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An individualized approach to sustained inflation duration at birth improves outcomes in newborn preterm lambs

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Tingay DG, Lavizzari A, Zonneveld CE, Rajapaksa A, Zannin E, Perkins E, Black D, Sourial M, Dellacà RL, Mosca F, Adler A, Grychtol B, Frerichs I, Davis PG. An individualized approach to sustained inflation duration at birth improves outcomes in newborn preterm lambs. *Am J Physiol Lung Cell Mol Physiol* 309: L1138–L1149, 2015. First published September 25, 2015; doi:10.1152/ajplung.00277.2015.—A sustained first inflation (SI) at birth may aid lung liquid clearance and aeration, but the impact of SI duration relative to the volume-response of the lung is poorly understood. We compared three SI strategies: *1*) variable duration defined by attaining volume equilibrium using real-time electrical impedance tomography (EIT; SI_{plat}); *2*) 30 s beyond equilibrium (SI_{long}); *3*) short 30-s SI (SI_{30}); and *4*) positive pressure ventilation without SI (no-SI) on spatiotemporal aeration and ventilation (EIT), gas exchange, lung mechanics, and regional early markers of injury in preterm lambs. Fifty-nine fetal-instrumented lambs were ventilated for 60 min after applying the allocated first inflation strategy. At study completion molecular and histological markers of lung injury were analyzed. The time to SI volume equilibrium, and resultant volume, were highly variable; mean (SD) 55 (34) s, coefficient of variability 59%. SI_{plat} and SI_{long} resulted in better lung mechanics, gas exchange and lower ventilator settings than both no-SI and SI_{30}. At 60 min, alveolar-arterial difference in oxygen was a mean (95% confidence interval) 130 (13, 249) higher in SI_{30} vs. SI_{long} group (two-way ANOVA). These differences were due to better spatiotemporal aeration and tidal ventilation, although all groups showed redistribution of aeration towards the nondependent lung by 60 min. Histological lung injury scores mirrored spatiotemporal change in aeration and were greatest in SI_{30} group ($P < 0.01$, Kruskal-Wallis test). An individualized volume-response approach to SI was effective in optimizing aeration, homogeneous tidal ventilation, and respiratory outcomes, while an inadequate SI duration had no benefit over positive pressure ventilation alone.

sustained inflation; neonatal resuscitation; lung mechanics; lung volume; variability; electrical impedance tomography; lung injury

THE MAJORITY OF EXTREMELY preterm infants require respiratory assistance in the delivery room (41). In part this is because many of these infants do not have the ability to generate the initial prolonged high transpulmonary pressures required to drive lung fluid from the main airways, allow alveolar aeration, establish functional residual capacity (FRC), and then maintain it during tidal ventilation, essential processes for efficient gas exchange and lung protection (19, 31). Recently, applying an initial sustained inflation (SI) at birth, consisting of an elevated pressure applied for longer than needed for usual tidal inflation, followed by sufficient positive end-expiratory pressure (PEEP), has been proposed as a method of generating the initial transpulmonary pressure needed at birth (10, 18, 20). SI has been extensively investigated in preterm animals (15, 26, 29, 32, 33, 35–38) and humans (10, 18, 34) with conflicting results. Some studies suggested SI improved aeration, FRC, and cerebral oxygen delivery (29, 32, 33), while others failed to demonstrate any benefit over standard respiratory support with sufficient PEEP (26, 35–37). SI was associated with increased lung injury in two studies (11, 12). These data suggest the optimal SI strategy still needs to be elucidated. Consequently, current International Liaison Committee on Resuscitation guidelines do not recommend its routine use in newborns (24).

All studies of SI have used predefined pressure, duration, and/or inflating volume goals (11, 12, 18, 26, 29, 32, 33, 35–38). These approaches assume that the lungs of preterm infants will behave in a consistent, predictable and uniform

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manner. This assumption is inconsistent with our understanding of the mechanical properties of the diseased lung (3, 23, 38). It is unlikely that a single duration, pressure, or volume will be appropriate for all individuals. A SI of predefined duration may be too short or too long, exposing the lung to the injurious consequences of inadequate aeration or excessively prolonged pressure. An alternative approach is to individualize a SI to the mechanical state of the lung and resultant volumetric behavior. It is well established that volume change during inflation behaves exponentially, requiring duration of five time constants to achieve steady-state volume (equilibrium). Although some previous studies reported whether or not lung volume had attained steady state during the SI (32, 33, 37), all did so using post hoc volume analysis. Recently, we found that the individual volume response to a predefined 30-s SI in preterm lambs was highly variable and in most recipients insufficient to attain volume steady state (37).

Advances in real-time imaging of the lung using electrical impedance tomography (EIT) now allow immediate display of global lung volume change at the bedside during birth transition in experimental animals (37, 38). This offers, for the first time, the ability to individualize SI application to ensure optimal aeration. We hypothesized that *1*) there would be intersubject variability in the time needed to obtain lung volume steady state (plateau) during a SI; *2*) the application of a variable duration "volumetric SI," defined by the attainment of a real-time volume equilibrium, would result in better short-term and lung injury outcomes than a SI that was too short for an individual lung; and *3*) a SI applied beyond volumetric steady state would expose the lung to the risks of regional overdistension.

The aim of this study was to compare, in preterm lambs, conventional ventilation without an SI with three SI strategies: *1*) an SI with a duration determined by the achievement of lung volume equilibrium; *2*) an intentionally short SI; and *3*) a SI prolonged beyond that required to reach equilibrium. The primary outcomes were the time to stable lung volume (*hypothesis 1*) and spatiotemporal patterns of aeration and tidal ventilation and oxygenation [expressed as alveolar-arterial difference in oxygen ($AaDO_2$)] and lung mechanics (*hypotheses 2* and *3*). In addition, hemodynamic parameters and the role of each strategy on regional early markers of lung injury and the interaction with regional aeration and ventilation were examined.

METHODS

The study was performed at the animal research facility of the Murdoch Childrens Research Institute (Melbourne, Australia) and approved by the Institute's Animal Ethics Committee in accordance with the National Health and Medical Research Committee (Australia) guidelines.

Experimental Instrumentation

Border-Leicester/Suffolk lambs, gestational age 127 ± 1 days (term ~147 days), were born to ewes exposed to betamethasone 11.4 mg im 24 and 48 h before delivery. Twin-pregnancy ewes were chosen to minimize maternal and environmental variability and optimize reduction. A single supplier cared for ewes and mating occurred during the same season to standardize environmental factors. The lambs were delivered via caesarean section under general anesthesia with isoflurane, intravenous propofol, and nitrous oxide using our previously described protocol (35, 37, 38). The fetal head was first exteriorized, the right jugular vein and carotid artery were cannulated, and the left carotid artery was encircled with a 4-mm transit-time flow probe (Transonic Systems, Ithaca, NY). Lambs were intubated with a 4.0 cuffed endotracheal tube (ETT), the chest was exposed and dried (35, 38), and the fetal lung fluid was passively drained to 10–15 ml/kg of anticipated birth weight before clamping the ETT. EIT needles were positioned equidistance around the chest in a transverse plane ~1 cm above the xyphisternum. The electrodes were secured in place with self-adherent bandage (Coban; 3M, St. Paul, MN) and connected to a Goe-MF II EIT system (CareFusion, Hoechberg, Germany), as previously described (4, 26, 35–38). After verification of the instrumentation, the lamb was delivered, weighed, and placed supine under an infant warmer. Temperature and peripheral oxygen saturation (SpO_2) probes were positioned, vascular lines and ventilator were connected, and a 10-s EIT recording of the unaerated lung was taken before unclamping the ETT. Throughout the study continuous infusions of ketamine (4–12 $mg \cdot kg^{-1} \cdot h^{-1}$) and midazolam (0.05–0.15 $mg \cdot kg^{-1} \cdot h^{-1}$) were used to maintain anaesthesia, analgesia and suppress any spontaneous respiratory effort.

