Ventilation/perfusion mismatch during lung aeration at birth

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Transition to newborn life is triggered by lung aeration, which stimulates a large increase in pulmonary blood flow (PBF). Current theories predict that the increase in PBF is spatially related to ventilated lung regions as they aerate after birth. Using simultaneous phase-contrast X-ray imaging and angiography we investigated the spatial relationships between lung aeration and the increase in PBF after birth. Six near-term (30-day gestation) rabbits were delivered by caesarean section, intubated and an intravenous catheter inserted, before they were positioned for X-ray imaging. During imaging, iodine was injected before ventilation onset, after ventilation of the right lung only, and after ventilation of both lungs. Unilateral ventilation increased iodine levels entering both left and right pulmonary arteries (PAs) and significantly increased heart rate, iodine ejection per beat, diameters of both left and right PAs, and number of visible vessels in both lungs. Within the 6th intercostal space, the mean gray level (relative measure of iodine level) increased from 68.3 ± 11.6 and 70.3 ± 7.5%·s to 136.3 ± 22.6 and 136.3 ± 23.7%·s in the left and right PAs, respectively. No differences were observed between vessels in the left and right lungs, despite the left lung not initially being ventilated. The increase in PBF at birth is not spatially related to lung aeration allowing a large ventilation/perfusion mismatch, or pulmonary shunting, to occur in the partially aerated lung at birth.

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would be spatially related to regional lung aeration, with the greatest increase in PBF occurring in aerated regions. The relationship between PBF and lung aeration was examined by imaging pulmonary vessels using an iodine-based contrast agent before lung aeration, during aeration of the right lung, and following aeration of both lungs in ventilated newborn rabbits.

METHODS

Experimental Procedure

All animal procedures were approved by the SPring-8 Animal Care and Monash University’s School of Biomedical Science’s Animal Ethics Committees. All studies were conducted in experimental hutch 3 of beamline 20B2, in the Biomedical Imaging Centre at the SPring-8 synchrotron, Japan.

Pregnant New Zealand White rabbits at 30 days gestation (term ≈ 32 days) were anesthetized using Rapinovet (12 mg/kg bolus iv; propofol, Schering-Plough Animal Health), and intubated, and anesthesia was maintained by isoflurane inhalation (1.5–4%; Isoflurane, Delvet, Australia). Fetal rabbits (n = 6) were partially delivered by caesarean section, sedated with sodium pentabarbital (pentobarbital; 0.1 mg ip), and a jugular vein catheter (24G; via tracheostomy) were inserted; the tip of the ET tube was directed into the right bronchus so that with ventilation onset only the right lung was ventilated (unilateral ventilation). During the surgical procedure, the kitten’s head remained covered with fetal membranes to prevent lung aeration and the umbilical cord remained intact. The kittens were then delivered, the umbilical cord ligated and placed upright in a perspex frame before the ET tube was connected to a purpose-built, time-cycled, pressure-controlled ventilator (16); image acquisition began as soon as possible after the kittens were positioned. Kittens were ventilated in air using a peak inflation pressure of 25 cmH2O and a positive end-expiratory pressure of 5 cmH2O. At the conclusion of the experiment (~10 min after ventilation onset for kittens), all animals were humanely killed with an overdose of sodium pentabarbitone (pentobarbital; 100 mg/kg) administered intravenously (doe) or intraperitoneally (kittens).

X-ray and Angiography Imaging

The energy was 33.2 keV, just above the iodine k-edge, and kittens were positioned 1.0 m upstream of the detector. The detectors used (either: EM-CCD C9100–02 or C9300–124F, Hamamatsu Photonics Hamamatsu, Japan or pco.edge, PCO AG, Germany) had a maximum effective pixel size of 31.8 μm (range 15.3–31.8 μm) and an active field of view of 21–29 (W) × 21–30 (H) mm2; images were acquired at frame rates of 5–10 Hz. During imaging, iodine boluses (Iopromide, 370 mg/ml iodine; Schering, Germany; 1.5 μl/g of kitten weight) were infused into the kitten via the jugular vein using a remote-controlled syringe pump (PHD2000, Harvard Apparatus). Iodine boluses were injected and images acquired for ~2 min before ventilation onset, during ventilation of the right lung (for ~3 min), and then during ventilation of both lungs (for ~2 min); the latter was achieved by retracting the tip of the ET tube. Subsequent iodine infusions allowed comparative analysis of pulmonary vasculature at each stage of ventilation.

