Inspiration regulates the rate and temporal pattern of lung liquid clearance and lung aeration at birth

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1Department of Physiology, 2School of Physics, 3Monash Centre for Synchrotron Science, and 4Department of Biological Engineering, Monash University, Melbourne; 5The Division of Newborn Services, Royal Women's Hospital, Carlton, Victoria, Australia; and 6Spring-8, Japan Synchrotron Radiation Research Institute, Sayo, Hyogo, Japan

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Siew ML, Wallace MJ, Kitchen MJ, Lewis RA, Fouras A, te Pas AB, Yagi N, Usugi K, Siu KK, Hooper SB. Inspiration regulates the rate and temporal pattern of lung liquid clearance and lung aeration at birth. J Appl Physiol 106: 1888–1895, 2009. First published April 2, 2009; doi:10.1152/japplphysiol.91526.2008.—At birth, the initiation of pulmonary gas exchange is dependent on air entry into the lungs, and recent evidence indicates that pressures generated by inspiration may be involved. We have used simultaneous plethysmography and phase-contrast X-ray imaging to investigate the contribution of inspiration and expiratory braking maneuvers (EBMs) to lung aeration and the formation of a functional residual capacity (FRC) after birth. Near-term rabbit pups (n = 26) were delivered by cesarean section, placed in a water plethysmograph, and imaged during the initiation of spontaneous breathing. Breath-by-breath changes in lung gas volumes were measured using plethysmography and visualized using phase-contrast X-ray imaging. Pups rapidly (1–5 breaths) generate a FRC (16.2 ± 1.2 ml/kg) by inhaling a greater volume than they expire (by 2.9 ± 0.4 ml·kg⁻¹·breath⁻¹ over the first 5 breaths). As a result, 94.8 ± 1.4% of lung aeration occurred during inspiration over multiple breaths. The incidence of EBMs was rare early during lung aeration, with most (>80%) occurring after >80% of max FRC was achieved. Although EBMs were associated with an overall increase in FRC, 34.8 ± 5.3% of EBMs were associated with a decrease in FRC. We conclude that lung aeration is predominantly achieved by inspiratory efforts and that EBMs help to maintain FRC following its formation.

Before birth, the fetal lungs are liquid filled and take no part in gas exchange, which occurs across the placenta (11, 14, 33). At birth, the liquid occupying the airways must be cleared to allow the entry of air and the onset of air breathing, but a thin film of liquid must remain to protect the epithelium from desiccation (1, 33). The process of lung aeration initiates major changes in cardiopulmonary physiology (5, 15) that are essential for the transition of the lung into an efficient gas exchange organ after birth and include a surface tension-mediated increase in lung recoil, increased oxygenation, a large increase in pulmonary blood flow, a reduction in intrapleural pressure, and closure of the ductus arteriosus (34). Although airway liquid clearance and lung aeration trigger many of these cardiopulmonary changes at birth, the factors regulating airway liquid clearance as well as the development and maintenance of an end-expiratory gas volume (functional residual capacity; FRC) are not clear. Previous studies have indicated that inspiration (16, 28, 38, 39) and factors regulating breathing activity (24, 43) are important for establishing and maintaining FRC, but the mechanisms involved and their relationship with airway liquid clearance are unclear. As a result, although failure to clear airway liquid is a major cause of respiratory morbidity in newborn infants (18), the underlying mechanisms are not well understood.

Adrenaline-induced activation of epithelial Na⁺ channels (ENaCs), leading to transepithelial Na⁺ uptake and lung liquid reabsorption, is thought to be the primary mechanism for airway liquid clearance at birth (18, 33), allowing the formation of a FRC. However, using phase-contrast (PC) X-ray imaging, we have shown that lung aeration is closely associated with inspiratory activity after birth, with little or no distal movement of the air-liquid interface during expiration and apnea (16). This indicates that transpulmonary pressures generated by inspiration may play a major role in both lung aeration and airway liquid clearance at birth (16), leading to the formation of FRC. However, the relative contributions of active liquid reabsorption and inspiratory activity to lung liquid clearance and lung aeration are unknown.

