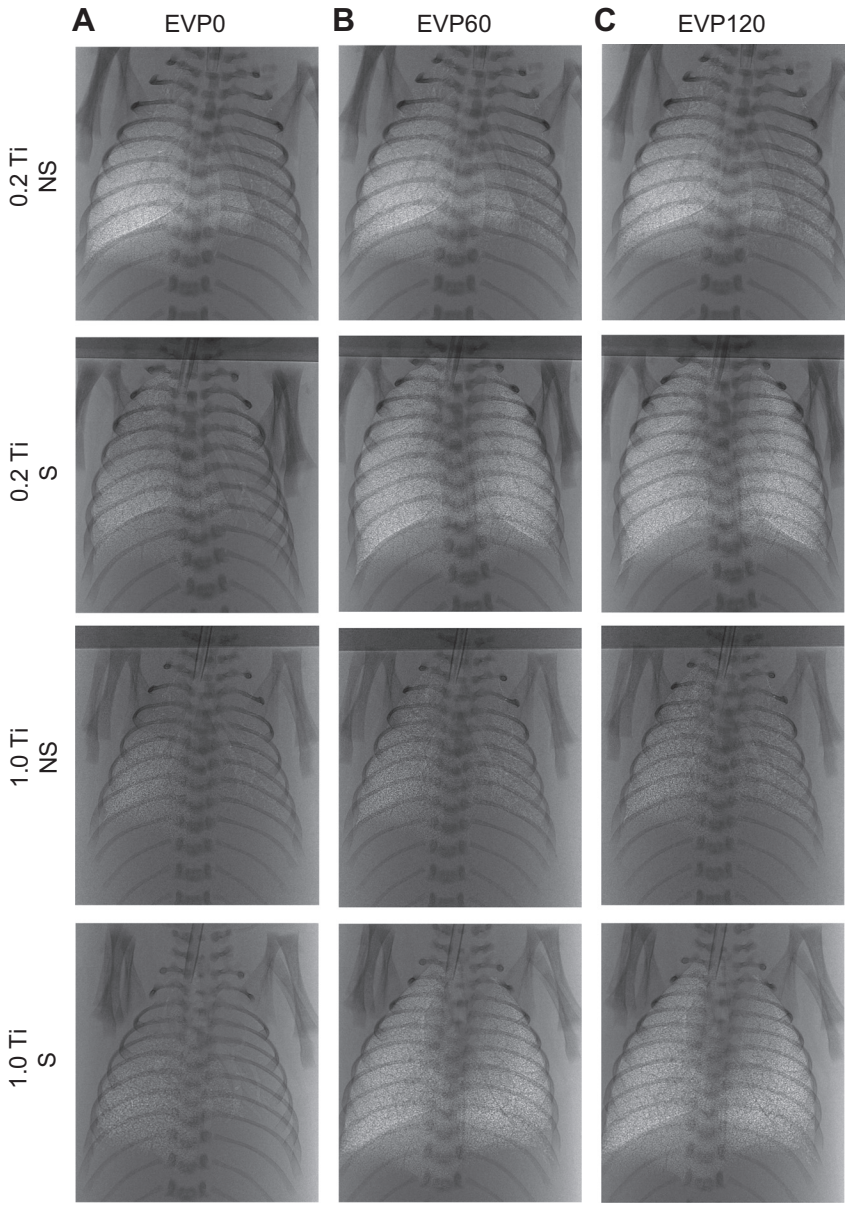


Fig. 1. Phase contrast X-ray images of preterm newborn rabbit kittens indicating the degree of lung aeration (white speckle pattern) in each group during the experimental ventilation period (EVP). *Top*: kittens ventilated with an inflation time (Ti) of 0.2 s with surfactant (S) or no surfactant (NS). *Bottom*: kittens ventilated with a 1.0-s Ti NS or S. Images were taken at the beginning of the EVP (EVP0; *A*) and at 60 s (EVP60; *B*) and 120 s (EVP120; *C*) after the start of the experimental ventilation period. At the beginning of the EVP, only the right lung was aerated.

inflation data were used to determine maximum values and the average values across five breaths at specific time periods throughout the experimental period (30 s, 60 s, 90 s, and 120 s after the onset of ventilation). We also measured peak inflation flows, expiratory flows, expiratory time constants (E_{TC}), and the ratio of the inflation time constant (I_{TC}) divided by the expiratory time constant during the first five breaths, when the target Vt was achieved, and during the last five breaths. These points were selected to ensure that the expired volumes were consistent between the groups, as these factors are tidal volume dependent. The I_{TC} and E_{TC} were calculated as the time taken to inspire or expire 95% of the total volume (respectively) for each breath.

The cumulative area under the airway pressure curve (pressure-time integral; PTI) was calculated to measure the cumulative pressure applied to the airways from the onset of iPPV during the experimental ventilation period. This was calculated by multiplying the mean airway pressure by the duration of the breath. The cumulative sum was then calculated to determine the total amount of pressure applied at any point during the experimental period to determine whether any differences between groups with different inflation times was simply due to the application of higher pressures for longer.

Statistical Analysis

All statistical analysis was undertaken in GraphPad Prism (Prism 8, GraphPad Software, Inc.), and a significance level of $P \leq 0.05$ was considered statistically significant. Data were tested for normality using a Shapiro Wilk test. Baseline data collected at the end of the preexperimental period and measurements at static time points were compared using a two-way analysis of variance (ANOVA). If an interaction factor was statistically significant, a Sidak's multiple comparisons test was performed. Measurements sampled at multiple time points were compared using a three-way ANOVA, or a mixed-effects ANOVA if values were missing for a kitten at any time points. If the main effect for a factor (either surfactant or Ti) was statistically significant, data were split based on that factor and a two-way repeated measures ANOVA was performed to identify any time-related differences between groups for the other factor. All data are presented as means ± SE.

