Positive end-expiratory pressure differentially alters pulmonary hemodynamics and oxygenation in ventilated, very premature lambs

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Polglase, Graeme R., Colin J. Morley, Kelly J. Crossley, Peter Dargaville, Richard Harding, David L. Morgan, and Stuart B. Hooper. Positive end-expiratory pressure differentially alters pulmonary hemodynamics and oxygenation in ventilated, very premature lambs. J Appl Physiol 99: 1453–1461, 2005. First published May 12, 2005; doi:10.1152/japplphysiol.00055.2005.—In mature lungs, elevated positive end-expiratory pressure (PEEP) reduces pulmonary blood flow (PBF) and increases pulmonary vascular resistance (PVR). However, the effect of PEEP on PBF in preterm infants with immature lungs and a patent ductus arteriosus is unknown. Fetal sheep were catheterized at 124 days of gestation (term ~147 days), and a flow probe was placed around the left pulmonary artery to measure PBF. At 127 days, lambs were delivered and ventilated from birth with a tidal volume of 5 ml/kg and 4-cmH2O PEEP; PEEP was changed to 0, 8, and 12 cmH2O in random order, returning to 4 cmH2O between each change. Increasing PEEP from 4 to 8 cmH2O and from 4 to 12 cmH2O decreased PBF by 20.5 and 41.0%, respectively, and caused corresponding changes in PVR; reducing PEEP from 4 cmH2O did not affect PBF. Despite decreasing PBF, increasing PEEP from 4 to 8 cmH2O and 12 cmH2O improved oxygenation of lambs. Increasing and decreasing PEEP from 4 cmH2O significantly changed the contour of the PBF waveform; at a PEEP of 12 cmH2O, end-diastolic flow was reduced by 82.8% and retrograde flow was reestablished. Although increasing PEEP improves oxygenation, it adversely affects PBF and PVR shortly after birth, alters the PBF waveform, and reestablishes retrograde flow during diastole.

ventilation; pulmonary blood flow; fetus; preterm birth

INFANTS BORN VERY PRETERM (<28 wk gestation) have surfactant-deficient and immature lungs, with a small surface area and a thick air-blood barrier that impairs gas exchange (16, 17). Consequently, very preterm infants usually require resuscitation at birth and assisted ventilation during their first weeks of extraterine life, although this increases the risk of injuring their lungs. It is important to identify ventilation techniques that maximize oxygenation of very preterm infants without injuring their lungs or adversely affecting the changes in pulmonary physiology that are essential for the transition to air breathing at birth. The application of positive end-expiratory pressure (PEEP) is important for maintaining lung volumes and improving oxygenation in very preterm infants (25) and reduces the risk of lung injury (2). However, little is known of the effects of PEEP on the transition of the lung into a functional gas-exchange organ at birth, particularly the increase in pulmonary blood flow (PBF), which is essential for surviving the transition to extraterine life.

During fetal development, pulmonary vascular resistance (PVR) is high (13, 15), and, as a result, the fetal lungs receive only ~12% of right ventricular output; most bypasses the lungs and passes directly into the systemic circulation via the ductus arteriosus (DA) (3, 14, 28, 31, 33). The high PVR and the presence of the DA confer unique characteristics to the contour of the PBF waveform in the fetus (31), particularly during diastole when the flow is largely retrograde and exits the pulmonary circulation via the DA (31). However, at birth PVR decreases, and as a result PBF increases (8- to 10-fold) (3, 19, 31), allowing the pulmonary circulation to accommodate the entire output of the right ventricle; the DA must also close to separate the pulmonary and systemic circulations (13, 31). As a result, the contour of the PBF waveform rapidly changes after birth with forward flow occurring throughout diastole. If PVR does not decrease soon after birth, the infant will experience gas-exchange problems, persisting pulmonary hypertension, and continued patency of the DA, all of which are prevalent in preterm infants with hyaline membrane disease or pulmonary hypoplasia (24, 40).