Measurements

The experimental protocol is summarized in Fig. 1*A*. SpO_2, heart rate (HR), arterial blood pressure (ABP; HP48S monitor; Hewlett Packard, Andover, MA), cerebral blood flow (CBF; TS420 Perivascular Flow Module, Transonic Systems, Ithaca, NY), airway pressure (P_{ao}), gas flow, and tidal volume (V_t) at the airway opening (Florian; Acutronic Medical Systems, Hirzel, Switzerland) were measured continuously from birth. Global and regional lung volume changes were acquired by EIT at 25 scans/s (35, 37, 38) and the unfiltered global lung volume change was displayed in real-time using the Thorascan software package (CareFusion). Arterial blood gases were performed at 5 min of life and every 15 min from birth. A 5-Hz oscillatory pressure was superimposed onto the ventilation waveform for 10 s on completion of the ventilation strategy at birth and immediately after every arterial blood gas sample to calculate lung mechanics using the forced oscillation technique (FOT) as described in Dellacà and colleagues (6, 7, 43).

Ventilation Strategies at Birth

Lambs were randomly assigned before the delivery to one of the following groups.

Control group. The control (no-SI) group received positive pressure ventilation (PPV) in a volume-targeted ventilation (VTV) mode from birth without a SI. Initially, PPV was delivered with a PEEP of 8 cmH_2O, inflating pressure (PIP) limit of 40 cmH_2O, inspiratory time of 0.4 s, rate of 60 inflations/min, and set V_t of 7 ml/kg (SLE 5000 infant ventilator; SLE Systems, Croydon, UK). These settings have previously been shown to be physiologically appropriate in our lamb population (35).

Short, fixed-duration SI of 30 s group. The short, fixed-duration SI (SI_{30}) of 30 s (Fig. 1*B* and Supplemental Video S1; Supplemental Material for this article is available online at the Journal website) group was unable to achieve lung volume plateau in our previous study (38) but described previously in preterm lambs (15).

Volume-plateau SI group. The volume-plateau SI (SI_{plat}) group received an SI individualized in each lamb and delivered until 10 s after a visually determined plateau by two investigators (C. Zonneveld and D. G Tingay) in the global EIT time-course volume signal on the Thorascan display (Fig. 1*B* and Supplemental Video S2).

Prolonged SI group. The prolonged SI (SI_{long}) group received an individualized SI in each lamb delivered until 30–40 s after visual observation of a plateau in the global EIT volume signal by the same investigators (Fig. 1*B* and Supplemental Video S3).

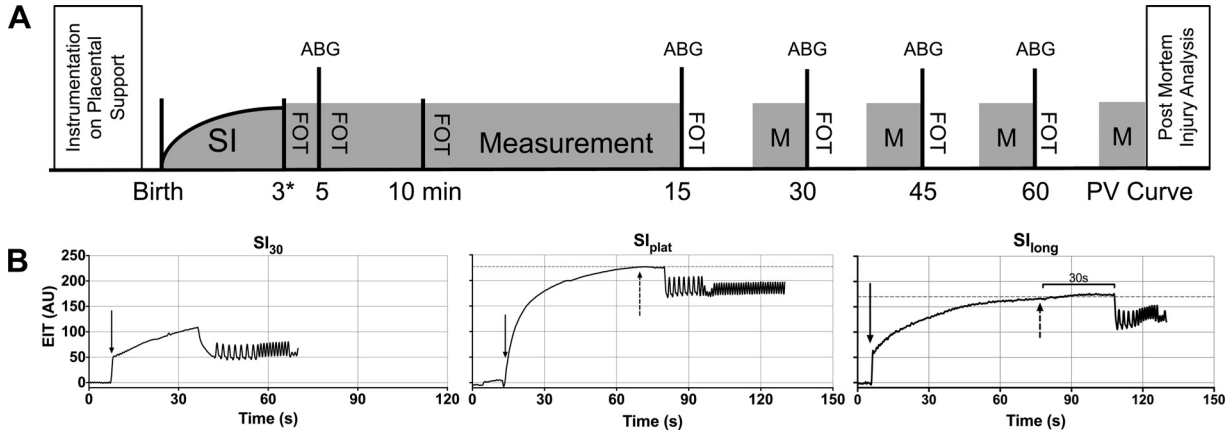

Fig. 1. *A*: Overview of experimental protocol. SI, sustained inflation as per study allocation or positive pressure ventilation (PPV); ABG, arterial blood gas; FOT, forced oscillation technique measurement of lung mechanics; M, measurement of physiological, ventilator, hemodynamic, and electrical impedance tomography (EIT) parameters. *Measurements were taken immediately after SI was completed or from birth (no-SI). *B*: representative tracing of the real-time global EIT time-course volume signal for each of the 3 SI groups. Unclamping of the endotracheal tube (ETT), as well as commencement of the SI, is indicated with the solid arrow. The point of signal plateau, as determined visually, in the SI_{plat} and SI_{long} groups is indicated with the dashed arrow and grey dashed horizontal line. EIT data are presented as shown on the Thorascan software in uncalibrated (arbitrary) units (AU). Real-time videos of each tracing are available in the Online Supplement (Supplemental Videos S1–S3).

All sustained inflations were delivered at 40 cmH_2O (8 l/min gas flow) using the Neopuff Infant T-Piece Resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand). On completion of the SI the lung was held at a PEEP of 8 cmH_2O for 5 s before clamping the ETT and transferring the lamb to the SLE5000 ventilator. PPV was then commenced as per the no-SI Group.

Ventilation Strategy and General Management after Birth

All lambs were initially ventilated using 0.21 $F_{I_{O_2}}$ until the first arterial blood gas and then adjusted to maintain a SpO_2 of 88–95%. Ventilator settings were adjusted after each arterial gas to maintain an arterial partial pressure of carbon dioxide (Pa_{CO_2}) of 45–65 mmHg by first altering V_t and then rate if hypocarbia at V_t 5 ml/kg. Body temperature was kept between 38 and 39°C (physiological for lambs), and hydration was maintained with a 0.18% NaCl, 4% glucose intravenous infusion.

At 60 min of life the animals were ventilated with 1.0 $F_{I_{O_2}}$ for 3 min and then received a lethal dose of pentobarbitone. The ETT was then disconnected to atmosphere until lung collapse. A static in vivo super-syringe pressure-volume (PV) curve was generated (maximum pressure: 40 cmH_2O) to determine the static mechanical properties of the respiratory system and calibrate the EIT signal (1, 26, 37). An additional 10 fetuses (fetal group) were euthanized at delivery as unventilated controls for injury analysis comparison.

Data Processing and Analysis

SpO_2, HR, ABP, body temperature, CBF, P_{ao}, and flow and expiratory V_t were recorded at 1,000 Hz (PowerLab; AD Instruments, Sydney, Australia), processed, and analyzed using Labchart 7 (AD Instruments). Together with these data, EIT data were monitored in real time and recorded (Thorascan, Carefusion, Germany) continuously for the first 15 min and subsequently for 2 min with each arterial blood gas. EIT signals were also recorded during the static in vivo PV curve. PIP, PEEP, and mean airway pressure (P_{aw}) were determined from the P_{ao} data, and dynamic compliance (C_{dyn}) calculated from the ΔP and V_t data. Total respiratory system reactance (X_{rs}) and resistance (R_{rs}) were computed from FOT recordings (6). X_{rs} is a measure of the elastic and inertive characteristics of the respiratory system that has been shown to be an accurate indicator of lung recruitment in our preterm lamb model (43). Static respiratory system compliance (C_{rs}) was determined from the PV curve (35). The alveolar-arterial difference in oxygen ($AaDO_2$) was calculated from the arterial blood gases.

Time-series images of the change in impedance were reconstructed from EIT data using a GREIT algorithm based on the correct anatomical shape of the lamb chest (2, 9) determined from computed tomography (CT) chest images in pilot 128-day gestation fetal lambs (37). Data acquired immediately before commencing ventilation were used as reference for image reconstruction (38). Due to the elongated shape of the lamb chest, the resulting 32 × 32 pixel EIT images contained 586 active (non-zero) pixels, which were used for all subsequent analysis. EIT images were analyzed using our previous described method after low-pass filtering to the respiratory domain with a cut-off at 130 inflations/min (38). The expiratory trough values of the EIT signal in individual image pixels were used to determine both global and regional changes in global end-expiratory volume from birth (ΔEEV) in the entire cross-sectional slice (global) slice of the thorax. The global ΔEEV signal was calibrated (ml/kg) to the change in impedance during the known-volume static PV curve (1, 17, 22).