RESULTS

Preexperimental Ventilation Characteristics

All four groups of kittens had similar preexperimental characteristics before commencing the experimental period. There were no significant differences in birth weight, the FRC achieved, or the maximum PIP required to achieve the set Vt at the end of the preexperimental ventilation period (Table 1).

Experimental Ventilation Period

Lung mechanics. Kittens ventilated with a Ti of 1.0 s recruited their target Vt (8 mL/kg) approximately eight times faster ($P = 0.001$), with significantly fewer inflations ($P = 0.0005$; Fig. 2*A*) and a lower PIP ($P = 0.01$; Fig. 2*B*), compared with those ventilated with a Ti of 0.2 s. There was no effect of surfactant on the number of inflations or PIP required to recruit Vt at either Ti (Fig. 2).

Kittens ventilated with a Ti of 1.0 s achieved a higher maximum dynamic lung compliance during the experimental ventilation period ($P = 0.003$; Fig. 3*A*) in significantly less time (1.0 s Ti kittens, 195.8 ± 33.1 s vs. 0.2 s Ti kittens, 336.7 ± 56.1 s; $P = 0.04$) and with significantly fewer inflations (1.0 s Ti kittens, 129 ± 22 inflations vs. 0.2 s Ti kittens, 482 ± 80 inflations; $P = 0.0002$). Interestingly, the temporal increase in dynamic lung compliance was very similar between groups over time, with all groups increasing in parallel (Fig. 3*B*). The primary difference between the groups was a ~0.2 $mL \cdot cmH_2O^{-1} \cdot kg^{-1}$ difference in dynamic lung compliance between kittens depending on whether they were ventilated with a Ti of either 1.0 s or 0.2 s (Fig. 3*B*). Exogenous surfactant did not affect the maximum lung compliance reached ($P = 0.86$; Fig. 3*A*) or the time ($P = 0.37$) or number of inflations ($P = 0.45$) required to reach maximum lung compliance. Similarly, neither Ti nor surfactant administration affected the expiratory time constant (95% of expired volume; Fig. 4*A*), although peak expiratory flow rates were increased with a longer Ti (64.6 ± 4.0 vs. 49.3 ± 2.8 $mL \cdot kg^{-1} \cdot s^{-1}$; $P = 0.004$). The ratio of I_{TC} to E_{TC} (I_{TC}/E_{TC} ratio; ratio of the time taken to reach 95% of the inspired volume versus time taken to reach 95% of the expired volume) was higher in kittens ventilated with a longer Ti, and surfactant administration did not influence this (Fig. 4, *A* and *B*).

The PTI, which is the cumulative area under the airway pressure curve (Fig. 4*C*), increased significantly faster in kittens ventilated with a 1.0-s Ti than kittens ventilated with a 0.2 s Ti (Fig. 5*A*; $P < 0.0001$). Surfactant had no effect on the rate of increase in PTI (Fig. 5*A*; $P = 0.14$). This was expected, as the PIP was held for a longer period of time in 1.0 s Ti kittens (Fig. 4*C*). To determine whether a faster increase in the PTI could explain a more rapid increase in lung compliance, we calculated the dynamic lung compliance at specific PTI levels. We found that for any given PTI, kittens ventilated with a 1.0 s Ti had a significantly higher dynamic lung compliance than kittens ventilated with a 0.2 s Ti (Fig. 5*B*). Surfactant had no effect on the dynamic lung compliance, with increasing PTI in kittens ventilated with a Ti of either 0.2 s ($P = 0.48$) or 1.0 s ($P = 0.24$; Fig. 5*B*).

FRC and Distribution of Ventilation

The supplemental movies demonstrate the effects of Ti and surfactant on lung aeration during the experimental ventilation period.

Supplemental Movie S1 (see https://doi.org/10.6084/m9.figshare.12673634) demonstrates that in the absence of surfactant, ventilation with a 0.2 s Ti resulted in little or no aeration of the previously unaerated lung. Instead, the majority of incoming air mostly enters the previously aerated lung.

Supplemental Movie S2 (see https://doi.org/10.6084/m9.figshare.12673652) demonstrates that in the presence of surfactant, ventilation with a 0.2 s Ti resulted in rapid aeration of the previously unaerated lung, which is in contrast to Supplemental Movie S1. Initially, surfactant blocked the airways but was rapidly cleared.

Supplemental Movie S3 (see https://doi.org/10.6084/m9.figshare.12673688) demonstrates that in the absence of surfactant, ventilation with a 1.0 s Ti also resulted in little or no aeration of the unaerated lung. During each inflation, although more incoming air appears to be distributed to the previously unaerated lung, this was not significant.

Supplemental Movie S4 (see https://doi.org/10.6084/m9.figshare.12673694) demonstrates that in the presence of surfactant, ventilation with a 1.0 s Ti resulted in rapid aeration of the previously unaerated lung, as per Supplemental Movie S2.