In the mature lung, raised airway pressure increases PVR and reduces PBF, largely because of compression of periairuleolar capillaries (4–6, 9). However, because of structural and mechanical differences that exist between the mature lung of term newborns and the immature lung of very preterm infants, particularly the separation of the pulmonary capillaries and the airway epithelium by interstitial cells (1), it is unclear whether constant positive airway pressures (e.g., PEEP) have similar effects on PBF in the immature lungs with a patent DA. Because the PBF waveform contour may be a sensitive indicator of downstream resistance in the pulmonary vascular bed (10), the effect of PEEP on the PBF waveform was also investigated to identify components most sensitive to PEEP. Thus our aim was to determine the effects of different levels of PEEP on PBF, PVR, oxygenation, and the PBF waveform in very preterm lambs immediately after birth. We hypothesized that high PEEP levels in preterm lambs would reduce PBF, increase PVR, and alter the PBF waveform, whereas low PEEP levels would not adversely affect these factors in structurally immature lungs.

METHODS

Aseptic surgery was conducted on 11 pregnant ewes (Border-Leicester × Merino) at 123–124 days of gestation (term is ~147...
days) as described previously (26). Polyvinyl catheters were implanted into the fetal carotid artery and jugular vein, the amniotic sac, and the left pulmonary artery. A 4-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was also placed around the left main pulmonary artery. Fetal well-being was monitored daily by measuring fetal arterial PO$_2$ (PaO$_2$), PCO$_2$ (PaCO$_2$), pH, and percentage oxygen saturation of hemoglobin (SaO$_2$) (ABL30, Radiometer, Denmark) by using samples collected from the carotid artery. This surgery was performed prenatally rather than during the caesarean delivery, to allow us to monitor longitudinal changes in PBF and PVR before birth, during delivery, and after birth.

**Experimental Procedures**

On day 126 of gestation, fetuses were divided into two groups, and control recordings (6 h) were made of arterial pressures and left pulmonary arterial flow in all fetuses. On day 127 of gestation, ewes and fetuses were anesthetized, the fetal head and neck were exposed via caesarean section, the trachea was intubated, and lung liquid was drained passively. The lambs were then delivered, dried, weighed, and ventilated (Babylag 8000 plus, Draeger, Lubeck, Germany) in volume guarantee mode with an expired tidal volume (VT) of 5 ml/kg and a PEEP of 4 cmH$_2$O for 20 min to allow time for changes in PBF to stabilize. Group 1 lambs (n = 5) were ventilated at either 0-, 8-, or 12-cmH$_2$O PEEP for 20-min periods in random order, returning to 4-cmH$_2$O PEEP after each PEEP level; total time of ventilation was ~2 h. Group 2 lambs (n = 6) were ventilated at 4 cmH$_2$O of PEEP throughout the study to determine time-related changes in PBF and PVR after birth. Lambs were hydrated via a continuous infusion of 5% dextrose (iv) and were lightly sedated (pentobarbital iv) throughout the study to prevent spontaneous breathing. During ventilation, the inspired gases were warmed and humidified, and the inspired oxygen fraction (FiO$_2$) was adjusted to maintain SaO$_2$ between 92 and 98%. The ventilator rate was adjusted to keep the arterial pH between 7.2 and 7.4 and the PaCO$_2$ between 35 and 55 Torr; arterial blood-gas measurements were taken every 10 min throughout the study and at least 5 min after any changes to FiO$_2$ and/or ventilator settings. Fetal systemic and pulmonary arterial pressures (Ppa), blood flow through the left pulmonary artery, peak inspiratory pressure, PEEP, respiration rate, VT, and heart rate were continuously recorded (Powerlab/SSP, ADIndustries, Castle Hill, Australia). On completion of an experiment, lambs were killed by an overdose of pentobarbitone sodium (130 mg/kg iv). The fetal chest and lungs were inspected for pneumothoraces, before the lungs were excised and weighed.

Although antenatal glucocorticoids and postnatal surfactant therapy are standard treatments for very preterm infants, they were not administered in this study because we wanted to examine the relationship between PBF and airway pressure in the most immature lungs possible. It is likely that these treatments will affect the relationship between airway pressure and PBF by altering lung structure and tissue mechanics. All experimental procedures on animals were approved by the Monash University Animal Welfare Committee.

