Intrauterine inflammation causes pulmonary hypertension and cardiovascular sequelae in preterm lambs

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Polglase GR, Hooper SB, Gill AW, Allison BJ, Crossley KJ, Moss TJ, Nitsos I, Pillow JJ, Kluckow M. Intrauterine inflammation causes pulmonary hypertension and cardiovascular sequelae in preterm lambs. J Appl Physiol 108: 1757–1765, 2010. First published March 25, 2010; doi:10.1152/japplphysiol.01336.2009.—Chorioamnionitis increases the risk and severity of persistent pulmonary hypertension of the newborn in preterm infants. Exposure of preterm fetal lambs to intra-amniotic (IA) lipopolysaccharide (LPS) induces chorioamnionitis, causes hypertrophy of pulmonary resistance arterioles, and alters expression of pulmonary vascular growth proteins. We investigated the cardiopulmonary and systemic hemodynamic consequences of IA LPS in preterm lambs. Pregnant ewes received IA injection of LPS (n = 6) or saline (controls; n = 8) at 122 days gestation, 7 days before exteriorization, instrumentation, and delivery of the fetus with pulmonary and systemic flow probes and catheters at 129 days gestation. Newborn lambs were ventilated, targeting a tidal volume of 6–7 ml/kg and a positive end-expiratory pressure (PEEP) of 4 cmH2O. At 30 min, all lambs underwent a PEEP challenge: PEEP was increased by 2 cmH2O at 10-min intervals to 10 cmH2O and then decreased similarly to 4 cmH2O. Ventilation parameters, arterial blood flows, and pressures were recorded in real-time for 90 min. LPS lambs had higher total protein in bronchoalveolar lavage fluid (P < 0.002), increased medial thickness of arteriolar walls (P = 0.013), and right ventricular hypertrophy (P = 0.012). Compared with controls, LPS lambs had worse oxygenation (P < 0.001), decreased pulmonary blood flow (P < 0.001), and higher pulsatility index (P < 0.001) and pulmonary (P < 0.005) and systemic arterial pressures (P = 0.005) than controls. Intra-amniotic LPS increased right-to-left shunting across the ductus arteriosus (P = 0.018) and decreased left ventricular output (P < 0.001). We conclude that inflammation and pulmonary remodeling induced by IA LPS adversely alters pulmonary hemodynamics with subsequent cardiovascular and systemic sequelae, which may predispose the preterm lamb to persistent pulmonary hypertension of the newborn.

Persistent pulmonary hypertension of the newborn; Doppler echocardiography; preterm birth; chorioamnionitis

DURING FETAL LIFE, THE LUNGS receive only 10–12% of combined ventricular output, and fetal gas-exchange requirements are met by the placenta (10, 38). Pulmonary blood flow (PBF) is low due to high pulmonary vascular resistance (PVR), and right ventricular output is shunted away from the lungs and into the systemic circulation through the ductus arteriosus (DA). At birth, airway liquid clearance and the onset of pulmonary ventilation initiate a major decrease in PVR and PBF increases 8- to 10-fold, allowing 100% of right ventricular output to enter the lungs (10, 30, 38). Failure of the reduction in PVR at birth has serious cardiorespiratory implications, including continued right-to-left shunting across the DA, reduced PBF, and decreased left ventricular output (LVO), all characteristics of persistent pulmonary hypertension of the newborn (PPHN) (2, 9). Preterm infants are especially at risk of the adverse effects of PPHN.

PPHN is idiopathic or may evolve in parallel with neonatal respiratory distress syndrome and subsequent bronchopulmonary dysplasia (BPD) (16, 21, 26). The recently noted causative association between antenatal inflammation/infection (often manifest clinically as chorioamnionitis) and preterm birth (36) has stimulated studies into the possible antenatal origin of cardiorespiratory diseases related to preterm birth. Whereas the presence of antenatal inflammation or infection has been linked to the development of BPD (39), its association with the development of PPHN is poorly defined. The presence of histological chorioamnionitis is associated with more severe PPHN in term neonates, as evidenced by more frequent use of inhaled nitric oxide and increased requirement for high-frequency oscillatory ventilation (42), as well as severity of hypoxic respiratory failure (43). There is a paucity of information regarding the affect of antenatal inflammation on pulmonary vascular development and postnatal consequences of such exposures, particularly in the preterm.