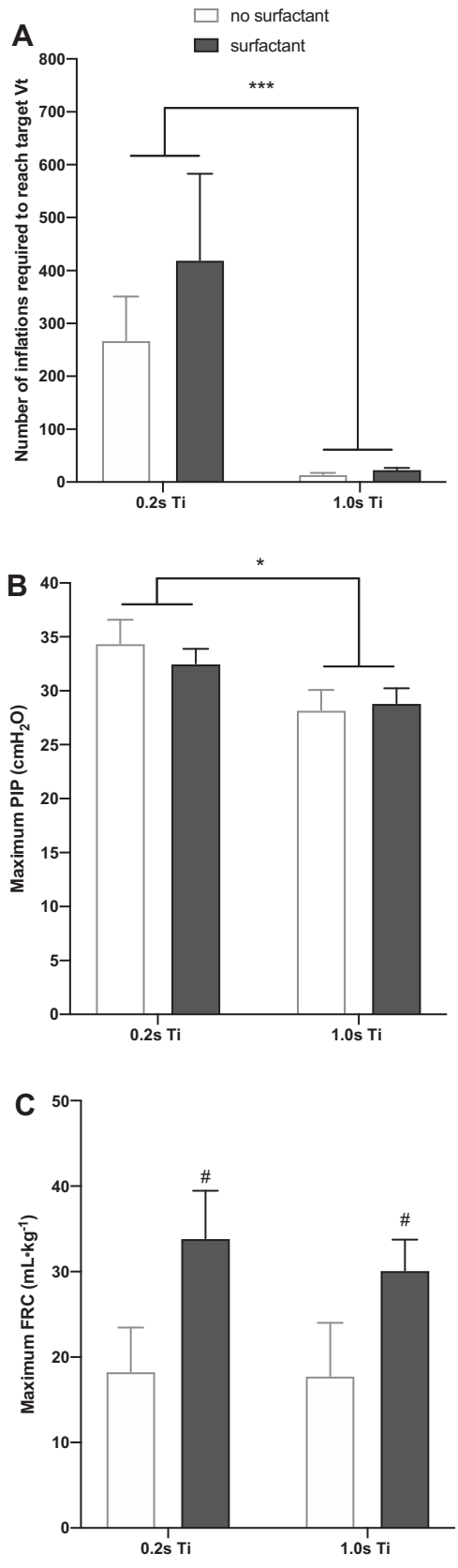
The maximum FRC achieved was similar in kittens ventilated with a Ti of 1.0 s and 0.2 s ($P = 0.69$; Fig. 2*C*). However, increasing the Ti significantly reduced the time taken to reach each stage of FRC recruitment ($P = 0.01$; Fig. 6*A*). Surfactant administration significantly increased the maximum FRC ($P = 0.02$; Fig. 2*C*), although kittens with surfactant ventilated with a 1.0 s Ti took longer to reach each stage of FRC recruitment than those without surfactant ($P = 0.03$; Fig. 6*A*).

Surfactant administration significantly increased FRC levels in the previously unaerated lung irrespective of whether the kittens were ventilated with a Ti of 1.0 s or 0.2 s (Fig. 6*B*). However, the increase in FRC in the previously unaerated lung was greater in kittens being ventilated with a Ti of 1.0 s, particularly during the early stages of bilateral ventilation (30 s, $P = 0.04$; 60 s, $P = 0.06$; 90 s, $P = 0.06$; 120 s, $P = 0.08$; Fig. 6*B*). Similarly, surfactant administration signifi-

Table 1. *Characteristics of rabbit kittens in each group at the end of the preexperimental ventilation period*

	0.2-s Ti, No Surfactant	1.0-s Ti, No Surfactant	0.2-s Ti, Surfactant	1.0-s Ti, Surfactant
Birth weight, g	31.3 ± 1.8	29.0 ± 1.5	32.7 ± 1.3	29.8 ± 1.4
No. of inflations	127 ± 15	130 ± 8	155 ± 13	153 ± 20
Vt, mL/kg	5.4 ± 0.2	5.9 ± 0.5	5.5 ± 0.2	4.9 ± 0.4
FRC, mL/kg	8.0 ± 2.9	8.9 ± 3.1	12.7 ± 5.4	9.2 ± 3.6

At the end of the preexperimental ventilation period, only the right lung was aerated. $P < 0.05$ considered statistically significant. FRC, functional residual capacity; Ti, inflation time; Vt, tidal volume.



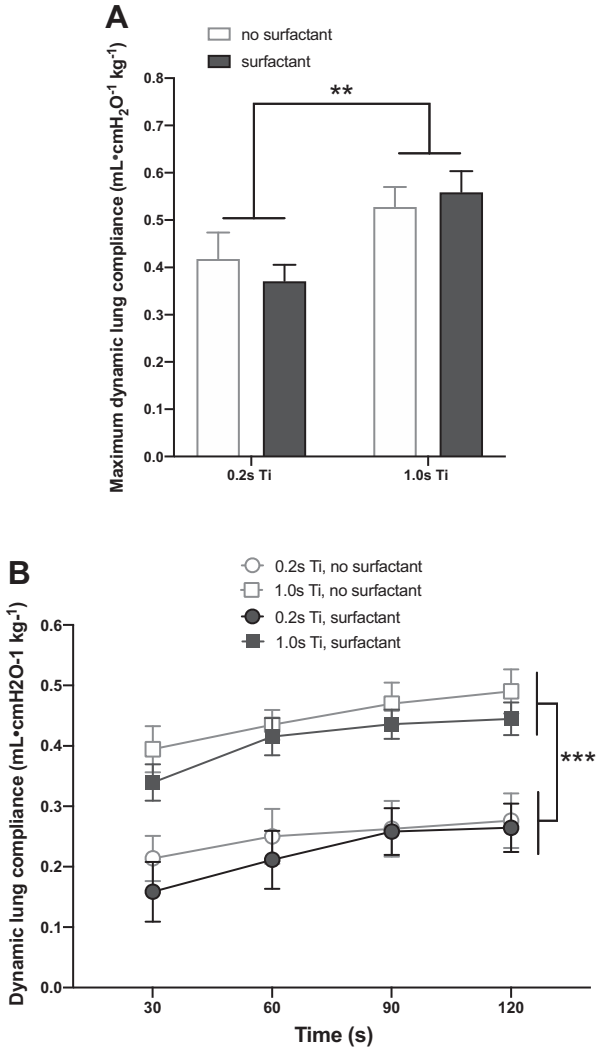


Fig. 3. Maximum dynamic lung compliance (*A*; mL·cmH_2O^{-1}·kg^{-1}) achieved and the time-dependent increase in dynamic lung compliance (*B*) during the experimental ventilation period in preterm newborn kittens. Kittens were ventilated with inflation times (Tis) of 0.2 (*A*, *left*; *B*, circles) or 1.0 s (*A*, *right*; *B*, squares), with (*A*, closed bars; *B*, closed symbols) and without (*A*, open bars: *B*, open symbols) surfactant. Values are means ± SE; **main effect of Ti, $P < 0.01$; ***main effect of Ti, $P < 0.001$.