Immediately after birth, the respiratory pattern of the newborn is highly variable but gradually becomes more regular over the first 60 min postpartum (9, 37). This pattern is commonly characterized by deep inspirations that are followed by a variety of expiratory braking maneuvers (EBMs), usually caused by closure of the glottis, which sustain elevated airway pressures and prolong expiration time (22, 23, 30). These maneuvers are thought to help develop and maintain FRC during the immediate newborn period when the lung is partially liquid filled and the chest wall is compliant (10, 28). However, the relative contribution of these breathing activities to lung aeration and the development of a FRC after birth are unknown.

Our aim was to define the relative contributions of inspiratory activity and EBMs to lung aeration and to the increase and maintenance of FRC after birth, which have not been previously assessed (8, 19, 29, 38, 39). We measured the breath-by-breath increase in lung gas volumes from birth in term rabbit pups delivered by cesarean section. Changes in lung gas volumes were measured using a water-filled plethysmograph while the lungs were simultaneously imaged using PC X-ray imaging, which can resolve the smallest air-filled structures of the lung (16, 20, 21, 25). PC X-ray imaging was used to demonstrate the relationship between lung aeration and inspiration by examining the breath-by-breath movement of the air-liquid interface as it traveled toward the distal terminal airway.
sacs. We hypothesized that pressures generated by inspiration are the primary determinant of lung aeration.

MATERIALS AND METHODS

Animals. All animal procedures were approved by the SPring-8 Animal Care and Monash University’s School of Biomedical Science’s Animal Ethics Committees. At 30 or 31 days gestational age (term = 32 days), pregnant New Zealand White rabbits were anesthetized using propofol (Rapinovet; 12 mg/kg bolus followed by 100 mg·kg⁻¹·h⁻¹ infusion iv). Propofol is short acting and was chosen to minimize breathing inhibition in pups, particularly since they can normally experience apnea for >1 min; propofol did not affect spontaneous breathing in dams. Pups were delivered by cesarean section and placed in a water-filled plethysmograph. Because the pups were active and able to initiate spontaneous breathing immediately after birth, the fetal membranes were kept intact over the pup’s nose and mouth to prevent lung aeration until imaging commenced (~1 min). After experiments, all animals were killed with an overdose of sodium pentobarbital (Nembutal; 100 mg/kg) administered intravenously (doc) or intraperitoneally (pups).

Plethysmography. The plethysmograph comprised an upright cylindrical Perspex (polymethyl methacrylate) water-filled chamber that was sealed at the top by a rubber diaphragm but was open to atmosphere via a water column. The pup’s body was placed in the water chamber (preheated to 37°C) with its head protruding through the diaphragm, which formed a water-tight seal around the pup’s neck. The increase in lung volume resulting from air inflation caused the displacement of water from the chamber into the water column, which was measured as an increase in pressure using a pressure transducer (DTX Plus TNF-R; Becton Dickinson) and recorded using a data acquisition system (PowerLab; ADInstrumments, Sydney, Australia) (Fig. 1). The system was calibrated by injecting 1 ml of water before each experiment.

PC X-ray imaging. PC X-ray imaging uses refractive index differences to enhance image contrast and is ideal for imaging soft tissues that weakly absorb X-rays but comprise media with markedly different refractive indexes (e.g., air and water) (20, 21, 25). Refraction at the air-tissue (water) interfaces causes significant phase shifts of the X-rays as they pass through the lung, producing interference patterns at a finite propagation distance beyond the lung (21, 25). With the use of this technique, the liquid-filled fetal lung becomes visible as air enters the airways and the air-liquid interface moves distally toward the alveoli (Fig. 2) (16, 25). Partially coherent synchrotron radiation (35) was used as the X-ray source because of its unique properties, particularly its brightness, which make it ideal for this type of imaging.

Pups were placed in the plethysmograph and imaged while their breathing activity was recorded. Studies were conducted in experimental hatch 3 of beam line 20B2, in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan, using previously described techniques (16, 21, 25). The X-ray energy was 25 keV, and the pups were located 2.0 m upstream of the detector (Hamamatsu C4742-95HR), which had a pixel size of 22.47 µm (2 × 2 binning mode) and an active area of 24.45 × 20.85 mm². A short exposure time (83 ms) was used to minimize motion blur, and images were acquired at 800-ms intervals. A preobject shutter prevented radiation exposure between image acquisitions, and the total radiation dose per pup was ~6 mGy.