Intra-amniotic injection of lipopolysaccharide (LPS) in pregnant sheep inhibits endothelial cell protein expression and results in vascular remodeling changes in small pulmonary resistance arteries in preterm lambs (14). Smooth muscle hypertrophy and increased adventitial collagen deposition within resistance arteries are evident 7 days after intra-amniotic LPS exposure (14). We hypothesized that abnormal fetal pulmonary vascular development due to intrauterine inflammation would negatively affect neonatal hemodynamic function. We investigated the effects of intra-amniotic LPS injection on cardiopulmonary and systemic hemodynamics during preterm delivery, the circulatory transition, and subsequent ventilation of preterm lambs.

MATERIALS AND METHODS

The experimental protocol was approved by the animal ethics committees of The University of Western Australian, Monash University, and The University of Sydney.

Antenatal and postnatal animal care. We performed ultrasound-guided intra-amniotic injection of 10 mg LPS (E. coli O55:B5; Sigma Aldrich; n = 6 animals) or 2 mL saline (8 control animals) in singleton
or twin-bearing pregnant ewes (Merino) at 122 ± 1 days of pregnancy (term is ~150 days) using an established technique (27). At 129 ± 1 days, animals were given general anesthesia (Attane isofluran, Bomec Animal Health, Hornsby, NSW, Australia). Polyvinyl catheters were inserted into the fetal carotid artery and main pulmonary artery. Ultrasonic flow transducers (4 mm; Transonic Systems, Ithaca, NY) were placed around the left pulmonary artery and superior vena cava (SVC), as described previously (31, 32). Left arterial PBF, SVC flow (indicative of blood flow returning from the head and upper limbs), and pulmonary and systemic arterial pressures were measured and recorded digitally in real time throughout the experiment (1 kHz; Powerlab, ADInstruments, Castle Hill, NSW, Australia). The fetal chest was closed, the fetal trachea was intubated orally, and lung liquid was drained passively. Chorioamnion tissue was sampled for assessment of inflammation.

Lambs were delivered, dried, weighed, and placed on a positive pressure ventilation device (Babylog 8000+, Dräger, Lübeck, Germany). Ventilation was initiated at peak inspiratory pressure (PIP) of 30 cmH₂O and a positive end-expiratory pressure (PEEP) of 12 cmH₂O; PEEP remained at 4 cmH₂O for the initial 30 min to allow stabilization of pulmonary hemodynamics. PIP was adjusted to maintain a tidal volume (VT) of ~7 ml/kg body wt (upper limit of 40 cmH₂O) and arterial Pco₂ (Paco₂) of 50—60 Torr; permissive hypercapnia was allowed to reduce barotrauma from the high pressures required to lower Paco₂ in this model. Inspired oxygen fraction was adjusted to maintain arterial oxygen saturation between 90% and 95%. The personnel delivering and caring for the lambs were blinded to the treatments that the animals had received. All lambs underwent a PEEP challenge to assess the cardiopulmonary hemodynamic response to pulmonary vasculature stress (31); PEEP was incremented in 2-cmH₂O steps at 10-min intervals to 10 cmH₂O followed by subsequent similar decreases back to baseline. Lambs were anesthetized with a continuous umbilical venous infusion of 0.05 mg/kg-1·min-1 remifentanil (Ultiva; GlaxoSmithKline, Port Fairy, Victoria, Australia) and 0.1 mg·kg-1·min-1 propofol (Repose, Norbrook Laboratories, Newry, Victoria, Australia). Lamb well-being examinations included regular blood-gas analysis (Rapidlab 865, Bayer Diagnostics) and postductal transcutaneous oxyhemoglobin saturation (Nellcor OxiMax N65; Tyco Healthcare, Lane Cove, NSW, Australia). Specific dynamic lung compliance was calculated as VT/kg birth weight/(PEEP · f · PaCO₂ where ΔP = PEEP and f is the respiratory frequency (11)). Arterial oxygenation was described using the alveolar-arterial difference in oxygen (33).