**Analytical Methods**

**PBF and PVR analysis.** In group 2 lambs, recordings were divided into 5-min periods, with 3 min from each being analyzed. In group 1 lambs, three 1-min recordings were analyzed at the 4-cmH$_2$O baseline period immediately preceding the next change in PEEP. Similarly, three 1-min recording periods were analyzed after the PEEP had been changed to 0, 8, or 12 cmH$_2$O; the recordings were allowed to stabilize for 5 min before they were analyzed. Finally, three 1-min recording periods were analyzed after the PEEP had returned to 4 cmH$_2$O. To determine the effect of PEEP history on PBF, the values recorded during the 3 min immediately before the change in PEEP were analyzed and compared with those immediately after the change in PEEP. PVR was calculated using the formula PVR = (Ppa − Pla)/Qp, where Pla is left atrial pressure, and Qp is flow through the left pulmonary artery; Pla is assumed to be 9 mmHg on the basis of our preliminary finding (unpublished) and those of previous studies (33). The lamb's oxygenation status was measured by calculating the alveolar-arterial difference in oxygen (A-aDO$_2$), using the formula A-aDO$_2$ = [P(barometric) − P(H$_2$O)] × FiO$_2$ − (PaCO$_2$/0.93) − PaO$_2$ (27) where P(barometric) is barometric pressure and P(H$_2$O) is water vapor pressure at 39°C; the respiratory quotient was set at 1.0 when FiO$_2$ was set at 1.0 or 0.93 for other FiO$_2$ settings as previous studies have shown this to be the correct value for sheep (32).

**PBF waveform analysis.** Changes in the contour of the PBF waveform in the left pulmonary artery were measured by selecting representative waveforms throughout 10 consecutive cardiac cycles from each lamb before birth, throughout the first 5 min after birth, and then during ventilation with either 0, 4, 8, or 12 cmH$_2$O of PEEP. The waveforms analyzed were collected after a 5-min stabilization period.

The waveform parameters examined (Fig. 1) include the systolic pulse amplitude, rate of pulse increase, rate of pulse decrease, peak systolic flow, end-diastolic flow, and end-diastolic flow, as well as the maximum, minimum, and average flow during diastole. In addition, waveforms from ~20 cardiac cycles from each fetus and at each level of PEEP were analyzed by fast Fourier transform analysis.

**Statistical Analysis of Data**

A two-way ANOVA for repeated measures was performed to determine the effect of PEEP on PBF, PVR, and PBF waveform characteristics. For each animal and at each PEEP level, the three recordings taken after the change in PEEP were averaged and, for graphical presentation of the PBF and PVR data, expressed as a percentage of the average of the three periods immediately preceding the change in PEEP. To determine the time-related change in PBF and PVR after birth in preterm lambs ventilated at 4-cmH$_2$O PEEP, a nonlinear regression analysis was performed by using the data analyzed at 5-min intervals. Data in the text are presented as means ± SE. The level of statistical significance was P < 0.05 for all statistical analyses.

**RESULTS**

**Physiological Status of Fetuses and Newborn Lambs**

All fetuses were healthy, according to their carotid arterial blood gas and acid-base status, before anesthesia; the mean in utero fetal arterial pH and blood-gas tensions were pH, 7.39 ±
ventilation parameters and FiO2, although some parameters were maintained within physiological ranges by altering the after birth.

Before birth, mean PBF was 18.7 ± 10.4 ml/min and, after birth, rapidly increased to 194.0 ± 17.1 ml/min within 5 min of the onset of ventilation at 4-cmH2O PEEP; this increase was not related to delivery or to umbilical cord occlusion per se (Fig. 2A). After birth, the increase in mean PBF was very rapid and increased by 97.4 ± 17.0 ml/min within 30 s of the onset of ventilation. By 6 min after the onset of ventilation, the increase in mean PBF had reached a plateau of 267.0 ± 19.9 ml/min above the value measured before delivery (Fig. 2B). These changes in PBF were associated with a large decrease in PVR from 3.2 ± 0.6 mmHg·min⁻¹·ml⁻¹ in

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Values are means ± SE. SAP, systemic arterial pressure; Ppa, pulmonary arterial pressure; FFT, fast Fourier transform analysis. a,b Values that do not share a common letter are significantly different from one another.
the fetus to 0.9 ± 0.2 mmHg·min⁻¹·ml⁻¹ at 30 s after birth
and to 0.2 ± 0.0 mmHg·min⁻¹·ml⁻¹ at 5 min after birth.