Image Analysis

Images were analyzed using ImageJ (v1.47; NIH) to compare the heart rate, iodine ejection per beat, number of visible pulmonary vessels, vessel diameters and change in mean grey level profiles within the left and right pulmonary arteries (PAs), the aorta and inferior vena cava (IVC) following iodine injection.

Vessel quantification. Visible blood vessels were counted using a composite image constructed from all X-ray images beginning from the first heartbeat immediately after iodine administration until after the iodine had left the pulmonary arteries. Images acquired during lung inflation were excluded due to motion blur and to ensure maximum possible overlap of vessels. Branches of each PA were classified according to branching generation; vessels distal to branching points were counted as individual vessels and were visible up to the 3rd order of branching.

Pulmonary artery vessel diameter. Changes in pixel gray level (intensity) along virtual lines transecting vessels perpendicular to the vessel wall were used to measure vessel diameter. For each line profile, the average background pixel gray level was calculated by measuring the average gray level along the line over 2–10 frames immediately prior to the first appearance of contrast within the vessel. This background gray level was subtracted from the pixel gray level along the transecting line following contrast injection and the resulting gray level plotted as a function of distance (pixels) along each line (Fig. 1). Vessel edges were identified when the pixel gray level decreased below 1 standard deviation (SD) of the background average on each side (Fig. 1, bottom panel). Nonuniform line profile plots, typically caused by iodine streaming towards the end of a heartbeat, were excluded from analysis. Vessel diameters from each frame were averaged over 10 frames to calculate a mean vessel diameter.

Changes in relative iodine levels within vessels. A virtual box was placed over the aorta, the IVC, the main PA immediately distal to the right ventricular outlet as well as over the left and right PAs at specific points along the main axial arteries. The latter were located within clear sections of the 6th, 7th, and 8th intercostal spaces, which provided the clearest, unobstructed view of the vessel during both the preventional and ventilation imaging stages. The changes in mean pixel gray level within each box were measured throughout an iodine injection sequence and expressed as a percentage of the background mean pixel gray level averaged over 2–10 frames before iodine injection. The temporal changes in relative iodine levels within the 3 virtual boxes along the left and right pulmonary arteries following iodine injection were integrated to provide a relative measure of iodine flow over time within these vessels. Heart rates were determined from the mean pixel gray level changes within the main pulmonary artery collected over several heart beats during an iodine injection sequence.

Statistical Analysis

Data are presented as means ± SE. Changes in vessel quantity, vessel diameter, and integrated relative iodine levels within vessels were analyzed using a two-way repeated-measures ANOVA. Post hoc analysis used the Holm-Sidak method. A P < 0.05 was considered statistically significant.

RESULTS

Animal Data

Six near-term rabbit kittens (from 5 does) underwent imaging before ventilation, during ventilation of the right lung (unilateral ventilation), and then during ventilation of both lungs (bilateral ventilation). Nonaerated regions of the lung are clearly evident by the absence of speckle pattern in the X-ray images. The gestational age of kittens at delivery was 30 days.

Observations from PC X-ray Videos

Compared with preventional, the flow of iodine (contrast agent) into both main PAs markedly increased (P < 0.05) in response to unilateral ventilation (Fig. 2; Supplemental Video 1, available with the online version of this article). As such, the number and amount of iodine flowing into the pulmonary vessels were markedly increased (Fig. 2; Supplemental Video...
increased significantly from 15 both lungs using angiography (Fig. 3). The number of vessels right lung markedly increased the number of vessels visible in
Numbers of Visible Vessels

1). The increase in iodine flow through vessels of the nonaerated left lung was very similar to the aerated right lung, displaying a uniform global increase in iodine visibility and, therefore, in PBF. This effect was consistently observed in all animals (n = 6) and was sustained following bilateral ventilation (Fig. 2).