Experimental procedure. Pups were delivered one at a time, and the umbilical cord was tied with the fetal membranes remaining intact over the pup’s nose and mouth to prevent the onset of gaseous ventilation. The pup was quickly placed in the water-filled plethysmograph (head out), ensuring the chest was in the path of the X-ray beam. The fetal membranes overlaying the pup’s mouth and nose were then removed, allowing the pup to breathe spontaneously; the plethysmograph recording began before the membranes were removed, and imaging commenced as soon as possible (~1 min) after their removal. Plethysmograph recordings and imaging continued until the pup’s lungs were fully aerated.

To determine whether the lungs could be aerated as a result of ventilation alone, in the absence of active energy-dependent endogenous mechanisms (such as Na⁺ reabsorption), fetal rabbits (n = 4) were killed before delivery by anesthetic overdose so that their lungs remained liquid filled. At ~2–3 h after death, these pups were intubated and mechanically ventilated in the plethysmograph while they were simultaneously imaged. Pups were initially ventilated with a peak inspiratory pressure (PIP) of 30 cmH₂O and a positive end-expiratory pressure (PEEP) of 5 cmH₂O; as the tidal volume (VT) increased, the PIP was reduced to achieve a VT of ~7 ml/kg.

Data analysis. All lung gas volumes were adjusted for body weight and were measured at 800-ms intervals, in synchrony with image acquisition, to demonstrate the temporal increase in FRC from birth. For each pup, the inspired and expired volumes were measured for the first 100 breaths after birth. Breaths were divided into blocks of five sequential breaths, and the average inspired (VTi) and expired (VTe) volume as well as the mean difference (VTi – VTe) was calculated. In some pups, the recordings did not extend for the first 100 breaths because they rapidly aerated their lungs and displayed prolonged (1–2 min) apneic periods. As a result, the number of pups per data point decreased from n = 26 at the beginning to n = 11 at >90 breaths.

To determine the relative contribution of respiratory activity to lung aeration and the increase in FRC at birth, we calculated the increase in end-expired lung volume by measuring the FRC immediately before and after each breath (Fig. 1). For each pup, these values were summed and then expressed as a percentage of the maximum FRC gained during the recording period; increases in FRC were only included when they increased above the last recorded maximum FRC value.

EBMs were defined as a cessation or marked slowing in expiratory flow that lasted for >100 ms, occurred before 85% of expiration was complete, and was followed by completion of expiration (Fig. 3). For each breath containing an EBM, the difference in FRC immediately before and after the breath was measured to determine the influence of EBMs on FRC. The incidence of EBMs in relation to the increase in FRC was also calculated.

Statistical analysis. Results are means ± SE, and all data were tested for normal distribution before analysis. Changes in tidal volumes with time were analyzed using a two-way repeated-measures analysis of variance (ANOVA), followed by a least significant difference post hoc test. If data were not normally distributed, a Friedman’s repeated-measures ANOVA on ranks was used, followed by a Stu-
dent-Newman-Keuls post hoc test. Changes in the incidence of EBM

Changes in the incidence of EBM were analyzed using a one-way ANOVA, and their effects on FRC were analyzed using a Student’s paired t-test.

RESULTS

Animal data. Recordings were collected from 26 spontaneously breathing newborn rabbit pups from a total of 10 pregnant rabbits; 20 of these pups were simultaneously imaged. All newborn pups were delivered at 31 days of gestational age except for three, which were delivered at 30 days of gestational age. The average pup weight was 44.7 ± 1.6 g.