cantly increased ventilation in the previously unaerated regions, as indicated by an increase in the proportion of incoming Vt entering the previously uninflated lung (Fig. 6*C*). However, there was no effect of Ti on ventilation in the previously uninflated lung (Fig. 6*C*).

The Vt entering the previously inflated lung did not exceed 7 mL/kg in any animal and remained in the ideal range (3–5 mL/kg) in the majority of kittens (Fig. 7*A*). Furthermore, neither surfactant administration nor Ti significantly influenced

Fig. 2. The number of inflations (*A*) and the maximum peak inspiratory pressure (PIP; cmH_2O) (*B*) required to achieve the target tidal volume (Vt) during the experimental ventilation period and the maximum functional residual capacity (FRC; mL/kg) obtained (*C*) in kittens ventilated with inflation times (Tis) of 0.2 s or 1.0 s, with and without surfactant. Values are means ± SE; *main effect of Ti, $P < 0.05$; ***main effect of Ti, $P < 0.001$; #main effect of surfactant, $P < 0.05$.

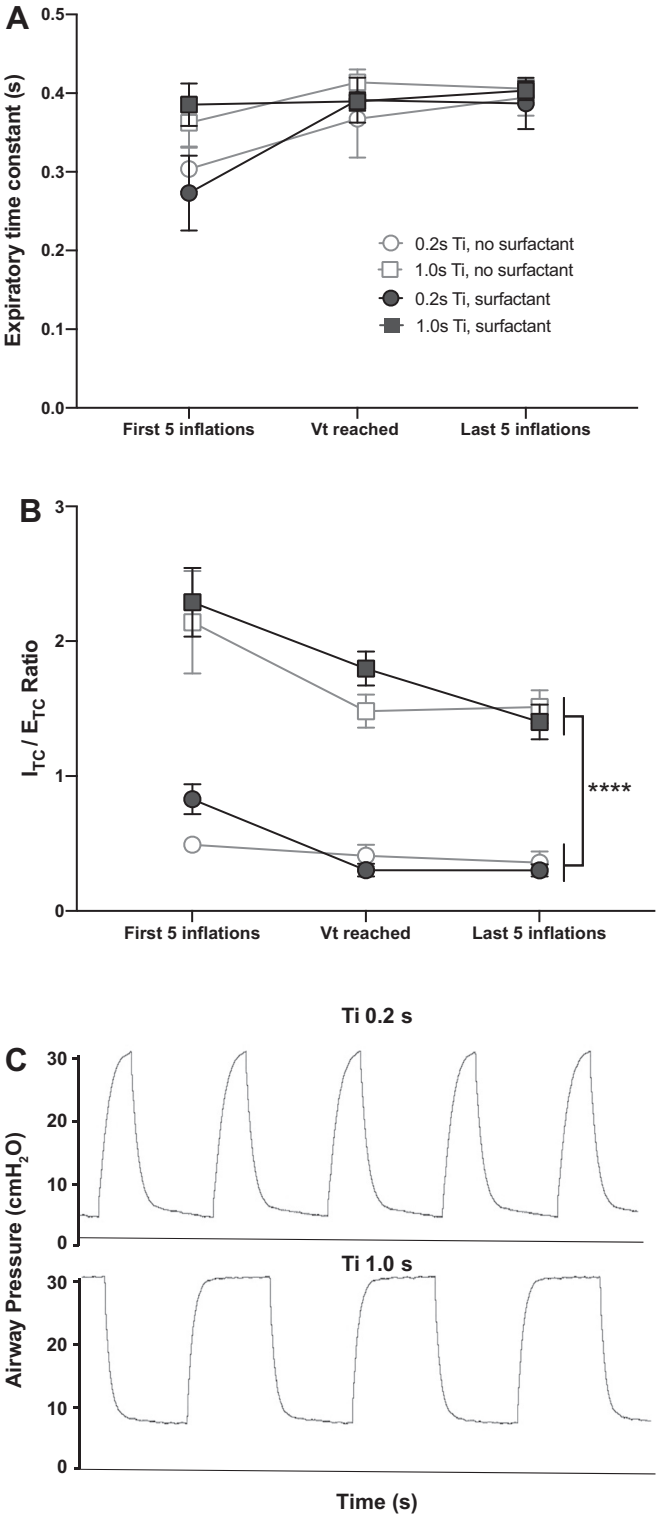


Fig. 4. Time-dependent changes in the expiratory time constant (E_{TC}; time taken to reach 95% of the total expired volume) (*A*) and ratio of the inflation time constant (I_{TC}; time taken to reach 95% of the total inspired volume) to the expiration time constant (I_{TC}/E_{TC}) (*B*) during the experimental ventilation period in kittens ventilated with inflation times (Tis) of 0.2 s (circles) or 1.0 s (squares), with (closed symbols) and without (open symbols) surfactant. Values are means ± SE; main effect of Ti, ****$P < 0.0001$. *C*: airway pressure profiles measured through the respiratory cycle in kittens ventilated with Ti of 0.2 s (*top*) or 1.0 s (*bottom*). Vt, tidal volume.