Internal Vessel Diameter

The axial PAs (both left and right) gradually narrow as they branch and penetrate into the more caudal lung lobes, penetrating at least into the 8th intercostal space (see Figs. 2 and 3). Internal vessel diameters of both the left and right axial PAs were measured in each intercostal space between ribs 6 to 8. Prior to ventilation, mean vessel diameters were ~550 μm (intercostal space 6), ~430 μm (intercostal space 7), and ~315 μm (intercostal space 8), which reflects the decreasing vessel diameter as it branches and penetrates into the distal lung regions (Fig. 4). Compared with preventilation values, the PA diameters in both lungs were significantly increased by unilateral ventilation in all intercostal spaces measured. In the 7th intercostal space, unilateral ventilation increased the vessel diameter from 432 ± 46 and 428 ± 31 μm to 490 ± 45 and 498 ± 31 μm in the left and right lungs, respectively; in the 8th intercostal spaces, vessel diameters increased from 291 ± 21 and 332 ± 13 μm to 352 ± 18 and 379 ± 18 μm in the left and right lungs, respectively. Compared with unilateral ventilation, bilateral ventilation significantly increased vessel diameter further to 555 ± 36 μm (left lung) and 559 ± 34 μm (right lung) in the 7th intercostal space and to 401 ± 23 μm (left lung) and 434 ± 23 μm (right lung) in the 8th intercostal space (Fig. 4).

Heart Rate and Beat-to-Beat Changes in Iodine Ejection

Compared with the preventilation period, unilateral ventilation increased the heart rate from 69 ± 7 to 98 ± 17 beats/min, which was increased further to 140 ± 14 beats/min following bilateral ventilation (P < 0.05; Fig. 5). The relative amount of iodine ejected per heart beat, as determined by the % change in gray level (below background) measured within the main pulmonary trunk, was increased by unilateral ventilation (P < 0.05; Fig 5). The amount of iodine ejected during the first and second heart beats was significantly increased from 23.2 ± 6.3% and 31.0 ± 5.1% to 54.8 ± 7.1% and 64.4 ± 5.0%, respectively, following ventilation onset (Fig. 5).

Temporal Changes in Iodine Levels Within the IVC and Aorta

The temporal increase in relative iodine levels (% change in pixel gray level below background) within the IVC following iodine injection was significantly reduced in response to both unilateral and bilateral ventilation (Fig. 6). At 0.8 s after iodine injection, the relative iodine level was 29.6 ± 6.2% (% change in pixel gray level below background) before ventilation onset and was reduced to 17.4 ± 7.1% in response to unilateral ventilation and to 5.7 ± 2.2% following bilateral ventilation of the lung (Fig. 6); this indicates less iodine flowing into the IVC following injection in response to ventilation. Within the de-
Fig. 2. Simultaneous PC X-ray images and angiograms of 4 newborn rabbits prior to ventilation (0LV), following unilateral ventilation of the right lung (1LV) and following ventilation of both lungs (2LV). Images were acquired 2–6 s after iodine injection.

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Ascending thoracic aorta, the temporal increase in relative iodine levels was significantly increased in response to unilateral ventilation and was increased further following bilateral ventilation (Fig. 6).

Change in Mean Pixel Gray Level Over Time Within the Pulmonary Artery

In response to unilateral ventilation, the integrated (over time) changes in relative iodine levels (% change in pixel gray level below background) within the left and right PAs following iodine injection were significantly and equally increased (Fig. 7). At the level of the 6th intercostal space, the integrated relative iodine level (%·s) increased from 68.3 ± 11.6 and 70.3 ± 7.5 %·s to 136.3 ± 22.6 and 136.3 ± 23.7 %·s in the left and right PAs, respectively. In the 7th intercostal space, the integrated relative iodine level increased from 59.2 ± 10.0 and 64.7 ± 8.6 %·s to 138.6 ± 34.8 and 139.1 ± 22.5 %·s in the left and right PAs, respectively. Bilateral ventilation tended to increase the integrated relative iodine level further, but these changes were not significant except for the region of PA within the 6th intercostal space (Fig. 7).

DISCUSSION

Our results confirm previous studies demonstrating that lung aeration at birth triggers a major hemodynamic response in the newborn, particularly an increase in PBF. However, contrary to what was hypothesized, we found that the relationship between lung aeration and the increase in PBF at birth was not spatially related. Specifically, we found that partial lung aeration triggered a marked increase in PBF in both aerated and unaerated lung regions, resulting in a major ventilation/perfusion mismatch in unaerated regions (Figs. 2 and 7; Supplemental Video 1). These findings were unexpected as, based on the mechanisms thought to be responsible for the increase in PBF at birth (e.g., increased oxygen and NO release), we hypothesized that the stimulus would be greatest in aerated compared with unaerated lung regions. However, in every parameter examined, we found that the response to unilateral ventilation was similar in pulmonary vessels supplying aerated and nonaerated lung regions. Although some differences were observed between unilateral and bilateral ventilation periods, as the observed changes were mostly similar between lungs, irrespective of whether they were previously aerated (right) or unaerated (left), these changes more likely reflect an “increasing time after birth” related response, for example, due to a gradual increase in oxygenation.