In group 2 lambs, ventilated at a constant PEEP of 4 cmH₂O,
mean PBF was reduced from 135.1 ± 12.3 ml·min⁻¹·100 g
lung wt⁻¹ at 5 min after birth to 64.1 ± 7.2 ml·min⁻¹·100 g
lung wt⁻¹ at 95 min after birth (r² = 0.986; P < 0.0001; Fig.
3A). This decrease in mean PBF was closely associated with an
increase in PVR from 0.18 ± 0.04 mmHg·ml⁻¹·min⁻¹·100 g
lung wt⁻¹ at 5 min to 0.31 ± 0.05 mmHg·ml⁻¹·min⁻¹·100 g
lung wt⁻¹ (r² = 0.921) at 95 min after birth (Fig.
3B). Although the Ppa was variable between lambs during this
period, no significant change was measured (Table 2).

Effect of PEEP on PVR and flow through the left pulmonary
artery. Increasing PEEP from 4 to 8 cmH₂O significantly
reduced mean PBF by 20.5 ± 3.5% (P = 0.006); the average
decrease in PBF was 33.4 ± 9.4 ml/min. Increasing PEEP from
4 to 12 cmH₂O significantly reduced PBF by 41.0 ± 4.4%
(P = 0.09); the average decrease in PBF was 58.2 ± 12.5
ml/min (Fig. 4A). The reduction in PBF caused by increasing
PEEP from 4 to 8 cmH₂O and from 4 to 12 cmH₂O was
associated with an increase in PVR. PVR was increased by
24.3 ± 4.5% (P = 0.009) by increasing PEEP from 4 to 8
cmH₂O and by 54.4 ± 8.6% (P = 0.05) by increasing PEEP
from 4 to 12 cmH₂O (Fig. 4B). However, when PEEP was
reduced from 4 to 0 cmH₂O, no significant change in PBF or
PVR could be detected; PBF and PVR were similar before
(224.6 ± 41.5 ml/min and 0.11 ± 0.02 mmHg·ml⁻¹·min⁻¹,
respectively) and after (227.5 ± 35.5 ml/min and 0.10 ± 0.01
mmHg·ml⁻¹·min⁻¹, respectively) reducing PEEP from 4 to 0
cmH₂O. Increasing PEEP from 4 to 8 cmH₂O or from 4 to 12
cmH₂O had no significant effect on Ppa (Table 2).

Effect of PEEP history on PBF. Increasing PEEP from 4 to
8 cmH₂O significantly reduced PBF by 39.0 ± 9.2 ml/min
during the first 3 min immediately after the change in PEEP.
However, within 3 min of reducing PEEP from 8- back to 4-
cmH₂O, PBF did not significantly increase compared with that
measured during the last 3 min of the 8-cmH₂O PEEP period;
PBF tended to increase by only 12.0 ± 14.7 ml/min (Fig. 5).
During the 8-cmH₂O PEEP period, PBF was similar during the
first 3 min (141.0 ± 21.3 ml/min) compared with the last 3 min
(143.0 ± 17.2 ml/min), indicating that PBF did not signifi-
cantly change during this period. Increasing PEEP from 4 to 12

Fig. 3. Changes in PBF (A), recorded from the left pulmonary artery, and
pulmonary vascular resistance (PVR; B) measured in lambs ventilated at
4-cmH₂O PEEP from birth. Time 0 is onset of ventilation. Equations indicate
the line of best fit.

Fig. 4. Effect of 0, 8, and 12 cmH₂O of PEEP on mean PBF (A) and PVR (B)
in very prematurely delivered, ventilated lambs. All values are expressed as the
percentage of the values measured at a PEEP of 4 cmH₂O immediately before
the change in PEEP to 0, 8, or 12 cmH₂O. *Significantly different from 4
cmH₂O PEEP, P < 0.05.
cmH₂O reduced PBF by 52.4 ± 14.2 ml/min within 3 min of increasing the PEEP. However, within 3 min of returning PEEP to 4 cmH₂O, PBF had only increased by 24.3 ± 7.8 ml/min (Fig. 5). At 12-cmH₂O PEEP, PBF was similar during the first 3 min (73.8 ± 20.6 ml/min) compared with the last 3 min (73.2 ± 20.6 ml/min) of the period.