Increase in FRC from birth. Changes in lung air volumes were measured from birth for an average of 13.0 ± 1.1 min. The mean maximum FRC attained was 16.2 ± 1.2 ml/kg, but this varied from 7.5 to 25 ml/kg in different pups. The temporal pattern for the increase in FRC also varied between pups. In some pups the increase in FRC was continuous and sigmoid-like (Fig. 4A), whereas in others, intermittent reductions in FRC occurred (Fig. 4B). The time taken for pups to achieve maximum FRC and aerate their lungs varied markedly between pups, ranging from a few seconds to more than 10 min, but was closely related to respiratory activity (see below).

Change in respiration rate from birth. The minute-by-minute spontaneous respiration rate was very variable between pups during the first 12 min after birth. In any 1-min period, pups were either apneic (which could last for >3 min) or had a rate of >50 breaths/min. When the data were combined from all pups, the respiration rate did not change over the first 12 min after birth (8 ± 1 breaths/min during 1st minute vs. 9 ± 2 breaths/min during 12th minute).

Fig. 2. Phase-contrast X-ray images (A–F) were acquired at the times indicated by the arrows (a–f) in Fig. 1. Images were acquired immediately before (A, C, and E) and after (B, D, and F) the 3rd, 4th, and 5th breaths after birth, respectively. In A, only the medial/basal lobes of the lung are partially aerated, with no aeration in the apical lobes, and no definition of the rostral margin of the diaphragm is apparent. Image in B was acquired 1.6 s after that in A and immediately followed the 3rd breath. This single breath resulted in aeration of the medial and basal lobes of the lung, thereby providing definition to the rostral margin of the diaphragm, as well as some aeration of the apical lobes. After the 5th breath (F), the lung is relatively well aerated. This process can be observed in real time by examining Supplemental Movie 1.
Breath-by-breath changes in \(V_{T1}\) and \(V_{TE}\) from birth. At the onset of air breathing, the \(V_{T1}\) varied between 5.8 and 25.3 ml/kg in different pups and gradually decreased \((P < 0.05)\) from a mean of 15.4 ± 2.1 ml/kg for the first 5 breaths to 8.5 ± 1.4 ml/kg for breaths 96 to 100 (Fig. 5A). Similarly, at the onset of air breathing, the \(V_{T1}\) varied between 5.2 and 21.7 ml/kg in different pups and decreased \((P < 0.05)\) from 12.5 ± 1.0 ml/kg for the first 5 breaths to 8.2 ± 1.2 ml/kg for breaths 96 to 100.

Breath-by-breath differences in \(V_{T1}\) and \(V_{TE}\). Over the first five breaths after birth, the \(V_{T1}\) was 2.9 ± 0.4 ml/kg per breath larger than the \(V_{T1}\) (see Figs. 1 and 2), so pups can accumulate, on average, a FRC of ~15 ml/kg during the first five breaths after birth, assuming that the FRC did not decrease between breaths (in only 15 of 26 pups), accounting for 5.2 ± 0.2 ml/kg \((P < 0.05)\) for breaths 6–10 and tended to decrease further, although not significantly, over subsequent breaths. However, as a positive difference persisted, neonatal rabbit pups continued to inspire larger volumes than they expired for the first 100 breaths.

Increase in FRC from birth: role of breathing activity. The increase in FRC associated with individual breaths accounted for 94.8 ± 1.4% of the maximum FRC achieved by each pup (Figs. 1 and 2). Only small increases in FRC could be detected between breaths (in only 15 of 26 pups), accounting for 5.2 ± 1.6% of the FRC attained (Fig. 1). The increase in lung aeration caused by individual breaths is clearly shown in Figs. 1 and 2 as well as in movie sequences (see Supplemental Movie 1; supplemental data for this article is available online at the Journal of Applied Physiology website), which simultaneously display the plethysmograph recording and images in near real time. Supplemental Movie 1 is from a different pup from that displayed in Figs. 1 and 2. By determining the duration and increase in FRC for each breath, we calculate that the average rate of lung aeration, which must equate to the rate of airway liquid clearance, during a single breath is 9.7 ± 0.8 ml·kg⁻¹·s⁻¹ (or ~35 l·kg⁻¹·h⁻¹).