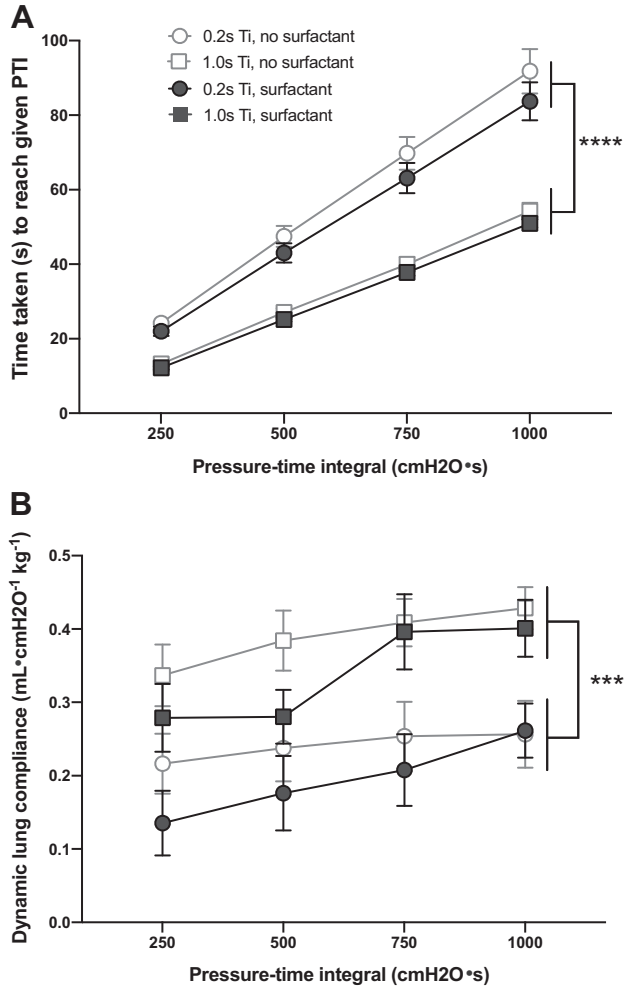


Fig. 5. Time taken for the airway pressure time integral (PTI; area under the curve) to increase to each successive level (*A*) and the increase in dynamic lung compliance for a given level of PTI (*B*) during the experimental ventilation period in preterm newborn kittens ventilated with an inflation time (Ti) of 0.2 s (circles) or 1.0 s (squares), with (closed symbols) and without (open symbols) surfactant. Values are means ± SE; ***main effect of Ti, $P < 0.001$; ****main effect of Ti, $P < 0.0001$.

the Vt entering the previously aerated lung during the first 120 s of ventilation (Fig. 7*B*).

DISCUSSION

After birth, lung aeration is driven by pressure gradients generated by inspiration or artificial inflations, which moves the liquid distally through the airways and across the distal airway wall (6, 22). However, when the air-liquid interface moves distally in an immature lung, the likelihood of it proceeding down both daughter airways at each airway branch is reduced (20). This results in partial lung aeration, which increases the risk of lung injury by over-distending aerated regions with tidal volumes intended for the entire lung (20). Although it is not known why partial lung aeration is more common in the immature lung, a sustained inflation and surfactant enhances the uniformity of aeration when given at the onset of lung aeration (22, 25). However, when the lung is partially aerated, it is unknown what approaches are most effective at improving the uniformity of lung aeration and