Lung and heart processing. At the end of the study, lambs were euthanized by pentobarbital sodium overdose (100 mg/kg iv; Vala-barb, Jurox, Rutherford, Australia). Tissue from the right lower lobe was frozen in liquid nitrogen for cytokine mRNA measurements and for assessment of wet-to-dry lung weight ratio. Total RNA isolated from frozen lung was used for quantitative RT-PCR using sheep-specific probes for IL-1β and IL-6, normalized to 18S (15). Bronchoalveolar lavage fluid was collected (12), and aliquots were used for measurement of protein (6). The right upper lobe of lung was inflation fixed in 10% buffered formalin at 30 cmH₂O for morphology. Measurements of arterial wall thickness were made using a smooth muscle actin immunostaining to demarcate the muscularis media (14) and were analyzed using Image-Pro Plus (version 4.5; Media Cybernetics, Bethesda, MD) on digitally acquired images. Measurements were made only for vessels with profiles that had perpendicular diameters that were different by no more than 33%. The adventitial fibrosis was evaluated by Masson’s trichrome staining on 5-μm paraffin sections. Ten arterioles per lamb with measurements of ≤50 μm external diameter accompanying the terminal bronchioles were scored by a blinded observer for collagen staining. Adventitial fibrosis was assessed using a semi-quantitative scoring system: score 0 = none, score 1 = mild, score 2 = moderate, and score 3 = severe. Hearts were removed at autopsy, and the ventricles were dissected and weighted. Right ventricular hypertrophy was quantified by measuring the right ventricle-to-left ventricle and septum weight ratios.

Left pulmonary arterial flow waveform analysis. Changes in the contour of the left pulmonary arterial waveform and pulsatility index were calculated as described previously (32, 34) from five consecutive cardiac cycles recorded before birth and at 10-min intervals throughout ventilation.

LVO and DA flow. LVO and DA flow direction were assessed using pulsed Doppler ultrasound (31). A 7-MHz ultrasound probe was directed anteriorly through a subcostal window to allow imaging of the left ventricular outflow tract. Five measurements of the Doppler-derived velocity time signal were recorded and averaged to determine the average velocity time integral (VTI), calculated as the area under the curve of the velocity time signal. The left ventricle outflow tract diameter was measured using calipers and a frozen two-dimensional view in a more transverse plane across the vessel. The initial diameter measurement was used to calculate all subsequent flows to decrease operator and measurement variation. Heart rate was calculated using the R-R interval. Flow in the LVO was calculated as: flow = VTI·π·heart rate·LVO-diameter²/4.

The DA was imaged from a left lateral view on the chest wall. The direction of flow in the DA was assessed using pulsed Doppler ultrasound. The proportion of time in the cardiac cycle of right to left shunting vs. left to right shunting was calculated using the ratio of left-to-right time/(left-to-right time + right-to-left time); pure left to right shunting is represented by a ratio of 1 and progressive increases in right-to-left time will reduce the ratio.

Measurements of both LVO and DA were calculated at a later stage from recordings, with the technician blind to the status of the animal. Echocardiograms were performed unblinded by two of the investigators (A. W. Gill, M. Kluckow). However, all analyses were performed at random, off line, at which time the investigators were blinded to subject group assignment and timing of the measurement.

Statistical analysis. Data are presented as means ± SE. A power calculation was performed using an estimated difference in PBF (based on previous experiments) of a minimum of 10 ml/kg with a maximum standard deviation of 6 ml/kg and allowed determination of differences of two standard deviations between group means with a calculated α-value of 0.808 for a type 1 error rate of 5%. Comparisons within groups were performed using two-way ANOVA with repeated measures (Sigmastat version 3.0, SPSS). Post hoc comparisons were performed using the Holm-Sidak method. An unpaired t-test was used to compare molecular indices. A Mann-Whitney rank sum test was used to compare morphometric indices. Statistical significance was accepted for P < 0.05.