Two types of functional EIT (fEIT) images were constructed from at least 20 s of continuous artifact-free data for each time point and the spatial distribution of ventilation and aeration was determined from these using the methods of Frerichs et al. (8). fEIT images of the end-expiratory minimum value were used to define aeration and the tidal amplitudes (minimum to maximum values) (8). Based on the CT data the uppermost three and lower most seven slices were excluded, as they contained no lung tissue. This allowed for relative V_t and aeration to be determined in 22 nondependent to dependent equal slices of the right and left hemithoraces. From these measurements, histograms of the gravity dependent distribution of V_t and aeration within the lung, expressed as a percentage of total, were created (37). Comparisons of relative regional aeration differences were made between three gravity-dependent lung regions (upper, middle, and lower) of equally weighted lung tissue (that is equal number of pixels) expressed as the percentage of total aeration (%).

Sustained Inflation ΔEEV Modeling

The duration of each SI was determined from the global time course ΔEEV signal and the final delivered volume (ml/kg) calculated. To determine the predicted time required to achieve a plateau

lung volume for each SI, a one-phase exponential model (23, 28, 38) was applied to each global EIT signal and the static time constant of the respiratory system (τ), predicted time to plateau EEV (t_{plat}, defined as 5τ), and predicted plateau EEV was calculated: $y = y_{plateau} \cdot [1 - e^{(-k \cdot x)}]$, where $y_{plateau} = EEV_{max}$ during SI, x = time (s), and k = reciprocal of τ.

Lung Injury Analysis

After autopsy, total left lung protein concentration was calculated on bronchoalveolar lavage fluid (14) using the Lowry method (21). Five hematoxylin and eosin (H&E)-stained sections from each of the upper, middle, and lower gravity-dependent thirds of the right upper lobe (fixed at 20 cmH_2O with 4% paraformaldehyde) were scored for lung injury (n = 15 total/lamb) by an investigator blinded to treatment allocation. A score out of 5 was assigned for each of *1*) alveolar wall thickness, *2*) detached epithelial cells, *3*) hyaline membranes presence, and *4*) alveolar collapse/atelectasis. On completion, 10% of slides were rescored blinded. Injury markers were compared with the values in fetal group. The highest and lowest scores from each of the five slides in each region for each lamb were removed from subsequent analysis. Gravity dependent and nondependent samples of the right lower lobe approximating the gravitational regions of EIT analysis were analyzed by quantitative real-time PCR for known early markers of lung injury (*CTGF*, *CYR61*, *EGR1*, *IL-1β*, *IL-6* and *IL-8* mRNA) (40) using *RSP29* as the reference gene and the $2^{-\Delta\Delta CT}$ method (40).

Statistical Analysis

A sample size of eight lambs per group would detect a clinically meaningful difference (SD) in $AaDO_2$ of 100 (100) mmHg and C_{dyn} of 0.1 (0.1) $ml \cdot kg^{-1} \cdot cmH_2O^{-1}$, assuming a power of 0.8 and alpha error 0.05. To account for potential error in antenatal parity assessment, the role of antenatal steroids and maternal and anticipated intersubject variability in SI volume response, mating was based on 12–13 lambs per treatment group. Data were tested for normality and analyzed with one-way ANOVA, Kruskal-Wallis test or two-way repeated-measures ANOVA (using time and ventilation strategy as factors), and Tukey's and Dunn's multiple comparison posttests as appropriate. Statistical analysis was performed with GraphPad PRISM 6 (GraphPad Software, San Diego, CA) and a $P < 0.05$ was considered significant.

RESULTS

Fetal Characteristics

Fifty-nine lambs were studied. The groups were well matched for birth weight, gestational age, fetal lung fluid drained, and fetal well-being (Table 1). One lamb in the no-SI group was excluded due to unrecognized oesophageal intubation. Five lambs developed pneumothoraces, one lamb at 33 min of life in the SI_{30} group, and two in each of the no-SI and SI_{30} groups after inflation to 40 cmH_2O during the static super-syringe PV curve. Two lambs in the fetal group were excluded due to fetal hypoxia.

SI Characteristics

Overall SI duration was 21.4 s longer in the SI_{long} group compared with $SI_{plat,}$ but this was not statistically different (Fig. 2*A*), with both groups being significantly longer than SI_{30}; $P < 0.0001$ (one-way ANOVA). The time needed to achieve the volumetric definitions of the SI_{plat} and SI_{long} strategies was highly variable; range: 36.1–131.6 s (SI_{plat}) and 76.5–145.2 s (SI_{long}) (combined coefficient of variability 59%). The one-phase exponential model was able to describe all the SI data with a good fit; median (IQR) R^2 of 0.950 (0.777, 0.988). With the use of this model, there was no difference in the t_{plat} between the three SI strategies. The model provided a $t_{plat} \leq 30$ s in only 9 (24%) sustained inflations.

Global and Regional Lung Aeration

At the end of SI_{30}, SI_{plat}, and SI_{long} the mean (SD) ΔEEV was 36 (21), 45 (36), and 50 (26) ml/kg, respectively ($P = 0.498$; one-way ANOVA; Fig. 2*B*), which compared with ΔEEV at 30s of 12 (7) ml/kg in the no-SI group ($P < 0.009$ and $P < 0.0003$ vs. SI_{plat} and SI_{long}, respectively). These differences were persistent at 100 s (or immediately after SI if >100-s duration). The modeling predicted that a median (IQR) 85 (75, 92)% of total ΔEEV occurred within the first 30 s and the additional 30 s beyond plateau a median (IQR) 3.3 (2.5, 4.8)% above EEV at t_{plat}.

Figure 3 shows the global ΔEEV over time. Strategy ($P < 0.0001$) but not time ($P = 0.508$) had a significant influence on ΔEEV (two-way ANOVA). There was no difference between SI_{long} and SI_{plat} groups or between no-SI and SI_{30} groups. The use of SI_{plat} or SI_{long} resulted in ~10 ml/kg higher ΔEEV at 5 min compared with no-SI and SI_{30} (all $P < 0.042$, Tukey's posttest) and remained significantly higher until 30 min (no-SI) and 15 min (SI_{30}), all permutations (all $P < 0.045$). The time-based decrease in SI_{plat} and SI_{long} groups did not reach statistical significance.

The relative gravity-dependent spatial distribution of aeration varied between strategies at 5 min of life (Fig. 4*A*). SI_{30} and SI_{plat} showed the most uniform distribution of aeration,

Table. 1. *Study groups characteristics*

	no-SI	SI_{30}	SI_{plat}	SI_{long}	Fetal
Number	12	13	12	12	10
Gestational age, days	127 (1.00)	127 (0.86)	127 (0.79)	127 (0.80)	127 (0.68)
Female *n*, %	5 (42%)	6 (46%)	7 (58%)	6 (50%)	6 (60%)
First born *n*, %	7 (64%)	7 (54%)	5 (42%)	6 (50%)	3 (30%)
Birth weight, g	3169 (519)	3119 (439)	3042 (420)	3016 (391)	2765 (432)
Fetal lung fluid, ml/kg	15.9 (8.6)	17.5 (6.0)	16.5 (10.0)	16.0 (8.5)	N/A
Arterial cord pH	7.33 (0.04)	7.35 (0.05)	7.33 (0.06)	7.34 (0.05)	7.20 (0.19)
Arterial cord Pa_{O_2}, mmHg	22.0 (5.1)	21.9 (5.4)	22.7 (3.7)	23.0 (5.0)	21.4 (14.7)
Static C_{rs}, $ml \cdot kg^{-1} \cdot cmH_2O^{-1}$	0.89 (0.23)*	1.02 (0.26)	1.18 (0.37)	1.21 (0.26)	N/A

All data means (SD). Strategies are as follows: variable duration defined by attaining volume equilibrium using real-time electrical impedance tomography (SI_{plat}); 30 s beyond equilibrium (SI_{long}); short 30-s SI (SI_{30}); and positive pressure ventilation without SI (no-SI). C_{rs}, static respiratory system compliance. *$P = 0.038$ no-SI vs. SI_{plat} and SI_{long}, by one-way ANOVA.