The images clearly show major differences in iodine flow into the PAs before and after lung aeration, which greatly alters their visibility, with only the primary branches (Figs. 2 and 3) being visible before lung aeration. As it is well established that PVR is high and PBF is low before lung aeration (1), the primary reason for this reduced flow of iodine is due to the low PBF, although other factors may have contributed. For instance, a reduced venous return likely contributed via a sub-

![Graph showing number of visible vessel branches](image-url)
stantial reduction in IVC flow caused by umbilical cord clamping, which causes umbilical venous return to cease. As umbilical venous return is an important contributor to IVC flow and preload for the fetal heart, these factors are dramatically reduced upon cord clamping and are only restored following the increase in PBF. This explanation is consistent with the finding that retrograde iodine flow along the IVC was substantially reduced following lung aeration, indicating that venous return and forward flow in the IVC was rapidly restored following ventilation onset and the associated increase in cardiac output (CO) (3). This is also consistent with previous studies showing that RV output is reduced by \( \sim 50\% \) following umbilical cord clamping and the loss of umbilical venous return (3, 6). Our finding that the amount of iodine ejected per beat from the RV and the heart rate were significantly lower prior to lung aeration and markedly increased with ventilation onset are entirely consistent with these previous observations.

The integrated changes (over time) in relative iodine levels measured at different positions along the main left and right axial PAs were used to assess the relative amounts of iodine passing through the PAs following iodine injection (Fig. 7). Although this analysis does not provide a quantitative measure of PBF, the changes measured must reflect increases in PBF resulting from decreases in PVR, which are known to occur following ventilation onset (3, 6). These findings are consistent with the increases in vessel diameters measured at specific points along these vessels. Although the vessel diameter changes are relatively modest (\( \sim 10\% \) and 20%), as the resistance to blood flow is inversely proportional to the vessel radius to the 4th power, these increases in diameter reflect substantial decreases in resistance; a 10% increase in diameter equates to a 40% reduction, whereas a 20% increase equates to a 60% reduction in resistance. These reductions in resistance are similar in magnitude to the reductions in PVR that have been reported previously with ventilation onset at birth (26, 27, 35).

![Fig. 4. Changes in internal vessel diameter along the main axial branches of the left and right pulmonary arteries at the level of the 6th (top panel), 7th (middle panel), and 8th (bottom panel) intercostal spaces, measured before ventilation onset (0LV), during unilateral ventilation of the right lung (1LV), and during ventilation of both lungs (2LV).](image1)

![Fig. 5. Changes in heart rate (top panel) and the change in relative iodine levels (% change in pixel gray level below background) measured within the main pulmonary artery (MPA) immediately distal to the right ventricular outlet during peak systole over consecutive heartbeats following iodine injection (bottom panel). The latter provides a relative measure of the amount of iodine ejected per ventricular contraction. Measurements were made before ventilation onset (0LV), during unilateral ventilation of the right lung (1LV), and during ventilation of both lungs (2LV). *\( P < 0.05 \), 0LV vs. 1LV; †\( P < 0.05 \), 0LV vs. 2LV. Within each graph, bars that do not share a letter are significantly different from each other (\( P < 0.05 \)).](image2)
Our finding that unilateral ventilation dilated pulmonary vessels and increased PBF in both aerated and unaerated regions of the lung indicates that the initial ventilation-induced increase in PBF is not spatially related to aerated lung regions. This was a surprising finding as the primary mechanisms thought to mediate the ventilation-induced increase in PBF should act locally to dilate adjacent blood vessels (1). For instance, the increase in tissue PO2 associated with aerating distal gas exchange regions was thought to stimulate endothelial NO release, which acts on vascular smooth muscle to dilate resistance vessels within the lung (15). Similarly, the entry of air and the formation of surface tension within the lungs is thought to decrease PVR by increasing alveolar wall recoil, which increases pulmonary capillary recruitment and expansion. Based on this mechanism, one would also assume that the decrease in PVR would be limited to aerated lung regions (13). Thus it is possible that an additional, currently unknown, mechanism is responsible for initiating the increase in PBF at birth, which is largely independent of oxygen and the localized effects of surface tension and increased lung recoil. One possible explanation is the activation of “J” receptors (24) in response to liquid accumulation within the tissue, which triggers a parasympathetic mediated decrease in PVR via vagal reflex. Whatever the mechanism, its activation only requires aeration of a small region of the lung and includes global vasodilation of the pulmonary vascular bed. Theoretically, this should benefit the infant, because lung aeration is often not uniform at birth and if the increase in PBF was dependent on complete lung aeration, then both CO and gas exchange will be compromised until this is achieved. As increasing PBF and restoring CO is arguably more important than simply achieving complete lung aeration immediately after birth, it is of greater benefit to the transitioning infant if the increase in PBF is not quantitatively linked to the degree of lung aeration.