RESULTS

Umbilical arterial blood gas and acid-base status immediately after delivery were normal for all lambs and not different between groups (data not shown). Fetal body weights were not different between groups (control group: 3.1 ± 0.6 kg; LPS group: 3.3 ± 0.2 kg).

Oxygenation. Arterial Po₂ was variable but did not differ between groups and did not change during the ventilation period (Table 1). There was a trend toward higher inspired oxygen fraction and Paco₂ in LPS lambs than in controls (P = 0.089 and P = 0.109 respectively; Table 1). Paco₂ deteriorated throughout the ventilation procedure in both groups, particularly at high PEEP (P = 0.030). Alveolar-arterial difference in oxygen was higher in LPS lambs than in controls (P < 0.001; Table 1), indicative of impaired ventilation perfusion and alveolar-arterial oxygen exchange.

Ventilation data. PIP decreased in both LPS and control lambs after the recruitment maneuver (32.1 ± 2.0 cmH₂O at 30 min to 21.0 ± 2.3 cmH₂O at 90 min; P < 0.001) but did not
mean PBF during diastole, postsystolic minimum PBF, and control animals. Despite comparable PIP, VT and minute ventilation were lower in LPS than in control lambs ($P < 0.001$ for all; Table 1). Consequently, specific measurement at 4 cmH2O PEEP. Peak inspiratory pressure, tidal volume, and minute ventilation, l · kg$^{-1}$ · min$^{-1}$ 8.4

Contraction, Torr

Ventilation and oxygenation parameters

Minute ventilation, l · kg$^{-1}$ · min$^{-1}$

Pulmonary arterial pressure was elevated before delivery in LPS lambs and remained elevated throughout the ventilation period (PEEP). Values are means ± SE. *Significant difference ($P < 0.05$) between LPS and control animals.

differ between the groups at any point (Table 1; $P = 0.479$). Despite comparable PIP, VT and minute ventilation were lower in LPS than in control lambs (Table 1). Consequently, specific dynamic lung compliency and ventilatory efficiency index were lower in LPS than in control lambs ($P = 0.022$ and $P = 0.002$, respectively; Fig. 1); no effect of time or interaction with time and/or PEEP was found.

PBF and arterial pressures. Left arterial PBF was similar between groups before delivery and after 10 min of ventilation. However, PBF was lower at 20, 30, and 40 min in LPS lambs than in controls ($P < 0.001$ for all; Fig. 2A); PBF was not different for the remaining ventilation period. Blood flow in the SVC was highly variable and not different between LPS and control lambs ($P = 0.840$; Fig. 2B). SVC flow increased with high PEEP and decreased with the subsequent decrease in PEEP, as has been shown clinically (17).

Carotid arterial pressure was not different before delivery or at birth between groups. Carotid arterial pressure increased throughout the ventilation period in LPS lambs but decreased during the same period in controls ($P = 0.005$; Fig. 2C). Pulmonary arterial pressure was elevated before delivery in LPS lambs and remained elevated throughout the ventilation period ($P < 0.001$; Fig. 3D); no effect of time or interaction with time and/or PEEP was found. There was a trend for higher heart rate in controls than in LPS lambs ($P = 0.070$; Table 1).

Left pulmonary arterial blood flow waveform analyses. Mean PBF during diastole, postsystolic minimum PBF, and end-diastolic PBF were all lower in LPS lambs than in controls ($P < 0.001$ for all; Fig. 3). Mean PBF during systole was not different between groups ($P = 0.208$). Pulsatility index, a sensitive measure of downstream resistance to blood flow (PVR), was significantly higher in LPS lambs than in controls ($P < 0.001$). Peak systolic flow and the PBF pulse amplitude (peak flow – postsystolic minimum flow) were higher in LPS lambs than in controls ($P < 0.001$ for both).