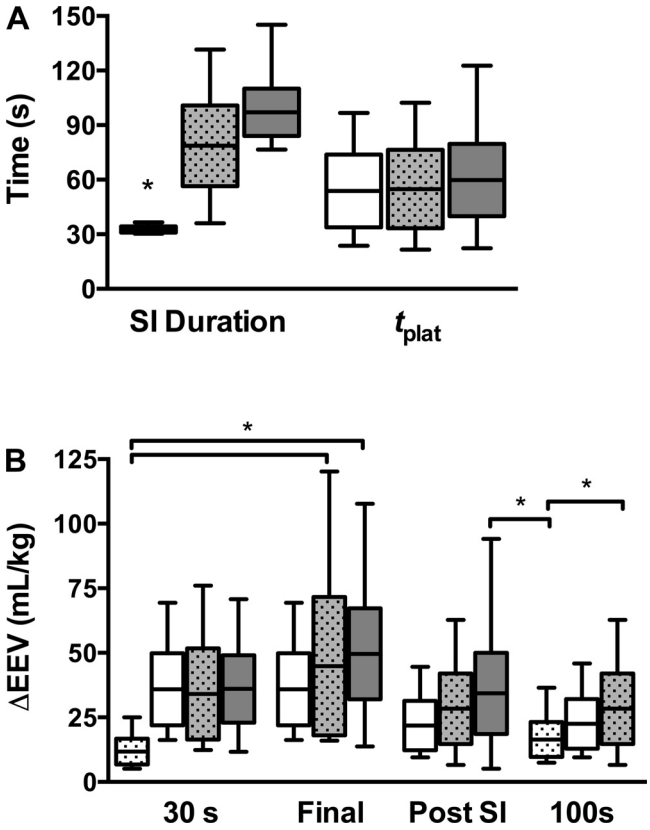

Fig. 2. *A*: actual SI duration and time to t_{plat} (determined from modeling) during the SI_{30} (white box), SI_{plat} (light grey hatched), and SI_{long} (dark grey). The time to stable change global end-expiratory volume measured by EIT (ΔEEV) during SI_{long} was mean (SD) 60 (30) s. $*P < 0.0001$, SI_{30} vs. SI_{plat} and SI_{long}. *B*: ΔEEV from birth for SI_{30}, SI_{plat}, SI_{long}, and no-SI (white hatched), using patterns as in *A*, at 30 s of life (30 s), the end of the SI if applicable (Final), during the first 10 inflations immediately post the SI (Post SI), 100 s of life, or immediately after SI if SI had not completed at 100 s (100 s). The mean (SD) ΔEEV at t_{plat} was 39 (19), 45 (36), and 42 (19) ml/kg for SI_{30}, SI_{plat}, and SI_{long}, respectively ($P = 0.498$, one-way ANOVA). Box represents 5-95th confidence interval and mean (line) and whiskers minimum and maximum. $*P = 0.009$ and $P = 0.0003$, no-SI vs. SI_{plat} and SI_{long}. dynamic compliance (C_{dyn}) calculated from the ΔP and V_t data. Total respiratory system reactance (X_{rs}) and resistance (R_{rs})

whilst no-SI ($P = 0.010$ and $P < 0.0001$) and SI_{long} ($P = 0.009$ and $P = 0.001$) had significantly less relative aeration in the nondependent (upper) regions compared with middle and lower (all Tukey's posttest). Heterogeneity was greatest in the no-SI group, with significantly different relative contributions to dependent ($P = 0.004$) and nondependent ($P = 0.045$) aeration compared with SI_{30}. There were no regional differences between other strategies.

Spatial aeration patterns changed with time, with all groups demonstrating gravity-dependent redistribution of

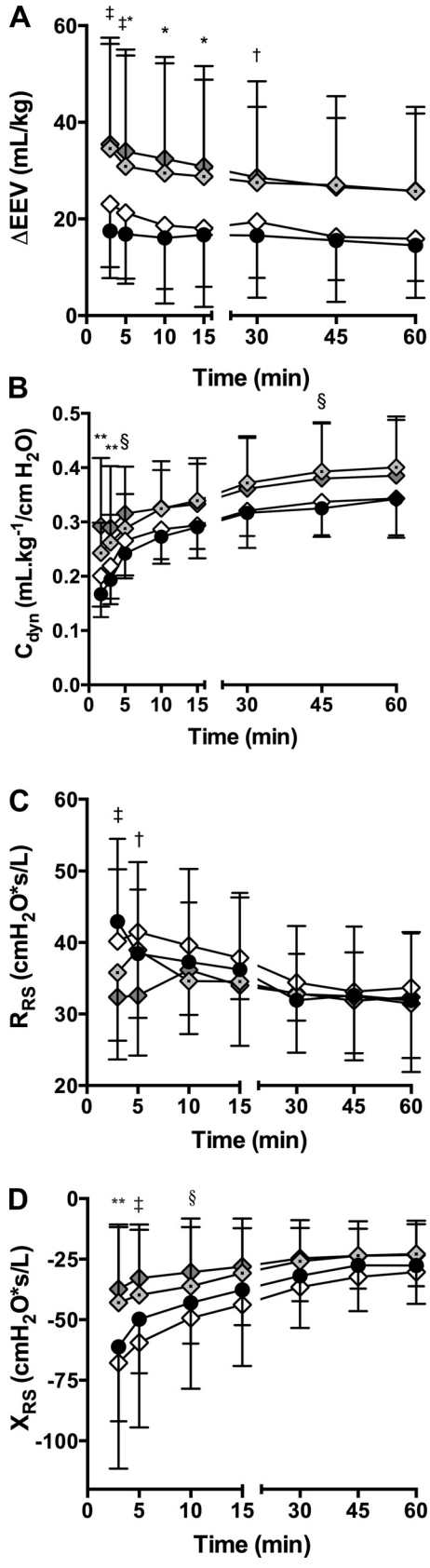

Fig. 3. *A*: ΔEEV from birth over time for no-SI (black circles), SI_{30} (open diamonds), SI_{plat} (grey diamond with dot), and SI_{long} (dark grey diamonds) groups. Change in dynamic compliance (C_{dyn}; *B*) and total respiratory system resistance (R_{rs}; *C*) and reactance (X_{rs}; *D*) using the same symbols as *A*. All data are means ± SD. $*P < 0.05$, SI_{long} vs. no-SI and SI_{30}; †$P < 0.05$, no-SI vs. SI_{long}; ‡$P < 0.05$, no-SI vs. SI_{long} and SI_{plat}; §$P < 0.05$, SI_{30} vs. SI_{long} and SI_{plat}; $**P < 0.05$, SI_{30} and no-SI vs. SI_{plat} and SI_{long}.