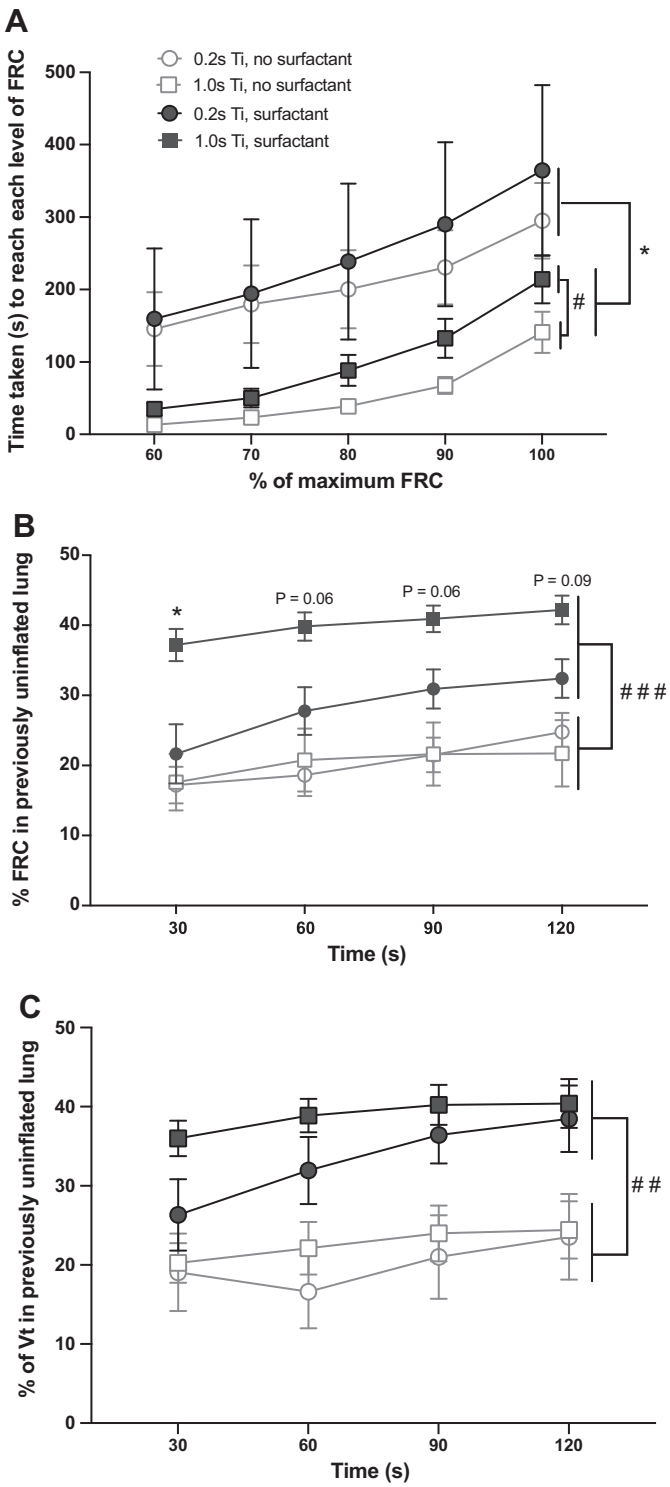


Fig. 6. Time taken to achieve each set percentage level of the maximum functional residual capacity (FRC; *A*), time-dependent increases in FRC within the previously unaerated lung (*B*), and time-dependent changes in the proportion of the tidal volume (Vt; *C*) ventilating the previously unaerated lung during the experimental ventilation period in kittens ventilated with inflation times (Tis) of 0.2 s (circles) or 1.0 s (squares), with (closed symbols) and without (open symbols) surfactant. Values are means ± SE; *main effect of Ti, $P < 0.05$; #main effect of surfactant, $P < 0.05$; ##main effect of surfactant, $P < 0.01$; ###main effect of surfactant, $P < 0.001$.

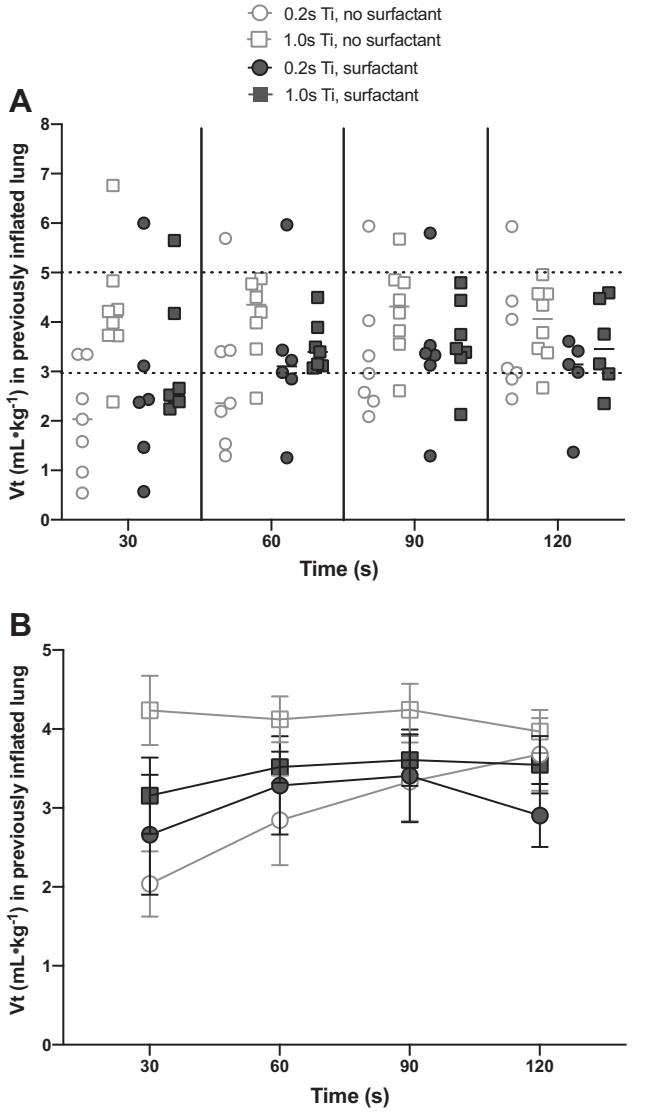


Fig. 7. The average (over 5 inflations) volume of air entering the previously aerated lung during a single inflation (*A*) and the time-dependent change in the tidal volume (Vt) entering the previously aerated lung (*B*) during the experimental ventilation period in preterm newborn kittens. Kittens were ventilated with inflation times (Tis) of 0.2 s (circles) or 1.0 s (squares), with (closed symbols) and without (open symbols) surfactant. Values are means ± SE; $P < 0.05$ was considered significant.

increasing the proportion of lung being ventilated. In this study, we found that surfactant greatly increased aeration of previously unaerated lung regions at FRC, which increased the percentage of lung being ventilated during iPPV in intubated newborn kittens. Although a longer inflation time did not increase the degree of lung aeration at FRC, it markedly improved lung mechanics independently from the effect of surfactant.