Retrograde flow (negative values of postsystolic minimum or end-diastolic left pulmonary arterial flow) was observed in all lambs after 30 min of ventilation but occurred earlier in the study in the LPS group: four of six LPS lambs exhibited retrograde flow after PEEP was increased to 6 cmH2O (by 40 min of ventilation; Fig. 3), whereas it was not observed in any of the control animals until PEEP was increased to 10 cmH2O PEEP (at 70 min of ventilation). Once apparent, retrograde

![Fig. 1. Ventilation efficiency index and compliance. Specific dynamic compliance (Cdyn,spec; A) and mean ventilatory efficiency index (VEI; B) in lipopolysaccharide (LPS; ) and control ( ) lambs during the ventilation procedure. Shaded regions indicate level of positive end-expiratory pressure (PEEP). Values are means ± SE. *Significant difference ($P < 0.05$) between LPS and controls. LPS lambs had lower Cdyn,spec and VEI than controls. Cdyn,spec improved over time in control lambs compared with LPS lambs ($P = 0.002$).](http://jap.physiology.org/content/108/6/1759/F1)

Table 1. Ventilation and oxygenation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control ($n = 8$)</th>
<th>LPS ($n = 6$)</th>
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<tr>
<td>pH</td>
<td></td>
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<tr>
<td>30 min</td>
<td>7.24 ± 0.08</td>
<td>7.16 ± 0.04</td>
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<tr>
<td>60 min</td>
<td>7.12 ± 0.08</td>
<td>7.12 ± 0.08</td>
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<tr>
<td>90 min</td>
<td>7.26 ± 0.01</td>
<td>7.14 ± 0.08</td>
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<tr>
<td>PaO2, Torr</td>
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<tr>
<td>30 min</td>
<td>54.4 ± 18.5</td>
<td>54.2 ± 1.5</td>
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<tr>
<td>60 min</td>
<td>39.1 ± 3.3</td>
<td>46.7 ± 6.4</td>
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<tr>
<td>90 min</td>
<td>65.7 ± 20.2</td>
<td>44.6 ± 15.3</td>
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<tr>
<td>PaCO2, Torr</td>
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<td>60 min</td>
<td>73.4 ± 16.7</td>
<td>75.6 ± 20.1</td>
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<td>40.1 ± 3.3</td>
<td>74.6 ± 11.8*</td>
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<td>FIO2, %</td>
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<td>30 min</td>
<td>54.6 ± 7.7</td>
<td>55.4 ± 6.9</td>
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<tr>
<td>60 min</td>
<td>59.0 ± 7.6</td>
<td>70.2 ± 9.1</td>
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<tr>
<td>90 min</td>
<td>56.7 ± 20.9</td>
<td>78.0 ± 13.5</td>
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<tr>
<td>A-aDo2,</td>
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<tr>
<td>30 min</td>
<td>348.4 ± 125.6</td>
<td>393.8 ± 48.9</td>
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<tr>
<td>60 min</td>
<td>371.9 ± 54.6*</td>
<td>499.2 ± 64.5*</td>
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<tr>
<td>90 min</td>
<td>395.1 ± 37.1*</td>
<td>554.8 ± 96.2*</td>
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<tr>
<td>Heart rate, beats/min</td>
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<tr>
<td>30 min</td>
<td>149 ± 7</td>
<td>128 ± 9</td>
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<tr>
<td>60 min</td>
<td>8.4 ± 1.0</td>
<td>6.5 ± 0.4*</td>
</tr>
<tr>
<td>Minute ventilation, 1 kg$^{-1}$ · min$^{-1}$</td>
<td>0.34 ± 0.01</td>
<td>0.25 ± 0.01*</td>
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Values are means ± SE. Shown are arterial gas measurements at 30 min (end of stabilization period), 60 min (10 cmH2O PEEP), and 90 min (final measurement at 4 cmH2O PEEP). Peak inspiratory pressure, tidal volume, and minute ventilation shown as mean values over the entire ventilation procedure. A-aDO2, alveolar-arterial oxygen difference; FIO2, inspired oxygen fraction; Pao2 and Paco2, arterial PaO2 and PaCO2, respectively; PEEP, positive end-expiratory pressure. *Significant difference ($P < 0.05$) between LPS and control animals.
flow persisted throughout the remainder of the study in all lambs, despite subsequent step-wise reductions in PEEP.