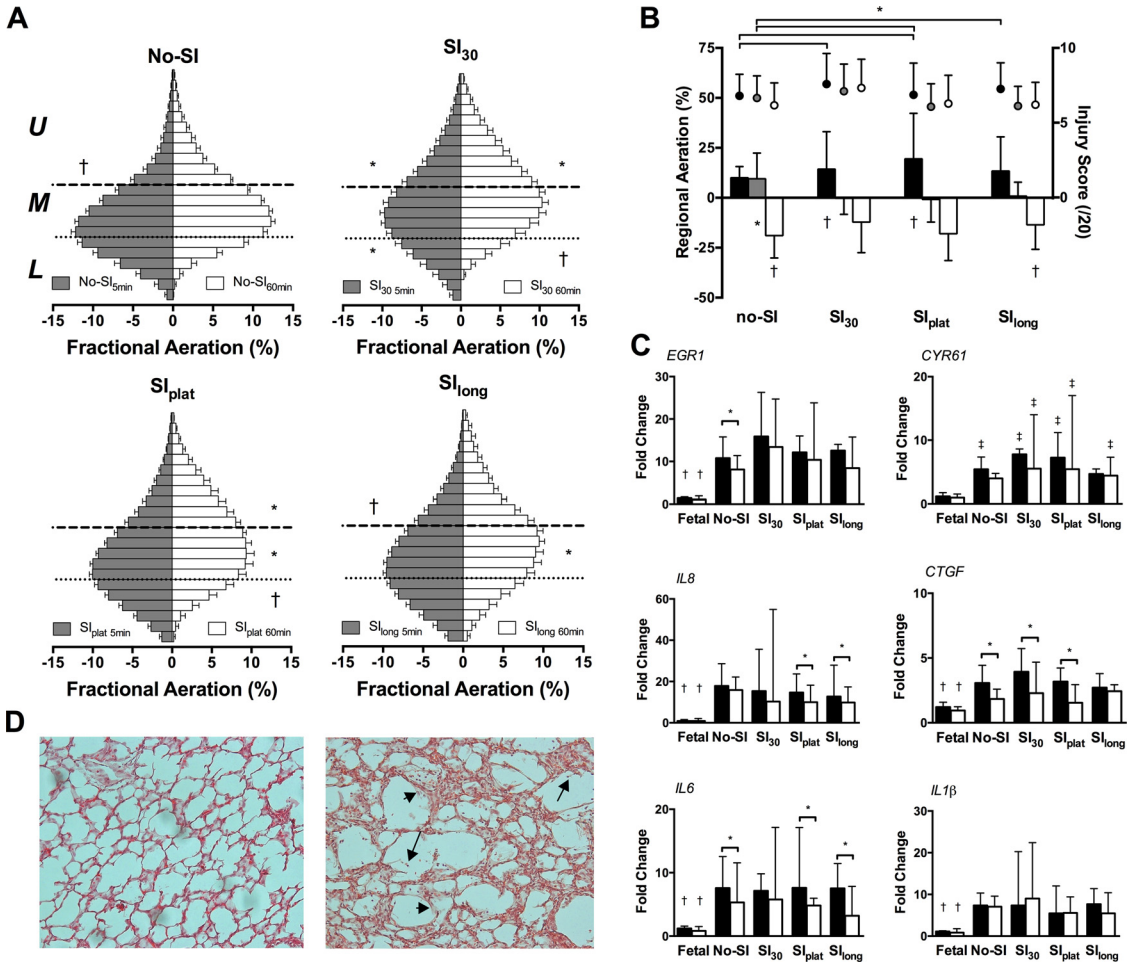

Fig. 4. *A*: functional (f)EIT images of the regional gravity-dependent distribution of aeration (expressed as %total aeration) at 5 and 60 min in each of the 22 equally sized slices for the right (dark bars) and left (white bars) hemithorax. The least gravity dependent slices of the thorax are at the top of each histogram and the most dependent at the bottom. Dashed and dotted lines delineate the upper (U), middle (M), and lower (L) equally weighted (by lung tissue) gravity-dependent regions. Data are means ± SD. *$P < 0.05$, against no-SI for that region; †$P < 0.05$, within strategy against both other regions (one-way ANOVA with Tukey's posttests). *B*: Change in relative regional aeration from 5 min at 60 min of life in the upper (most gravity non-dependent; black bars), middle (grey bars) and lower (most gravity dependent; white bars) equally sized thirds of the lung. Corresponding lung injury score (hematoxylin and eosin sections) shown using circles with same color scheme for regions. Data are means ± SD. *$P < 0.05$, between strategies for that region; †$P < 0.05$, within strategy against both other regions (one-way ANOVA with Tukey's posttests). *C*: expression of *EGR1*, *CYR61*, *CTGF*, *IL-1β*, *IL-6*, and *IL-8* mRNA in the nondependent (black bars) and dependent (white bars) regions. mRNA data median and IQR. †$P < 0.05$, between fetal vs. all other strategies for region; ‡$P < 0.05$ vs. fetal for region (Kruskal-Wallis test with Dunn's multiple comparison). *D*: representative hematoxylin and eosin-stained right upper lobe lung tissue sections in an unventilated fetus (*left*) and a mechanically ventilated lamb with lung injury (*right*) demonstrating hyaline membranes (arrowheads) and detached epithelial cells (arrows).

aeration towards the nondependent lung by 60 min (Fig. 4, *A* and *B*) compared with 5 min (all $P < 0.01$; Tukey's posttest). Within each strategy the no-SI, SI_{30}, and SI_{plat} groups had heterogeneity of aeration at 60 min and SI_{long} had the most spatiotemporal uniformity. Between strategies, relative aeration was greater in the SI_{30} and SI_{plat} within the upper region compared with no-SI ($P = 0.019$ and $P = 0.012$, respectively) and greater in the no-SI group compared with SI_{plat} ($P = 0.013$) and SI_{long} ($P = 0.042$) within the middle region at 60 min.

Gas Exchange, Ventilator, and Hemodynamic Parameters

Only the SI_{plat} and SI_{long} groups achieved target SpO_2 within the first 5 min of life, with the other groups requiring up to 15 min (all $P < 0.034$, Tukey's posttest; Fig. 5*A*). Target SpO_2 in the SI_{30} and no-SI groups was achieved at the expense of a higher $AaDO_2$ (Fig. 5*B*; all $P < 0.032$, Tukey's posttest). ΔP was lower in the SI_{plat} and SI_{long} groups compared with no-SI and, to a lesser extent, SI_{30} during the first 15 min (Fig. 5*C*). Before 5 min of life, the set V_t of 7 ml/kg could not be obtained in the no-SI and SI_{30} groups (Fig. 5*D*). Pa_{CO_2} was also significantly higher in the no-SI group at 5 min compared with the other groups (all $P < 0.004$; Fig. 5*E*). There was no difference in CBF (Fig. 5*F*), HR, or ABP (data not shown) during the entire study period.

Lung Mechanics

C_{dyn} was higher in the SI_{long} and SI_{plat} groups compared with both SI_{30} and no-SI at all time points ($P < 0.001$, two-way ANOVA with Tukey's posttests; Fig. 3*B*). R_{rs} was lower in the SI_{plat} and SI_{long} groups at 3 min ($P = 0.003$–