Reducing PEEP from 4 to 0 cmH₂O had no significant effect on PBF within 3 min; PBF was similar before (224.5 ± 41.5 ml/min) compared with after (227.5 ± 35.5 ml/min) the reduction in PEEP (Fig. 5). In contrast to 8- and 12-cmH₂O PEEP, PBF tended to decline (to 190.7 ± 18.1 ml/min) during ventilation at 0-cmH₂O PEEP, although this was not significant. However, within the first 3 min of increasing PEEP back to 4 cmH₂O, PBF was significantly reduced by 26.1 ± 5.4 ml/min (to 164.5 ± 21.2 ml/min). As a result, PBF was 60.0 ± 25.7 ml/min lower after the return to 4-cmH₂O PEEP compared with PBF during the 4-cmH₂O PEEP period immediately before the PEEP was decreased to 0 cmH₂O.

**Analysis of the PBF Waveform**

The effects of birth and PEEP on the contour of the blood flow waveform in the left pulmonary artery are shown in Fig. 6; the characteristics analyzed are shown in Fig. 1. The characteristics associated with both the systolic and the diastolic periods were markedly altered by the onset of ventilation, although PEEP mainly affected diastole. The amplitude, the rate of increase, and the rate of decrease in PBF associated with the systolic pulse rapidly declined from 552.2 ± 40.4 ml/min, 140.6 ± 15.4 ml/s², and 72.3 ± 5.6 ml/s² in the fetus to 232.2 ± 24.1 ml/min, 46.0 ± 4.4 ml/s², and 37.0 ± 5.0 ml/s², respectively, at 10 s after initiation of ventilation. No further significant changes were observed over the subsequent 10 min. In contrast to the changes that occur at birth, PEEP had no significant effect on the pulse amplitude or the rate of increase and decrease in PBF associated with each pulse; these were similar at 0, 4, 8, and 12 cmH₂O (Table 2). Because the heart rate was not significantly altered by PEEP, the pulse frequency was also similar at all PEEP levels (Table 2).

End-systolic blood flow through the left pulmonary artery (see Fig. 8B) was significantly increased from −74.7 ± 31.2 ml/min in the fetus to 92.2 ± 36.5 ml/min after birth at 4-cmH₂O PEEP; negative flow values indicate retrograde flow away from the lungs. Increasing the PEEP from 4 to 8 cmH₂O significantly reduced end-systolic PBF from 92.2 ± 36.5 to 26.6 ± 39.7 ml/min (see Fig. 8B); retrograde PBF, a characteristic of PBF in the fetus, was reestablished in two of the five

Fig. 5. Effect of changing the PEEP from 4 to 0 and back to 4 cmH₂O (left), from 4 to 8 and back to 4 cmH₂O (middle), and from 4 to 12 and back to 4 cmH₂O (right) on mean PBF. In each panel, the PBF is a percentage of the mean PBF measured during the initial 4-cmH₂O PEEP period (means ± SE). For each panel, the first bar represents the flow measured during the initial 4-cmH₂O PEEP period, the second bar represents the flow measured during the first 3 min after the change in PEEP, the third bar represents the flow measured during the last 3 min of the PEEP period, and the last bar represents the flow measured after the change back to 4-cmH₂O PEEP. a,b,cValues that do not share a common letter are significantly different from one another.

Fig. 6. Effect of birth and different levels of PEEP on the contour of the PBF waveform measured in the same animal. The fetal recording was made immediately before the induction of anesthesia.
lambs. Increasing PEEP from 4 to 12 cmH2O caused all five lambs to reestablish retrograde flow at end systole; end-systolic flow decreased from 92.2 ± 36.4 to −32.2 ± 9.6 ml/min (see Fig. 8B).

Immediately after birth, end-diastolic flow through the left pulmonary artery increased from −60.2 ± 11.0 ml/min in the fetus to 92.0 ± 15.0 ml at 70 s after the initiation of ventilation but then did not change significantly over the next 10 min (Fig. 7B). Increasing PEEP from 4 to 8 cmH2O and from 4 to 12 cmH2O significantly reduced end-diastolic flow from 79.7 ± 20.5 to 44.2 ± 23.4 ml/min (8-cmH2O PEEP) and to 13.7 ± 6.2 ml/min (12-cmH2O PEEP), respectively (Fig. 8A). Reducing PEEP from 4 to 0 cmH2O also significantly reduced the end-diastolic flow to 47.3 ± 21.7 ml/min (Fig. 8A). The minimum, mean, and maximum PBF during diastole were also significantly affected by both birth and the PEEP level (Table 2, Fig. 8C); the mean PBF during diastole significantly increased from −32.4 ± 2.6 ml/min in the fetus to 116.7 ± 26.9 ml/min in the newborn ventilated at 4-cmH2O PEEP (Fig. 8C and Table 2). Increasing PEEP from 4 to 8 cmH2O and from 4 to 12 cmH2O reduced the diastolic minimum, average, and maximum PBF; the mean PBF during diastole was reduced from 116.7 ± 26.9 to 71.8 ± 29.7 ml/min at 8-cmH2O PEEP and to 31.3 ± 6.2 ml/min at 12-cmH2O PEEP (Fig. 8C and Table 2).