Currently, international guidelines do not specify a Ti range for use in the delivery room during noninvasive iPPV of preterm infants at birth, but instead recommend a ventilation rate of 40–60 inflations per minute (17). Australian and European guidelines recommend ranges of 0.3–0.5 s for Ti and 0.5–0.8 s for Te (13, 27), which have presumably been extrapolated from time ranges commonly used in the neonatal inten-

sive care unit (NICU). Indeed, respiratory strategies used in the delivery room are commonly extrapolated from the NICU, despite the physiology being very different (8), and may also be influenced by pediatric and adult ventilation practices. For instance, a Ti that is approximately half the Te is commonly chosen in adults to avoid gas trapping resulting from incomplete expiration (19). However, this assumes that immature lungs of preterm infants are highly compliant like pediatric and adult lungs (2). As such, it assumes they have expiratory flows that are markedly slower than inspiratory flows, typically taking twice as long to complete expiration compared with inspiration. However, the immature lung is considerably stiffer than the adult lung (23), particularly when the lung is partially liquid-filled, leading to much higher expiratory gas flows. Thus, a 1:2 Ti-to-Te ratio is probably unnecessary, which is consistent with the finding that ventilation of kittens with a Ti of 1.0 s and a Te of 0.5 s (2:1 Ti-to-Te ratio) did not cause gas trapping. Indeed, Ti had no effect on FRC (Fig. 2*C*), and the expiratory time constant (0.40 ± 0.01 s; Fig. 4*A*) was found to be less than the set Te (0.5 s). We also found that maximum expiratory flow rates are only marginally less than maximum inspiratory flow rates (72.0 ± 6.6 vs. 57.4 ± 3.6 $mL \cdot kg^{-1} \cdot s^{-1}$) and that surfactant did not increase lung compliance or reduce expiratory gas flows and increase expiratory times as a result. This is contrary to our hypothesis and contrary to what would be expected when surfactant is given much later after birth. It also suggests that a Ti-to-Te ratio of at least 1:1 is appropriate, assuming that the Ti is not too short (i.e., <0.4 s), and that early surfactant administration should not influence this consideration.

For comparison, the maximum expiratory time constants we measured in our very preterm kittens (0.40 ± 0.01 s; Fig. 4*A*) are considerably shorter (~6-fold) than what we measured in adult sheep (2.5 ± 0.1 s) ventilated with the same Vt per kg body weight (unpublished observations). Thus, if we had ventilated these immature kittens with a Ti of 0.4 s and a Te of 0.8 s, they would have completed expiration in ~0.4 s and then remained at FRC for a further 0.4 s, or 33% of the respiratory cycle. As liquid can reenter the airways at FRC and restrict gas exchange (22), leaving the lung at FRC for one-third of the respiratory cycle potentially reduces the time available for gas exchange. When combined with a reduced gas exchange surface area caused by partial lung aeration, this would likely necessitate the use of a much higher inspired oxygen level than would otherwise be required.

A Ti of 1.0 s improved lung mechanics, compared with a Ti of 0.2 s, by increasing the maximum dynamic lung compliance, reducing the time and the number of breaths required to achieve the target Vt, and reducing the maximum PIP required to achieve this Vt. This is partly due to the faster rate of lung aeration and FRC accumulation in kittens receiving a Ti of 1.0 s, which is consistent with the faster increase in dynamic lung compliance. However, the maximum FRC achieved was not affected by Ti, which was surprising, as longer inflations increase FRC when given at the start of lung aeration (25). Nevertheless, combining a longer Ti with surfactant significantly increased the FRC in previously unaerated lung regions (Fig. 6*B*), whereas the longer Ti did not affect the Vt entering the previously unaerated lung in the absence of surfactant.

When the lung is partially aerated, during each inflation air preferentially enters and inflates aerated lung regions because airway resistance is ~100-fold less when the airways are air-filled than when they are liquid-filled (24). Liquid in the liquid-filled airways will only move distally when airway pressures exceed the pressure required to overcome the resistance to moving this liquid through the airways. This is commonly referred to as the lung's "opening pressure," which is not an appropriate term as it implies that the lung is closed. "Clearance pressure" is perhaps more appropriate. Nevertheless, a longer Ti means that the airway pressure remains above the clearance pressure for longer, which increases the efficiency of airway liquid clearance. This is clearly evident in the videos of aerating lungs (Supplemental Movies), but it is important to note that this increase in gas volume occurs very slowly and is mostly undetectable using conventional flow sensors. Indeed, kittens ventilated with a Ti of 1.0 s spent at least five times longer at PIP than kittens ventilated with a Ti of 0.2 s (0.40 ± 0.03 vs. 0.08 ± 0.02 s; Fig. 4*C*). Although some consider that spending a longer time at PIP increases the risk of overinflation in aerated lung regions, resulting in air leak, the evidence is not conclusive, and its current applicability is questionable (9). Indeed, the studies were all performed in the NICU during an era when surfactant and antenatal steroids were not common, using Tis that varied between 0.7 and 2.0 s and included a mixture of pressure and volume-limited ventilation strategies (9). We found that a longer Ti did not increase the Vt entering the previously aerated lung region (Fig. 7), which is consistent with our previous finding that sustained periods at PIP increase aeration of previously unaerated lung regions and do not continue to expand aerated regions (25).

As kittens ventilated with a Ti of 1.0 s received higher airway pressures per unit time, we measured the cumulative PTI for each kitten and compared dynamic lung compliances at the same PTI. For any given PTI, ventilation with a Ti of 1.0 s resulted in a significantly greater dynamic lung compliance (Fig. 5*B*). This indicates that it is not the PTI per se that is important for airway liquid clearance and the associated increase in lung compliance, but the time spent above the clearance pressure. It is important to note that this clearance pressure is likely to decrease as more of the lung aerates. However, a longer Ti must have an additional effect on dynamic lung compliance that is more difficult to explain. It cannot readily be explained by a surfactant-mediated reduction in surface tension (Fig. 3), an increase in FRC (Fig. 2*C*), or an in increase the proportion of the unaerated lung being ventilated (Fig. 6, *B* and *C*). It is possible that a shorter Ti influences airway dynamics, particularly in the smaller muscularized airways, causing a reactive constrictor response. This is consistent with the finding that peak expiratory flows were higher in kittens ventilated with a Ti of 1.0 versus 0.2 s (64.6 ± 4.0 vs. 49.3 ± 2.8 $mL \cdot kg^{-1} \cdot s^{-1}$, respectively). Indeed, it is difficult to rationalize how different inflation times that result in the same Vt could impact expiratory flow rates, other than having an effect on airway resistance that persists into the expiratory phase of the respiratory cycle. This also explains why peak expiratory flow rates are higher with long Tis, despite having a higher lung compliance, because normal expectations would be that as lung stiffness decreases, passive expiratory flows should also decrease, not increase. As such, it is possible that the primary effect of longer Tis on dynamic lung compliance is due to an effect on airway resistance that extends into expira-