The contention that the initial ventilation-induced increase in PBF is not oxygen dependent is also supported by previous studies (15, 33). For example, in fetal sheep ventilated in utero, the majority (~70%) of the increase in PBF was achieved by ventilating fetuses with a hypoxic gas mixture (35). Switching...
ventilation to 100% O₂ increased PBF further, indicating that increasing oxygen can contribute, but they concluded that increasing oxygen is not the dominant factor and an unknown “effect of ventilation” was primarily responsible. Similarly, in lambs delivered and ventilated at birth, using strategies that either increased or decreased oxygenation levels resulted in a similar increase in PBF (33). In our study, we found that some of the factors measured increased, or tended to increase, between the periods of unilateral and bilateral ventilation, which may reflect increased oxygenation of the kitten. Indeed, it is well established that the pulmonary vasculature is sensitive to changes in oxygen tension from early in gestation, with both fetal hypoxia and hyperoxia causing vasoconstriction and vasodilation of the pulmonary vasculature bed, respectively (21). However, the fact that the increase was similar in both lungs, irrespective of whether the lung had previously been aerated (right) or unaerated (left), indicates that this mechanism is time-related and may be independent of the mechanism that was initially triggered by lung aeration.

Increased cardiac function, resulting from both an increase in HR and contractility, may have also contributed to the time-related increases of our indirect measures of PBF. With the onset of lung aeration, the infused iodine primarily entered the right ventricle and was immediately ejected, within one or two heartbeats, into either the pulmonary vasculature or the descending aorta via the ductus arteriosus. As a result, both the proportion of iodine entering the RV and the amount of iodine that was ejected per beat increased (Fig. 5), indicating that RV output had increased. This finding is consistent with the finding of an increase in HR (Fig. 5) and is also consistent with numerous previous studies (2, 6, 23). However, recent studies have also indicated that the increase in PBF is a critical determinant of the increase in cardiac function (HR and contractility) after birth, which increases before an increase in oxygenation can be detected (3). As preload for both ventricles is greatly diminished following umbilical cord clamping, causing large reductions in both stroke volumes and HR, it is not until PBF increases that preload for the LV is restored (3, 6). The important role of PBF in CO at birth was recently demonstrated by a study that delayed umbilical cord clamping until after ventilation had commenced and PBF had increased. This procedure completely abolished the reduction in CO associated with umbilical cord clamping, indicating that the source of preload can immediately switch from the umbilical circulation to PBF, without disrupting CO (3). As such, the increase in RV output and increase in HR we observed in response to unilateral ventilation is more likely to be a consequence of the increase in PBF than a cause.

We have used simultaneous angiography and PC X-ray imaging to investigate the spatial relationship between lung aeration and the increase in PBF at birth. Although we hypothesized that lung aeration and the increase in PBF would be spatially related, our finding that they are unrelated demonstrates that our current understanding of the mechanisms driving the initial increase in PBF requires reevaluation. Indeed, we found that partial lung aeration can lead to large ventilation/perfusion mismatches in unaerated lung regions. This suggests that factors such as P[subscript O₂] and mechanical expansion, which are well-established factors thought to reduce PVR at birth, may have nonlocal effects in the lungs. However, it is not known how these factors could lead to reductions in resistance simultaneously across the entire lung, particularly in unaerated regions, when perfusion is initially so low. It is possible that other physiological factors, such as vasodilators, simultaneously reduce resistance across the aerated and unaerated regions, but these are generally thought not to be dominant factors (2, 10). Again, while these factors may potentiate any response, it is hard to envisage how they could initiate such an effect when pulmonary perfusion is initially so low.

Our observations of a global increase in PBF within the lungs were observed to occur within ~40 s of ventilation onset, which indicates the presence of a rapidly acting highly potent vasoactive process. This may be the result of a previously unsuspected mechanism that provides the initial stimulus for the increase in PBF at birth. In any event, it highlights our lack of understanding of the mechanisms that regulate this crucial process, despite the fact that it underpins the transition to newborn life.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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