Fast Fourier transform analysis of PBF waveforms demonstrated that birth and the level of PEEP caused significant changes in the power spectra. The power of the signal that was present in the first harmonic significantly increased from 35.6 ± 1.8% in the fetus to 64.8 ± 4.1% after birth in preterm lambs ventilated at 4-cmH2O PEEP (Table 2). Increasing PEEP from 4 to 8 cmH2O and from 4 to 12 cmH2O reduced the power in the first harmonic from 64.8 ± 4.1 to 44.4 ± 4.0%, respectively.

**DISCUSSION**

Our findings indicate that increasing the level of PEEP applied to ventilated, very preterm lambs significantly reduces blood flow through the left pulmonary artery by increasing PVR, despite a significant increase in oxygenation. This finding is consistent with previous findings in newborns (4–6) and adults (7, 12, 23, 34) and indicates that the relationship between airway pressure and PVR described in the mature lung also applies to the immature lung of very preterm lambs. Furthermore, we have shown that components of the PBF waveform are very sensitive to increasing airway pressure, particularly during diastole. It is possible, therefore, that changes in PBF during diastole, particularly end-diastolic flow, in addition to blood oxygenation levels, may provide important
information on the level of PEEP that can be effectively applied to preterm infants, without adversely affecting lung circulatory physiology.

In the mature lung, the decrease in PBF and increase in PVR caused by increasing PEEP is thought to be due to an increase in alveolar pressure above capillary pressure, resulting in compression of perialveolar capillaries (6, 7, 20, 30, 33, 37). We consider that a similar mechanism is responsible for the PEEP-induced increase in PVR observed in this study. Consistent with this concept, previous studies have shown that changes in lung volume (and luminal pressure) are closely related to changes in PBF and PVR in the immature lung of fetal sheep (15, 38). Similarly, reductions in intraluminal pressure associated with fetal breathing movements, as well as single deep inspiratory efforts, are associated with marked increases in PBF and decreases in PVR (26). Thus it would appear that, both before and after birth, intraluminal pressure is an important determinant of PVR. Another factor that may contribute to the PEEP-associated increase in PVR is increased vasoconstriction of muscularized arterioles (6, 14, 18, 36, 37), although an associated increase in oxygenation with increasing PEEP (Ref. 27; Fig. 2C) would be expected to oppose increased vasoconstrictor activity (14, 33, 34).

The blood flow waveform within the left pulmonary artery changed markedly after birth (see Fig. 6), and components of this waveform were very sensitive to changes in PEEP. With the onset of ventilation, the waveform components associated with both systole and diastole were significantly altered, compared with before birth. The rate of increase, decrease, and amplitude of the systolic pulse were all significantly reduced by the onset of ventilation, although these changes were likely due to an increase in diastolic PBF. Indeed, mean PBF during diastole increased from $-34.5 \pm 8.8$ to $154.2 \pm 23.7$ ml/min within the first 70 s after delivery, whereas peak systolic flow only increased from $316.9 \pm 26.5$ to $383.5 \pm 58.8$ ml/min; as a result, the pulse amplitude was significantly reduced (Table 2). In contrast, little effect of PEEP was observed on the PBF waveform components associated with the systolic pulse, despite marked changes in PBF during diastole. This indicates that increasing PEEP (up to 12 cmH$_2$O) does not replicate all the PBF waveform characteristics of the fetus.