Increase in FRC from birth: role of EBMs. An analysis of FRC values measured immediately before and after a breath with an EBM (see Fig. 3) demonstrates that EBMs are associated with both increases and decreases in FRC (Fig. 6A). However, the proportion of EBMs associated with an increase in FRC was greater than that associated with a decrease \((65.2 ± 5.3 \text{ vs. } 34.8 ± 5.3\%)\), and the mean volume increase per EBM was greater than the mean volume decrease \((1.7 ± 0.2 \text{ vs. } 1.0 ± 0.2 \text{ ml/kg})\). As a result, EBMs were associated with a small but significant increase in FRC of 0.7 ± 0.3 ml/kg per EBM (Fig. 6A). However, the incidence of EBMs increased as the FRC increased after birth, with >80% detected after the FRC had reached 80% of the maximum FRC for each pup (Fig. 6B).

Lung aeration and liquid clearance in fetal rabbits ventilated after death. In fetuses ventilated 2–3 h after death, the images show that the lungs aerate, down to the distal air sacs (Fig. 7; see Supplemental Movie 2), in a manner similar to live, spontaneously breathing pups (<5 min). The increase in FRC was also very similar to the pattern displayed by some spontaneously breathing pups (compare Figs. 4A and 7A) and increased with each breath (Fig. 7A). However, the temporal change in \(V_t\) was very different in ventilated dead fetuses compared with spontaneously breathing pups, gradually increasing as the lungs aerated (Fig. 7C).

**Fig. 3.** Plethysmograph recording (i) of a single breath from a spontaneously breathing newborn rabbit pup that included an expiratory braking maneuver (EBM). The EBM is indicated by the horizontal arrow and is followed by completion of the breath and the return of lung volume to FRC, which in this instance was similar to that recorded before the breath. Images in ii are consecutive images (A and B) collected at the times indicated by the arrows (a and b) and demonstrate that lung aeration remains constant throughout the EBM.
DISCUSSION

Using simultaneous PC X-ray imaging and plethysmography, we have investigated the role of inspiration and EBMs in lung aeration and the establishment and maintenance of FRC from birth. We found that lung aeration primarily (~95%) resulted from inspiration, as indicated by larger inspired volumes compared with expired volumes and the distal movement of the air-liquid interface with each breath, which confirms previous suggestions (28). In contrast, the FRC rarely increased in the absence of breathing activity (Fig. 1; Supplemental Movie 1), and after an FRC was established, rabbit pups continued to inspire larger volumes than they expired for at least the first 100 breaths after birth. This indicates that pups can lose some FRC between breaths and that each inspiration helped to reestablish FRC (Fig. 1A). Although we found that EBMs resulted in a small net increase in FRC, most EBMs (>80%) occurred after a significant proportion of the FRC was established. Thus term rabbit pups initially clear their lungs of liquid and develop a FRC primarily as a result of inspiration, and once a FRC is established, they maintain it by continuing to inhale more than they exhale and by using EBMs.

Lung liquid clearance and aeration at birth. The suggested mechanisms for lung liquid clearance at birth include mechanical forces and an osmotic gradient generated by Na⁺ reabsorption (37). Because uterine contractions can increase fetal spinal flexion, particularly after membrane rupture, the resulting increase in thoracic pressure (12) can increase liquid loss via the nose and mouth (39), particularly after membrane rupture. Although increased spinal flexion may contribute to some lung liquid loss during labor (15, 26, 37), pups in this study were delivered by cesarean section before labor, so it is unlikely that this mechanism contributed to airway liquid clearance.

Adrenaline (and vasopressin)-induced activation of ENaCs, particularly amiloride-sensitive ENaCs, are thought to play a major role in airway liquid clearance at birth (32, 40, 41). However, although considerable evidence supports a role for Na⁺ uptake in alveolar fluid clearance, it is likely that additional mechanisms are involved. Indeed, blockade of ENaCs with amiloride delays, but does not prevent, lung liquid clearance at birth (31). Similarly, although α-ENaC (but not β- or γ-ENaC) null mice have impaired lung water clearance at birth, they can survive for up to 40 h after birth and must establish some pulmonary gas exchange (17).