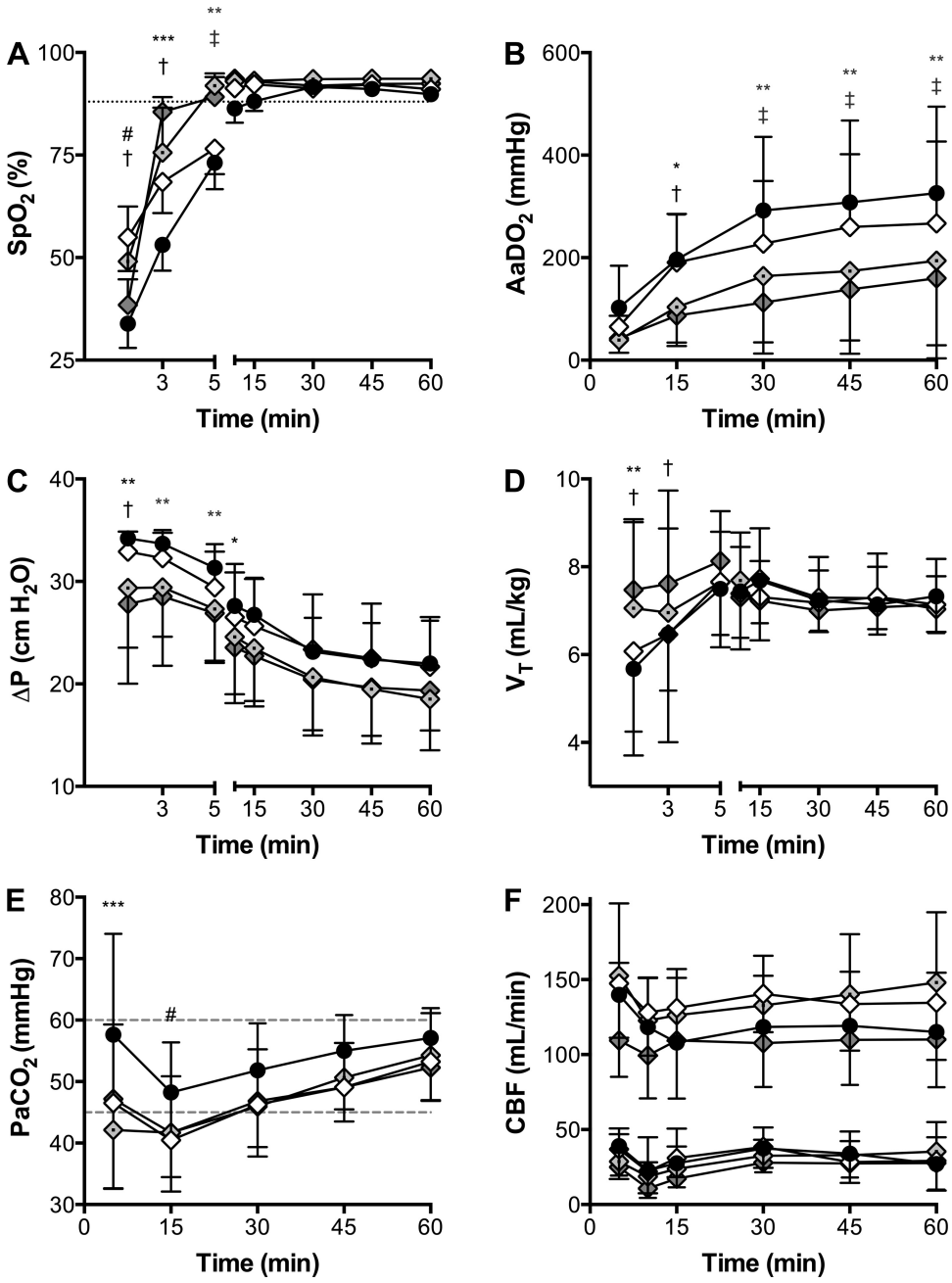

Fig. 5. Change in peripheral oxyhemoglobin saturation (SpO_2; *A*), alveolar-arterial difference in oxygen ($AaDO_2$; *B*), pressure amplitude (ΔP; *C*), tidal volume (V_t; *D*), Partial pressure of arterial carbon dioxide (Pa_{CO_2}; *E*), and minimum and maximum cyclical cerebral blood flow (CBF; *F*) for the 4 recruitment strategies using the same symbols as Fig. 2. The first time points for SpO_2, ΔP, and V_t represent data at 100 s (no-SI and SI_{30}) or during the first 10 inflations after the SI (SI_{plat} and SI_{long}). All data are means ± SD, except SpO_2 (means ± SE). *$P < 0.05$, no-SI vs. SI_{long}; †$P < 0.05$, SI_{30} vs. SI_{long}; ‡$P < 0.05$, SI_{30} vs. SI_{plat}; **$P < 0.05$, no-SI vs. SI_{plat} and SI_{long}; *** $P < 0.05$, no-SI vs. all SI strategies; #$P < 0.05$, SI_{30} vs. SI_{plat}.

0.047; Fig. 3*C*), and SI_{long} vs. SI_{30} at 5 min ($P = 0.010$). Both SI_{long} and SI_{plat} groups showed higher X_{rs} at 3 min, and remained so for SI_{long} vs. SI_{30} until 10 min ($P = 0.001$–0.049; Fig. 3*D*). Static C_{rs} was higher in SI_{plat} and SI_{long} compared with no-SI (Table 1).

Gravity-Dependent Distribution of Tidal Ventilation

Figure 6 shows the gravity-dependent spatial distribution of V_t at 5 and 60 min. All groups behaved differently, with the no-SI group showing increased temporal heterogeneity of V_t within the lung (most gravity-dependent third $P = 0.016$ and least dependent $P = 0.046$, paired *t*-tests). Relative V_t was similar throughout the lung at 5 min following SI_{30} but significantly decreased within the dependent regions by 60 min ($P = 0.023$). There were no temporal changes in V_t distribution within the SI_{plat} group, but the nondependent lung always contributed a significantly greater portion to total V_t ($P = 0.032$ at 5 min and $P = 0.048$). There were no spatiotemporal differences in V_t following the SI_{long}. The relative contribution to V_t in the most gravity-dependent third of the chest was less at 60 min in the SI_{30} group compared with SI_{long} ($P = 0.010$, one-way ANOVA with Tukey's posttest); all other permutations of regions and strategies were not different.

Lung Injury

There was no difference in the total protein count between groups (Table 2). All groups had a higher lung injury score than the Fetal controls (all $P < 0.001$, Kruskal-Wallis test with

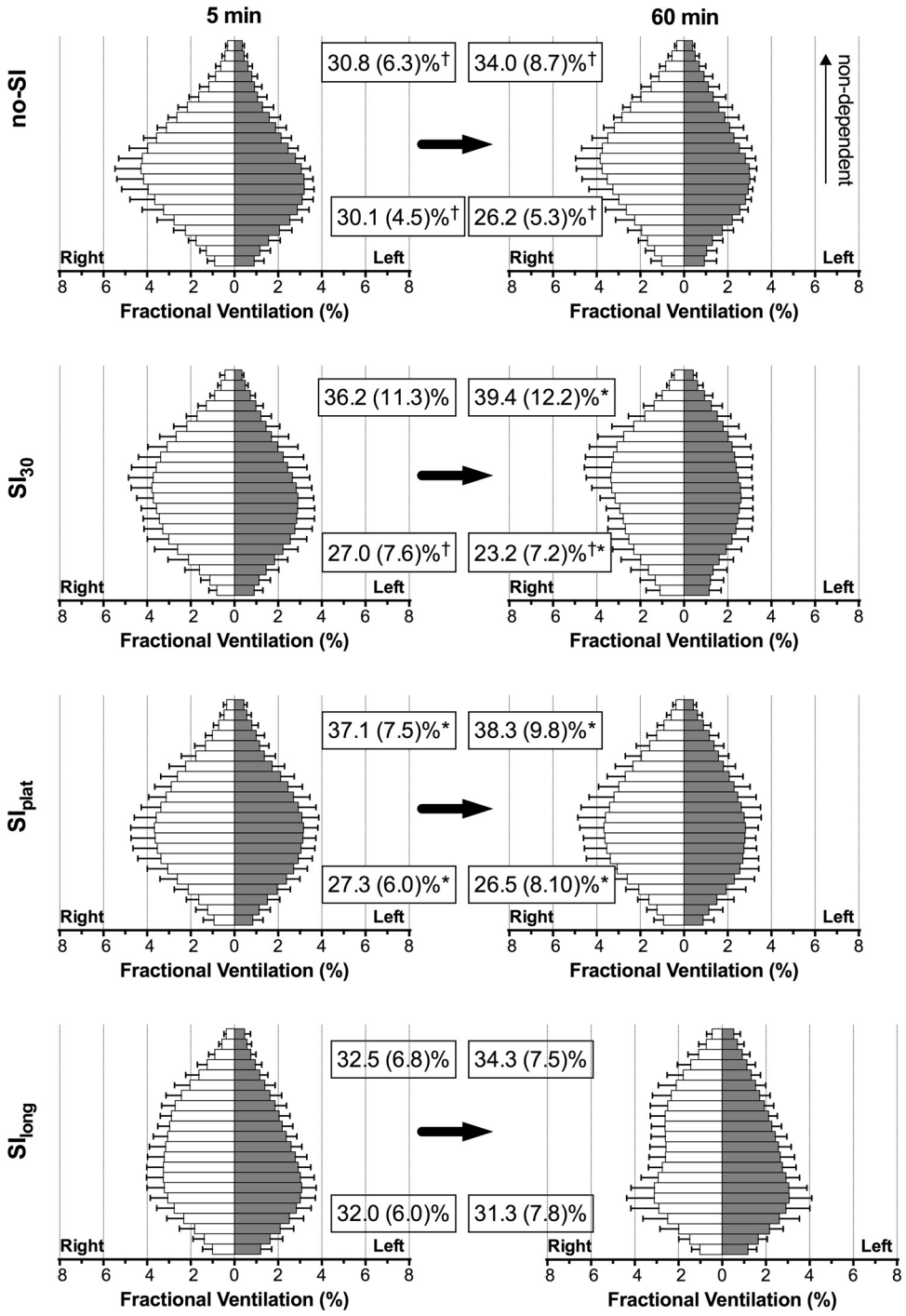

Fig. 6. fEIT images of the regional gravity-dependent distribution of V_t (expressed as %total V_t) at 5 and 60 min using the same format and orientation as Fig. 4*A*. The relative contribution of the equally weighted most- and least-dependent thirds of each lung slice to total V_t are shown in each histogram. All data mean and SD. Specific P values are listed in the text with $*P < 0.05$ (details in RESULTS), between gravity-dependent region for a strategy at that time point and $\dagger P < 0.05$, within gravity-dependent region between 5 and 60 min for a strategy.