**Doppler echocardiography measurements.** LVO was lower in LPS lambs than in controls \((P < 0.001)\); LVO appeared to decrease with increasing PEEP in controls, whereas this effect was absent in LPS lambs (Fig. 4A). The proportion of left-to-right shunt time vs. total cardiac cycle was lower in LPS lambs at maximum PEEP than in controls \((P = 0.018\); Fig. 4B), indicative of greater right-to-left ductal shunt in the LPS lambs. The ratio was not different at 90 min.

**Inflammation.** The presence of chorioamnionitis was visually confirmed by thickened and edematous fetal membranes that were present in all LPS lambs but absent in controls. Controls had no clinical indicators of chorioamnionitis. Total protein in the bronchoalveolar lavage fluid was higher in LPS lambs than in controls \((146.6 \pm 6.9 \mu g/\mu l, LPS group; 410.5 \pm 9.7 \mu g/\mu l; P < 0.002)\). There was no difference in the expression of IL-1\(\beta\) (control group: 1.0 \(\pm\) 0.6; LPS group: 1.3 \(\pm\) 0.8; \(P = 0.628\)) and IL-6 (control group: 1.0 \(\pm\) 0.6; LPS group: 0.2 \(\pm\) 0.1; \(P = 0.236\)) mRNA in lung tissue between LPS and control lambs.

**Lung and heart measurements.** Wet-to-dry lung weight ratios were not different between LPS and control animals (control group: 8.9 \(\pm\) 0.2, LPS group: 8.1 \(\pm\) 0.6; \(P = 0.190\)). Resistance arterioles (<50 \(\mu m\) external diameter) were thicker in LPS lambs than in controls (control group: 4.5 \(\pm\) 0.2 \(\mu m\), LPS group: 5.6 \(\pm\) 0.1 \(\mu m\); \(P = 0.013\)). The adventitial fibrosis score was higher in LPS lambs than in controls (control group: 0.6 \(\pm\) 0.3, LPS 1.2 \(\pm\) 0.4; \(P = 0.01)\) with increased collagen distribution in the adventitia evident (Fig. 5). Total heart weight, corrected for body weight, was not different between groups (control group: 6.8 \(\pm\) 0.4 g, LPS group: 7.8 \(\pm\) 1.3 g; \(P = 0.22\)). Right ventricular hypertrophy was evident in LPS lambs, with a higher right ventricle-to-left ventricle and septum ratio (control group: 0.36 \(\pm\) 0.1, LPS group: 0.44 \(\pm\) 0.02; \(P = 0.012\)).

**DISCUSSION**

The prevalence of chorioamnionitis in preterm deliveries and the paucity of information about its effect on the pulmonary vasculature led us to investigate the effects of intrauterine
Fig. 3. Left main pulmonary arterial blood flow waveform analysis. Mean systolic PBF measured through the left main pulmonary artery (A), mean diastolic (B), peak systolic (C), postsystolic minimum (min) PBF (D), end diastolic PBF (E), and pulsatility index (F) in LPS (○) and control (●) lambs before delivery (fetal) and during 90 min of ventilation. Shaded regions indicate level of PEEP. Areas below the dashed line (0) indicate retrograde flow. Values are means ± SE. *Significant difference (P < 0.05) between LPS and controls. LPS lambs had lower mean diastolic PBF and postsystolic minimum PBF than control animals, indicative of higher pulmonary vascular resistance, as confirmed by the higher pulsatility index.
inflammation by intra-amniotic LPS injection on pulmonary function and early postnatal cardiovascular function in preterm lambs. We have shown for the first time that antenatal exposure to LPS for 7 days and consequent pulmonary vascular remodeling results in pulmonary hypertension, higher PVR, increased right-to-left shunting through the DA and adverse PBF waveforms. Furthermore, we have shown significant cardiovascular and systemic sequelae of intrauterine inflammation, including right ventricular hypertrophy, reduced LVO, and higher systemic arterial pressures.