Fig. 4. Changes in lung air volume, measured in 2 spontaneously breathing rabbit pups from birth, demonstrating the temporal pattern for the increase in FRC. Values were measured at 800-ms intervals, simultaneously with image acquisition; the outlier points are lung volume measurements coinciding with breathing activity. The temporal pattern for the increase in FRC depicted in A is relatively continuous and sigmoidlike, whereas the pattern depicted in B demonstrates an overall increase in FRC, interspersed with reductions in FRC.

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Fig. 5. Breath-by-breath analysis of the change in mean inspired (VTi; A) and mean expired tidal volumes (VTe; B) as well as the mean difference between the inspired and expired tidal volumes (VTi − VTe; C) for the first 100 breaths after birth. The data were divided into groups of 5 breaths, and each data point represents the mean ± SE for those 5 breaths. For each graph, values that do not share a common letter are significantly different from each other (a,b,c,dP < 0.05).
We consider that the entry of air into the lung after birth must equate to airway liquid clearance, because otherwise the liquid would have to remain within and line the distal airways following lung aeration. This would form a thick liquid layer that would increase the barrier for gas exchange, and the airways would have to greatly expand to accommodate the increase in gas volume (~16 ml/kg) in addition to the preexisting liquid volume (>20 ml/kg) (11, 14, 33). Thus the airways would have to expand to a volume of ~36 ml/kg at rest, increasing to 50–60 ml/kg at end inspiration. This would place the lung at the top of its pressure-volume curve, decrease its compliance, reduce the inspirotary reserve volume, and expose the lung to volutrauma. Furthermore, if liquid remained in the airways, there is no mechanism to prevent liquid from refilling the airways during expiration caused by recoil of the remaining gas. Because pups can ventilate with little effort following the first few breaths and are well oxygenated (pink in color), clearly this does not occur. Furthermore, lung compliance markedly increases during lung aeration in ventilated pups (Fig. 7B), as indicated by an increasing Vt that eventually requires a reduction in inspiratory pressure with lung aeration (PIP data not shown).

Our data indicate that ~95% of lung aeration occurs during inspiration in spontaneously breathing pups, which is consistent with our previous observations (16). We suggest that transepithelial hydrostatic pressures associated with inspiration create the driving pressure for liquid to leave the airways and enter the interstitial tissue compartment. Because these pups were born by cesarean section and did not undergo the stress of labor, it is unlikely that mechanisms normally induced by labor (3, 32, 42) were activated in these pups. It is not surprising, therefore, that little liquid clearance could be attributed to these mechanisms, which is consistent with the finding of airway liquid clearance in ventilated dead fetal rabbits (Fig. 7; Supplemental Movie 2). Stress-induced mechanisms such as Na⁺ reabsorption could not be activated in these fetuses (at >2 h after death), and the finding that FRC increased with each breath indicates that hydrostatic pressures alone are capable of aerating the lung, presumably by clearing the airways of liquid. We calculate that liquid is cleared at ~35 l·kg⁻¹·h⁻¹ during a spontaneous breath (~300 ms in total), which is ~1,000 times greater than liquid reabsorption rates measured during high-dose adrenaline infusions (13, 32, 41, 42).

It is unlikely that airway liquid clearance at birth is simply determined by transepithelial hydrostatic pressure gradients, because otherwise some liquid should reenter the airways during expiration. We propose that liquid cleared from the airways at birth crosses a semipermeable membrane to enter the interstitial tissue compartment (2), the rate of which could be facilitated by an increase in epithelial pore sizes (6, 7). Retention of the liquid within the interstitial tissue compartment explains the transient increase in interstitial tissue pressure, to ~6 cmH₂O (27), and chest wall expansion (16) that occurs shortly after birth. Although the interstitial tissue pressure gradually declines to become subatmospheric within 6 h (27), the higher pressures (of ~6 cmH₂O) at rest after birth should promote water movement back into the airways. Thus other mechanisms must help retain water within the interstitial tissue compartment, which may include transepithelial osmotic pressures generated by Na⁺ reabsorption (33) and oncotic pressures (7), as well as elevated airway pressures caused by EBMs. Whatever the mechanisms, they likely play an important role in lung liquid clearance, because if the liquid is not retained within the interstitial tissue compartment, it cannot be cleared from the lungs via the lymphatics and blood vessels (2).