Dunn's multiple comparison test). Lung injury scores were higher in the SI_{30} group vs. no-SI ($P < 0.01$), SI_{plat} ($P < 0.0001$), and SI_{long} ($P < 0.001$), due to variable regional differences between groups (Fig. 4*B*). The no-SI group demonstrated greater injury scores in the middle zone compared with SI_{plat} ($P = 0.040$).

All groups exhibited greater expression of all mRNA markers compared with the Fetal group (Fig. 4*D*), except SI_{long} (*CYR61* upper region) and no-SI (*CYR61* lower). There were no differences in mRNA expression between strategies. Within each strategy there were regional differences in the expression of *EGR1* (no-SI; $P = 0.042$), *CTGF* (all groups $P < 0.005$ except SI_{long}, $P = 0.064$), *IL8* (SI_{plat}, $P < 0.034$, and SI_{long}, $P < 0.001$), and *IL6* (all groups $P < 0.010$ except SI_{30}; $P = 0.733$, Kruskal-Wallis test, Dunn's multiple comparison).

DISCUSSION

Our preterm lamb study found that achieving volume equilibrium during a SI resulted in the best pulmonary mechanics and aeration. An inadequate SI duration provided no short-term benefit over PPV with sufficient PEEP alone. This study has

Table 2. *Total protein and histological injury data by gravity-dependent lung region*

	UVC			No-SI			SI_{30}			SI_{plat}			SI_{long}		
	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L
n	50	50	50	55	55	55	65	65	65	60	60	60	50	50	50
Total score (/20)	5.3 (1.3)	6.0 (1.4)	5.4 (1.5)	6.8 (1.4)[a]	6.7 (1.5)[a,d]	6.2 (1.5)[a]	7.6 (2.0)[a,d,e]	7.1 (1.8)[a,d,e]	7.3 (1.9)[a,b,d,e]	6.9 (2.1)[a]	6.1 (1.5)	6.3 (1.9)[a]	7.3 (1.7)[a]	6.1 (1.7)	6.2 (1.5)[a]
Alveolar wall thickness (/5)	2.7 (0.7)	2.6 (0.7)	2.6 (0.8)	3.1 (0.8)[a]	3.3 (0.7)[a]	3.1 (0.7)[a]	3.3 (0.9)[a]	3.2 (1.0)[a]	3.4 (1.0)[a,d]	3.0 (0.7)[a]	3.0 (0.7)[a]	3.1 (1.0)[a]	3.3 (0.9)[a]	3.2 (0.7)[a]	3.3 (0.7)[a]
Detached epithelial cells (/5)	1.6 (0.8)	2.0 (0.9)	1.8 (0.9)	2.3 (0.8)[a,c,d,e]	2.2 (0.8)	1.9 (1.0)	2.9 (1.4)[a,b,e]	2.6 (1.3)[a,d,e]	2.5 (1.5)[a,b,d,e]	2.3 (1.5)[a]	1.8 (1.0)	1.8 (1.1)	2.7 (1.1)[a]	1.6 (0.9)	1.7 (1.0)
Hyaline membranes (/5)	0.3 (0.5)	0.6 (0.6)	0.3 (0.5)	0.8 (0.6)[a,d,e]	0.6 (0.5)[e]	0.6 (0.5)[a]	0.7 (0.5)[a]	0.7 (0.5)[e]	0.8 (0.5)[a,b,d,e]	0.4 (0.5)	0.6 (0.5)	0.5 (0.5)[a]	0.5 (0.5)	0.4 (0.5)	0.4 (0.5)
Atelectasis (/5)	0.7 (0.6)	0.7 (0.6)	0.8 (0.7)	0.6 (0.5)	0.6 (0.7)	0.5 (0.6)	0.7 (0.7)	0.6 (0.8)	0.6 (0.6)	1.2 (1.0)[a,b,c,e]	0.7 (0.7)	0.8 (0.7)	0.8 (0.5)	0.9 (0.7)[b,c]	0.7 (0.7)[b,c]
Total protein, mg/ml		0.54 (0.26)			0.72 (0.46)			0.89 (0.54)			0.66 (0.55)			0.74 (0.73)	
Total score (/20)		5.6 (1.4)[a]			6.5 (1.5)[a]			7.3 (1.9)[a,b,d,e]			6.4 (1.9)[a]			6.5 (1.6)[a]	

All data are mean (SD). Regional injury: U, upper; M, middle; L, lower. UVC, unventilated control. [a]$P < 0.05$ vs. UVC; [b]$P < 0.05$ vs. no-SI; [c]$P < 0.05$ vs. SI_{30}, [d]$P < 0.05$ vs. SI_{plat}; [e]$P < 0.05$ vs. SI_{long}, one-way ANOVA with Tukey's posttests.

important clinical implications that have not been reported previously. While acknowledging the limitations inherent in the use of an animal model, we note that this is the first study to demonstrate that an individualized SI strategy using direct physiological feedback is possible and beneficial. These results suggest that predefined, standardized SI protocols based on fixed times and pressures may not always be beneficial and have the potential to be harmful. Our findings suggest that the use of an intentionally prolonged SI maybe a better approach than one that is too short.

The need to rapidly transition from a fluid-filled to aerated lung at birth provides a sound physiological rationale for the use of a SI as the first step in infants unable to generate adequate respiratory effort at birth (13, 39). Despite this the results of animal and human studies have been inconclusive (5, 11, 15, 18, 26, 29, 32, 33, 35–37). Notwithstanding variability in study design, all these studies adopted an a priori set SI duration based on time. Our data suggest that these conflicting results may be related to intersubject variability in the time constants of the respiratory system at birth and the subsequent volume response. The high variability in final EEV attained (26) during each SI restricts the utility of targeting a predefined absolute lung volume, as has been previously proposed (29). In a highly variable system, utilizing the relative time-based response is the only method of ensuring steady state has been achieved. Our use of such a dynamic, individualized approach to a SI with real-time monitoring, rather than targeting static a priori parameters, represents an important shift in the conceptualization of managing respiratory interventions at birth. Considering the volumetric response is arguably a more translatable approach to the use of animal models, which have different lung mechanics to humans, but whose lungs follow the same mechanical concepts. In our model, obtaining lung volume steady-state during a SI influences the efficacy and safety of the intervention. Previous studies of SI at birth have not reported absolute volumes or whether lung volume equilibrium, and thus optimal aeration, were attained within each individual subject (15, 26, 29, 35, 36). We contend that our study can be considered the first to systematically investigate time as a SI parameter. The observation that the volumetric behavior of the lung at birth when exposed to an inflating pressure is exponential was not surprising (23, 28, 38) and validates the utility, and research potential, of EIT to measure ΔEEV.