In a neonate with a fully or partially open DA, the factors governing PBF are likely to be complex. During the ejection phase of the cardiac cycle, PBF must be influenced by ventricular contractility as well as the stiffness of the main pulmonary trunk, the resistance to flow through the DA (including downstream resistance in the systemic circulation), as well as downstream PVR (31). However, during diastole, PBF is largely determined by PVR, which is responsible for generating the backward traveling compression and expansion waves that reflect off the pulmonary vascular bed and influence the PBF waveform during middle to late systole (10); other influences include the elastic recoil of the main pulmonary arteries as well as flow through the DA (either right-to-left or left-to-right, if present). Because increasing PEEP is likely to affect PBF by increasing PVR, it is not surprising that the characteristics of the PBF waveform most sensitive to changes in PEEP occur during diastole when PVR is the dominant factor. Indeed, it appears that end-diastolic flow is more sensitive to changes in resistance than can be detected using the calculated value of PVR, which is derived from an average of the PBF measured throughout the cardiac cycle.

In our ventilated preterm lambs, the relationship between PEEP and end-diastolic flow was biphasic and not linear. End-diastolic PBF was highest with 4-cmH$_2$O PEEP and was reduced when PEEP was either reduced from 4 to 0 cmH$_2$O or increased from 4 to 8 and 12 cmH$_2$O. Thus, in these immature lungs, a modest level of PEEP (4 cmH$_2$O) is likely to have a beneficial effect on PVR (as determined by end-diastolic flow), which is most probably due to a reduction in the number of vasoconstricted atelectatic regions. Indeed, during zero PEEP, A-aDO$_2$ was increased by $52.1 \pm 12.9\%$ ($P = 0.07$), and, as a result, it was necessary to increase $F_O_2$ to 1.0; despite this, $S_aO_2$ was significantly reduced during zero PEEP (Table 1). Although we could not detect a reduction in mean PBF after a reduction from 4- to 0-cmH$_2$O PEEP, the mean PBF tended to decrease from $227.5 \pm 35.5$ to $190.7 \pm 18.1$ ml/min during 0 cmH$_2$O PEEP, which is consistent with a gradual increase in PVR over this time. On the other hand, increasing PEEP to 8 and 12 cmH$_2$O caused detectable changes in mean PBF and PVR, although the magnitude of these changes was modest compared with the changes in end-diastolic flow (Fig. 8). Thus it appears that a small amount of PEEP is important for maintaining both arterial oxygenation levels and a low PVR in ventilated preterm lambs, but that too much PEEP increases PVR, despite a significant increase in arterial oxygenation. Furthermore, our findings indicate that end-diastolic flow may be a very sensitive and useful indicator of PVR, as it is for umbilical vascular resistance in growth-restricted fetuses (35).

It is likely that the change in PBF and PVR associated with increasing PEEP is not uniform across the entire lung. Previous studies have demonstrated that, in late gestation, the distribution of PBF is “lobular” and that perfused regions cycle at random across the lung, with only $\sim 43\%$ of the fetal lungs perfused at any one time (22). Thus the high PVR in the fetus may, in part, be due to infinite resistance in a significant proportion of the vascular bed, with a small number of vascular channels offering a low-resistance pathway (21). Because the perfusion is more uniform in the postnatal lung, it is possible that an increase in the number of low-resistance regions may greatly contribute to the overall decrease in PVR and increase in PBF at birth (21). Similarly, the PEEP-associated increase in PVR observed in our study may result from an increase in the numbers of regions offering high resistance to flow, which may be due to localized overexpansion of these regions. Other studies have reported a PEEP-related redistribution of blood flow within the lung from the core of the lung to its periphery and from the nondependent ventral lung to the dependent dorsal regions (11, 18, 29, 39). However, it is unlikely that these findings are directly applicable to our study, because the Ppa are much higher in our ventilated preterm lambs shortly after birth and the DA is likely to be patent.