tion or even throughout the entire ventilation period. Nevertheless, the expiratory time constants were similar in kittens ventilated with a Ti of 1.0 s and 0.2 s, indicating that the expiratory curve was initially steeper but flatter toward the end of expiration; the time-related changes in the I_{TC}/E_{TC} ratio shown in Fig. 4 simply reflect the temporal changes in Vt recruitment. This indicates that the inherent recoil properties of the lung are similar in all groups and not influenced by surfactant because otherwise we would have expected the expiratory flow to be lower and the expiratory time constant to be longer in a more compliant lung.

Exogenous surfactant did not influence the rate of Vt recruitment, rate of increase in dynamic lung compliance, or the maximum lung compliance achieved and tended to increase the time taken to recruit each percentage of the maximum FRC in these preterm kittens (Fig. 6*A*). However, as the maximum FRC achieved was considerably greater in surfactant-treated kittens, the latter finding is not surprising. Furthermore, as surfactant can temporarily block the small endotracheal tube, requiring a transient increase in pressure to clear this blockage, this likely contributes to the initial lower lung compliance and longer FRC recruitment times in surfactant-treated kittens. Nevertheless, the markedly higher FRC in surfactant-treated kittens supports the concept that reducing surface tension at airway branches increases the probability that air will pass down both daughter airways, thereby enhancing lung aeration. Although we have previously shown that surfactant greatly increases the uniformity of lung aeration when given at the start of lung aeration in preterm kittens (20), we now confirm that surfactant is equally effective when given after the onset of lung aeration. This is indicated by increased aeration of previously unaerated lung regions and likely has greater clinical relevance, as very preterm infants commonly start breathing before there is an opportunity to administer surfactant (16, 18). These findings have many potential benefits, which first require verification in infants. Surfactant increases the proportion of lung that is ventilated and thereby reduces the risk of overinflating and injuring previously aerated regions. It also increases the surface area available for gas exchange, thereby reducing the alveolar/blood oxygen concentration gradient required to achieve adequate oxygen and carbon dioxide exchange. This likely explains why surfactant increases oxygenation and reduces the required fraction of inspired oxygen ($F_{I_{O_2}}$) in preterm infants, thereby reducing the risk of hyperoxia-induced lung injury.

We chose to study inflation times that are both above (1.0 s) and below (0.2 s) the currently recommended values to readily identify the effect of Ti on lung aeration and lung mechanics and distinguish between the relative contributions of surfactant and Ti on lung function in preterm kittens. Nevertheless, previous studies have investigated Tis that ranged between 0.3 and 2.0 s in preterm infants (9). Furthermore, although these settings are now not commonly used with mechanical ventilators, newborns are likely exposed to these inflation times in the delivery room when iPPV is given noninvasively. It is well established that achieving a sufficiently long Ti using a bag and mask or even a T-piece resuscitation device is difficult, particularly in a stressful situation. For example, a recent observational study found the Ti provided varies widely between different practitioners and is considerably shorter than the target Ti in the majority of cases (target Ti 2–3 s, range 0.3–4.0 s, median Ti 1.5 s) (4). Inadvertently short inflation times are common; however, our results suggest that a longer Ti is preferable.

This study was specifically designed to investigate the abilities of surfactant and longer inflation times to fully aerate the partially aerated lung in preterm newborns who require intubation and mechanical ventilation shortly after birth. In developing the model, we chose to initially restrict lung aeration to the right lung for several reasons: *1*) so that the initial degree of lung aeration at the beginning of the experiment was restricted to a maximum of 60% of the entire lung so that the starting point was similar in all animals between groups and *2*) to improve visualization of lung aeration in the previously unaerated lung in the videos. As such, although the degree of lung aeration at our experimental starting point (i.e., 60% of the entire lung) is expected to be similar to very preterm infants shortly after birth, the pattern of lung aeration would likely be very different in preterm infants. Lung aeration would be expected to occur more randomly across the entire lung in very preterm infants depending on factors such as gravity that result in dependent/nondependent lung regions.

Conclusions

Our findings clearly show that surfactant is crucial for improving the total volume of air retained in the lung at end-expiration (FRC) and the proportion of the lung being ventilated in preterm kittens. A longer Ti improved dynamic lung compliance, possibly by reducing airway resistance, and the rate of lung aeration independently from the effects of surfactant. It also increased peak expiratory flows and improved aeration in previously unaerated lung regions in the presence of surfactant. As a result, when the independent effects are combined, surfactant and a higher Ti resulted in higher FRCs, higher dynamic lung compliances, increased aeration of previously unaerated lung regions, and an increased proportion of the lung being ventilated during iPPV. Thus, short inflation times of 0.3 s–0.5 s may not be the most effective way of supporting very preterm infants immediately after birth when the lung is partially liquid-filled, which requires further investigation in infants. Instead, increasing the Ti and maintaining the respiratory rate by reducing the Te may be a better solution that could be examined. Indeed, as expiratory flows during the immediate newborn period are not dissimilar to inspiratory flows and are considerably higher than adult expiratory flows, a 1:2 Ti-to-Te ratio at birth makes little sense and warrants further investigation.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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