Intra-amniotic LPS initiates an inflammatory response within the lungs that increases white blood cell counts and cytokines IL-1β, IL-6, and IL-8, which persist for days (20). Four days after LPS injection, the lungs have decreased expression of multiple markers of vascular development, indicative of reduced microvasculature development, which is a feature in the lungs of infants with BPD (1). By 7 days, the medial thickness of the arteriolar walls is increased (as reproduced in this study), alveolar size is increased, and alveolar numbers are decreased relative to controls (14, 19).

The increased muscularization of the resistance vessels and reduced microvasculature development may explain the increased pulmonary arterial pressure that we observed before delivery and during subsequent ventilation. Analysis of the PBF waveform showed significant decreases in mean diastolic PBF and the minimum value after systolic pulse and increased pulsatility index in LPS lambs compared with controls. These waveform findings suggest increased PVR (30, 40), further supported by the earlier increase in R-L shunting through the DA in LPS lambs compared with controls (Fig. 4B).

All lambs had patent DAs throughout the protocol, as expected for lambs at this stage of development. In previous studies, we showed that the normal transition for 129-day preterm ventilated lambs involves a significant initial left-to-right shunt across the DA, which contributes 50% of PBF between 10 and 30 min after birth; DA flow significantly reduces in a time-related fashion but continues to shunt left-to-right and still contributes ~25% of PBF at 2 h (7). Right ventricular hypertrophy observed in LPS lambs is a further consequence of the increased pulmonary arterial pressure and
pulsatility index and is further evidence of chronic pulmonary hypertension in this model.

Increased capillary leak and the resulting pulmonary edema, altered tissue mechanics, and increased vascular resistances are possible mechanisms whereby antenatal LPS exposure may contribute to cardiorespiratory failure in preterm neonates. Increased protein was observed in the bronchoalveolar lavage fluid of LPS lambs, indicative of increased protein leakage. However, wet-to-dry lung weight ratios (a simple index of interstitial tissue fluid content) were not different between LPS and control groups. This finding supports results from the chronically ventilated preterm lamb BPD model, which suggested that increased lung microvascular pressure rather than increased permeability was responsible for the increased vascular and airway resistance (4). Vascular dysfunction may be responsible for increased vascular resistance, as evidenced by the loss of the pulmonary vasodilator response to inhaled nitric oxide, which is attributed to diminished abundance of endothelial nitric oxide synthase (23) and soluble guanylate cyclase (5) in the pulmonary circulation (14). Although this explanation remains plausible, no differences in endothelial NOS and VEGF receptor 2 expression between LPS lambs and controls have been shown previously at 7 days (14). Other potential pathways of vascular remodeling, including the RhoA/ROCK pathway and transforming growth factor β1-mediated connective tissue growth factor (CTGF), have been shown to be important in the development of pulmonary hypertension in various models, including that of inflammation (22, 24, 25). The vasodilator response of the vessels will need to be investigated further to fully explore these concepts.

Increased pulmonary pressure and resistance have significant implications for cardiovascular function, as indicated by the reduced LVO found in LPS-exposed lambs. The reduced LVO may be a function of increased right-to-left shunting away from the lungs, resulting in reduced PBF and thus decreased left atrial filling. In our study, systolic arterial pressure progressively increased in LPS lambs; this may represent an autoregulatory increase in blood flow to maintain oxygen delivery to the brain. SVC flow, used clinically as a measure of blood flow from the upper body and brain (18), was not different between groups despite reduced LVO in LPS lambs. This indicates that cerebral autoregulation was intact despite the prematurity of these lambs. The effects of chronic LPS exposure on the structure and vasoreactive function of the systemic vasculature are unknown. Alterations to the systemic vasculature may affect the ability of the cerebral circulation to protect against hemodynamic or ischemic injuries that result from altered blood flow from the cardiopulmonary circulation. The increased systemic pressure could contribute to impaired LVO in LPS lambs.