Establishing and maintaining a FRC. Numerous mechanisms have been postulated to explain how newborns create and maintain a FRC after birth, and EBMs, which slow expiratory flow and increase airway pressures (22, 23), are a likely candidate. We found that spontaneously breathing rabbit pups establish most of their FRC by inhaling larger volumes than they exhale, resulting in the accumulation of FRC with each breath. Surfactant recruitment to the air-liquid interface must also play an important role in this process by reducing lung recoil and stabilizing the airways as they recoil during expiration. Because pups did not begin to adopt EBMs until most (>80%) of the FRC had been established, EBMs are unlikely to be a major contributing factor to FRC formation.
during the early stages. However, a possible limitation of this study is our inability to detect subtle changes in expiratory flow using water-based plethysmography. The high viscosity and inertia of water (vs. air) movement between the chamber and water column must limit the sensitivity of detecting brief and/or small changes in flow and may increase the work of breathing. As a result, it would be difficult to detect EBMs that were short in duration (<100 ms) and/or caused small reductions in expiratory flow, which is the reason for choosing the classification described in the Materials and Methods. Furthermore, although unlikely, it is possible that the rubber seal around the pup’s neck may have influenced the duration and number of EBMs made by the pups. Nevertheless, we detected up to 40 EBMs per pup over the 13-min recording period, with the incidence in any one pup depending on whether the FRC increased rapidly or slowly. Furthermore, in some instances the technique was sensitive enough to detect the pup’s heart beat (Fig. 1).

Our finding that EBMs were associated with an overall increase in FRC is consistent with the suggestion that they help to maintain FRC after birth (23, 30). Although we also recorded numerous EBMs (34.8 ± 5.3%) that were associated with a reduction in FRC, it is possible that this reduction would have been greater in the absence of an EBM. The mechanism responsible for a reduction in FRC following an EBM is unknown but could be associated with abdominal muscle contractions. This would pressurize the airways when the glottis is closed but might reduce FRC if the glottis opens while thoracic pressure remains high. As the chest wall gradually stiffens after birth, it becomes the primary factor maintaining FRC and basal lung expansion at rest. Since this process can take weeks to accomplish (4, 36), it is likely that newborns continue to use mechanisms such as EBMs to maintain their FRC during this time.

Reductions in FRC were common during lung aeration (see Fig. 4B), and it is interesting that these reductions were usually (almost always) associated with a series of large breaths that followed a prolonged apneic period (Fig. 4B). Although the mechanism is unknown, it is possible that apnea-induced augmented breaths led to expiratory muscle activity, particularly abdominal muscle contraction, causing high expiratory gas flows that reduced FRC. Other explanations could include the reentry of lung liquid into the airways or a temporary disruption to the surfactant monolayer, thereby increasing lung recoil. In any event, it is clear that FRC can vary markedly in the immediate newborn period and is likely to be determined by the sum of numerous opposing forces.

Although average breathing rates were relatively low (8–12 breaths/min) in our study, this resulted from intermittent and prolonged apneic periods interspersed with breathing periods at up to 50 breaths/min. It is possible that propofol anesthesia given to the mother influenced respiratory rates in pups during the immediate newborn period, although it had no effect on breathing rates in the mother. However, the pups were mostly active at birth, were able to rapidly aerate their lungs using spontaneous inspiratory efforts, and were able to sustain their respiratory needs, as indicated by their pink coloring at the end of the imaging/recording period.

Our study has investigated the relative contribution of breathing activity and EBMs to lung aeration and the formation and maintenance of FRC after birth. We found that the majority (~95%) of lung aeration occurs during breathing activity,
suggesting that transepithelial pressures generated by inspiration provide the predominant pressure gradient for airway liquid removal. EBMs were observed at increasing frequency with increasing FRC after birth, indicating that EBMs are unlikely to play a significant role in the initial formation of FRC. However, they are likely to play an important role in maintaining FRC after it has developed, particularly while the lung tissue retains a significant proportion of the reabsorbed lung liquid and the chest wall is compliant.

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