A nonuniform pattern of blood flow distribution across the lung may also help explain the effect of PEEP “history” on PBF that we observed. We found that after ventilation with 8- or 12-cmH$_2$O PEEP, returning PEEP to 4 cmH$_2$O failed to restore PBF to values observed before the initial increase in PEEP, even after adjustment for the slow decline in PBF found in group 2 animals. This effect of PEEP history has been observed previously and was considered to be due to a lung volume-induced increase in pulmonary vascular tone (6). It is
also possible that the effect of PEEP history is related to volume hysteresis within the lung and results from ventilating the lung at different regions of the pressure-volume curve before and after the increase in PEEP. That is, although increases in airway pressure may facilitate recruitment of previously unventilated regions, it may also increase the number of overdistended regions offering high resistance. Thus, after the return to low PEEP from a high level of PEEP, a significantly greater number of regions may remain overexpanded with high resistance, compared with the period before the increase in PEEP, despite the same pressure. Little is known of the effect of volume hysteresis on PBF, particularly in very preterm infants, but as volume recruitment strategies and a desire to ventilate on the expiratory limb of the pressure-volume curve are becoming popular in neonatal ventilation, it is important to investigate this relationship. On the other hand, after a period of zero PEEP, increasing PEEP to 4 cmH2O significantly reduced PBF and increased PVR. The mechanisms involved are unknown but may relate to the failure or delay in reopening of blood vessels closed because of hypoxic vasoconstriction in atelectatic regions, in combination with overdistension of previously perfused regions.

Previous studies have shown that increasing PEEP significantly improves oxygenation levels (27), which is consistent with our finding of a 47.4 ± 13.2% reduction in A–aDO2 in response to an increase in PEEP from 4 to 12 cmH2O. In the immature lung, PEEP is thought to enhance alveolar recruitment, leading to an increase in surface area and improvements in regional gas exchange (34). It is surprising that increasing PEEP markedly improved oxygenation levels at the same time it reduced PBF and increased PVR, because increased oxygenation levels would be expected to promote vasodilation of the pulmonary vascular bed; increased oxygenation reduces PVR and increases PBF through a variety of mechanisms, including vasodilation and changes in Ppa (8, 14). However, our aim was to maintain SaO2 values between 92 and 98% (Table 1), and, although the inspired oxygen content was reduced and PaO2 tended to increase with increasing PEEP, the SaO2 values were not significantly different (between 4-, 8-, and 12-cmH2O PEEP). It is difficult to explain the coincident increase in oxygenation and decrease in PBF with increasing PEEP, but it probably indicates that ventilation-perfusion matching markedly improves with increasing PEEP. In all lambs, the arterial pH remained at the lower end of our target range (Table 1), most likely because of a mild respiratory acidosis caused by underventilation. We chose to ventilate these very preterm lambs at a fixed VT to maintain consistency between ventilation periods at different PEEP levels. However, we chose a low VT (5 ml/kg) that mildly underventilates these preterm lambs (28), because we were concerned that a larger VT would cause lung injury at the higher PEEP levels. Because the pH and PaCO2 were the same for all PEEP levels, it is unlikely that they significantly affected our results, but they clearly demonstrate that, although increasing PEEP significantly improves oxygenation and reduces PBF, it has no effect on CO2 clearance.

In lambs ventilated continuously at 4-cmH2O PEEP for 2 h, PBF gradually decreased and PVR gradually increased. The mechanisms responsible are unknown but may include a gradual decrease in the release of vasodilators (e.g., bradykinins and prostaglandins) that promote pulmonary vasodilation at birth (36). It is also possible that, with the gradual recruitment of respiratory units over time, a constant 4-cmH2O PEEP may have led to a gradual increase in the proportion of overdistended regions with high resistance. Whatever the reason, the gradual decrease in PBF with time, as well as the effect of PEEP history on PBF, were the reasons why we randomized the order in which the different PEEP levels were applied and why we returned to 4-cmH2O PEEP between each test. This allowed the change in PBF and PVR associated with the change from 4- to 0-, 8-, and 12-cmH2O PEEP to be directly compared with both the preceding and following 4-cmH2O PEEP period by using a paired analysis.

In conclusion, we have demonstrated that high levels of PEEP can have significant adverse effects on pulmonary hemodynamics in very prematurely delivered lambs, indicating that increased intra-alveolar pressures increase PVR and reduce PBF in a structurally immature lung. The increase in PVR associated with increased PEEP caused marked changes in the PBF waveform, particularly during diastole. The changes in the PBF waveform caused by increased PEEP, particularly at 12 cmH2O, were more characteristic of the fetal state than a newborn, as demonstrated by the return of retrograde flow during diastole. It is possible, therefore, that characteristics of the PBF waveform could provide important information on the level of PEEP that is appropriate for individual, very premature infants. However, as we also observed an effect of PEEP history on PBF, the relationship between airway pressure and PBF is complex and may depend on the part of the pressure-volume curve at which the infant is ventilated.

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