SI durations of 30 s or less have been extensively explored in newly born lambs (15, 26, 35–38). In a previous study by our group, aeration was found to be still ongoing by 30 s on post hoc EIT analysis in some lambs (37). The recent availability of real-time EIT imaging allowed this observation to be further investigated in this study. A 30-s duration was likely to be sufficient for volume equilibration in only 24% of recipients, and the SI_{30} group had no clinical benefit over PPV alone. More importantly, the SI_{30} group had the worst lung injury profile within the limitations of interpretation due to study design. Preterm lung injury is associated with heterogeneous regional volume states (30, 42). Unlike other studies we compared aeration and ventilation alongside regional injury, confirming that all strategies caused complex regional lung injury patterns. The differing spatiotemporal aeration and ventilation profiles suggest that multiple injurious states, such as atelectasis, overdistension, and tidal shear forces, were likely occur-

ring simultaneously. The gravitational changes over time (spatiotemporal changes) in aeration were the most striking finding and consistent with a previous study in preterm lambs without antenatal steroid exposure (37). Following any SI approach, the initial spatial benefits in aeration were not sustained, with all three strategies showing temporal gravity-dependent changes in aeration. The observation of more histological lung injury in the SI_{30} group and no increase in the SI_{long} group is potentially important in this context. It is simplistic to consider that the duration of a SI, and thus aeration, will have an incremental effect on the lung. The combination of poorer initial ΔEEV, indicating partial recruitment, combined with the greatest spatiotemporal heterogeneity of aeration and ventilation, and thus risk of atelectasis, overdistension, and tidal shear forces simultaneously, would explain the higher injury profile in the SI_{30} group. Within the dynamic process of aeration and ventilation in early life, lung protection is defined by dynamic volume state considerations, and considering it in terms of aeration or not is too simplistic.

Targeting lung volume equilibrium (or longer), irrespective of the time needed, resulted in better outcomes than a SI that did not achieve steady state lung volume, due to the longer transpulmonary pressure and better lung-liquid clearance (as evident by the initial improvements in R_{rs}). The finding that applying an intentionally too long SI did not also result in greater lung injury was unexpected. The benefits in oxygenation and lung mechanics obtained from the volumetric approaches were due to increased aeration in the least dependent regions, indicating these regions needed longer to aerate and our intention of significant overdistension may not have been achieved. Our approach of visually targeting the volumetric response globally will not identify these differences, something that could be addressed via targeting regional volumetric stability in future studies. The SI_{long} strategy was the only strategy that did not result in any spatiotemporal differences in V_t, indicating that the subtle differences in regional aeration seen were practically relevant if not statistically significant. The lack of difference in SI duration between SI_{plat} and SI_{long} is partially explainable by the intentional individualized SI design but also suggests that the SI_{long} strategy was insufficient to cause generalized overdistension. Practically, when clinicians are uncertain, it may be more prudent to apply a slightly longer SI rather than a shorter one.

The SI durations required in the SI_{plat} and SI_{long} to achieve volumetric, and arguably, physiological optimization of the initial aeration for lambs in our study are unlikely to be required in human infants, whose smaller lung volumes and different chest mechanics will dictate shorter absolute time constants and thus time to volume stability. However, the concepts identified in this study, and the models used, are translatable (23, 28). The application of excessive transpulmonary pressure to the thorax, and overdistension, may have cardiovascular consequences (25). We were not able to identify any differences in HR, ABP, and CBF. As pulmonary artery blood flow and ductal status were not measured, our hemodynamic findings should be interpreted with caution. Pulmonary blood flow was not adversely impacted in a similar preterm lamb study comparing a 40-s SI against no-SI, although global and regional SI volumes were not reported (29). The same study found that cerebral blood flow was elevated in the no-SI group and correlated with hypoxia. Our study, with larger group sizes, reassuringly did not identify any difference in CBF. Clinically translatable measures of cerebral oxygenation, such as near-infra red spectroscopy, have been used to describe SI at birth (26) and warrant consideration in future studies, ideally in humans.

The main focus of our study was the interaction among SI strategy, spatiotemporal volume patterns, oxygenation, and mechanics. Fundamentally, any clinical strategy addressing these important short-term measures also needs to be lung protective for meaningful clinical translation. Preterm lung injury is a complicated multifactorial process, and our study period and design only allowed assessment of early markers of injury, limiting interpretation. This was intentional to allow isolation of the inflammatory events occurring in early life from those of the injurious cycle of ongoing mechanical ventilation, but the relatively short study duration, although still longer than many previous SI animal studies (26, 29, 32, 33, 36), was unlikely to be sufficient to result in posttranscription protein changes within the lung and correlation with the histology findings. We chose accepted markers of preterm lung injury known to be upregulated within 30 min of injurious ventilation (11, 12, 14, 40), but clear patterns for specific mechanisms of injury were not apparent. This may have been influenced by the sample size of each treatment group and the large variability in mRNA expression due to the multiple influences beyond those variables controlled in our study. Our study was powered to assess short-term outcomes and, although considerably larger than all other previous preterm respiratory transition studies (12, 15, 25–27, 29, 32, 33, 35–38, 40), was insufficient for evaluation of some of the mRNA parameters. This highlights the difficulty in defining acute preterm lung injury and the need for new approaches. Despite these limitations, the spatial injury findings are potentially important, especially when considered with the EIT data. In addition our sample size of 12/group would allow detection of a 1.8 (2.0) difference in lung injury score. The observation that injury changes were more prominent in the dependent lung, the region most susceptible to temporal volumetric changes, is in keeping with our understanding of lung injury in the surfactant-deficient lung. This suggests that our integrated regional volume state, mechanics, and injury methodology could be used in longer term studies primarily designed to consider injury pathways.

This study has some additional limitations. To limit confounding factors between groups, the lambs were anesthetized and ventilated with cuffed ETT, not a common clinical scenario but consistent with other similar animal studies at birth (15, 26, 27, 29, 32, 33, 36–38, 40). Measuring dynamic regional lung volumes during birth transition is difficult. Although limited to the neonatal research setting, EIT offers the only practical solution at present, and our study demonstrates potential utility to guide (42) rather than simply monitor therapy but is not without well-described limitations (16). Applying individual EIT electrodes is a time-consuming and operator-dependent process that has limited clinical utility. However, three new EIT systems are now commercially available. These use a single nonadhesive belt and have very short application times that would potentially allow for use in the delivery room during noninvasive ventilation. Although the calibration of EIT signals to known volume measurements is validated (1, 22), we only calibrated the global signal (17) and

limited our interpretation of regional volumetric behaviour to relative rather than absolute changes.

In conclusion, our study suggests that the high variability and unpredictability of the mechanical properties of the lung limits the efficacy of the current standardized approaches to a sustained inflation at birth. An individualized approach to SI, tailored to the volumetric response of the lung over time to an applied pressure was effective in optimizing aeration, homogeneous tidal ventilation, and bedside respiratory outcomes, with potentially less lung injury. The optimal strategy to delivering a sustained inflation to the preterm lung, and the most suitable real-time monitoring system to guide it, still need to be further investigated. In the meantime, this study suggests a SI that is as long, or longer, than the time required for volume equilibrium at birth may confer less risk of injury than one that is too short.

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DISCLOSURES

Politecnico di Milano University (the institution of C.E. Zannin and R. L. Dellacà) owns a patent on the use of forced oscillation technique for the detection of lung volume recruitment/derecruitment.

AUTHOR CONTRIBUTIONS

Author contributions: D.G.T., E.Z., M.S., R.L.D., A.A., and P.G.D. conception and design of research; D.G.T., A.L., C.E.E.Z., A.E.R., E.P., D.B., and M.S. performed experiments; D.G.T., A.L., C.E.E.Z., A.E.R., E.Z., E.P., D.B., and B.G. analyzed data; D.G.T., C.E.E.Z., A.E.R., E.Z., R.L.D., A.A., B.G., and P.G.D. interpreted results of experiments; D.G.T., A.L., C.E.E.Z., A.E.R., E.Z., E.P., and B.G. prepared figures; D.G.T. and A.L. drafted manuscript; D.G.T., A.E.R., E.P., R.L.D., F.M., A.A., B.G., I.F., and P.G.D. edited and revised manuscript; D.G.T., A.L., C.E.E.Z., A.E.R., E.Z., E.P., D.B., M.S., R.L.D., F.M., A.A., B.G., I.F., and P.G.D. approved final version of manuscript.

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