All lambs received a ramp increase and decrease in PEEP to stress the cardiopulmonary circulation; we have previously shown that this strategy adversely affects cardiopulmonary hemodynamics (31). Increasing airway pressure causes significant reductions in PBF and increases PVR by increasing interstitial tissue pressure (reducing transmural pressure), thus reducing capillary caliber (30). Increasing PEEP also results in decreased LVO (Fig. 4A), likely as a result of decreased pulmonary venous return (35). The thinning of interstitial tissue caused by LPS (41) results in an increased translation of airway pressure to the vasculature, resulting in a greater reduction to PBF and LVO than shown in controls at higher airway pressure (Figs. 2A and 4A). LPS lambs also had lower LVO and higher DA ratio, pulmonary arterial pressure, and A-aDO2 than control lambs at birth, indicating that severe PPHN was already present in these lambs. The postnatal application of high PEEP in LPS lambs exacerbated the cardiopulmonary consequences of the antenatal environment. We have shown previously that PBF and PVR do not return to normal after subsequent reductions in PEEP, suggestive of a "history" effect of high airway pressure on the pulmonary vasculature (31, 32). PBF and LVO remained lower in LPS lambs than in controls when PEEP was decreased to 4 cmH2O. This may indicate the reduced ability of the resistance arterioles to regulate changes in blood flow when subjected to increased muscularization and increased adventitial fibrosis, which led to an exaggerated response to vasoconstrictors and decreased vasodilation in this model (14). These changes to the pulmonary circulation would result in sustained reduced pulmonary venous return, thus explaining the maintenance of low LVO in LPS lambs after the PEEP maneuver.

Despite similar peak inspiratory and mean airway pressures, LPS lambs had lower VT, worse oxygenation, lower ventilator efficiency index, and lower dynamic compliance than controls. This is similar to previous findings (11) and indicative of respiratory and vascular distress. Although we targeted PAo2 between 45 and 55 Torr, LPS lambs had worse PAo2 at 90 min due to the upper limit of PIP being reached. The higher requirements of ventilatory pressure in LPS lambs are also similar to those shown in a previous finding (11). The resulting moderate respiratory acidosis may have contributed to the higher pulmonary resistance at 90 min. Interestingly, lambs exposed to LPS for 7 days have higher saturated phosphatidylcholine and surfactant proteins A, B, and C than controls (3) but in this study had worse respiratory distress than surfactant-deficient controls. The cause of the worsened ventilation requirements is unknown, given the consistent findings of improved static compliance after LPS (11, 13) and greater surfactant, but may relate to the simplification of the lung parenchyma (41) coupled with the vascular remodeling. Surfactant was not given to these lambs at birth, as we aimed to explore the deleterious effects of PEEP on PBF in a model of preterm respiratory distress syndrome. Furthermore, our earlier study showed that, despite improved oxygenation, administration of surfactant does not prevent the deleterious effects of PEEP on PBF (8). A limitation of our present study is that the lambs were caesarean delivered, which would prevent exposure of lambs to many of the birth-related physiological events. Ewes in this study did not receive antenatal betamethasone, as our group (8) has previously shown that the pulmonary structural remodeling resulting from antenatal betamethasone exposure causes an abnormal transition in pulmonary hemodynamics at birth.

IL-1β expression and IL-6 expression were not increased at 7 days in LPS lambs. In the lungs, IL-1β and IL-6 are increased within hours of intra-amniotic LPS exposure with peak levels measured at 1–2 days (15). The increase in cytokines is resolved by 4 days (15); thus a 7-day exposure to LPS should not result in increased expression of cytokine mRNA per se. However, ventilation, particularly injurious ventilation in the presence of inflammation, is known to increase cytokine expression (29). The lack of a difference in lung cytokine mRNA
expression between the groups suggests that each group received a similarly injurious ventilation strategy. Recent prospective observational studies in humans have shown that the presence of histological chorioamnionitis is associated with more severe PPHN, indicated by greater use of inhaled nitric oxide and an increased requirement for high-frequency oscillatory ventilation (42) as well as the severity of hypoxic respiratory failure (43). Our study has shown that 7-day exposure to LPS in utero caused increased pulmonary arterial pressure and vascular resistance, resulting in increased right-to-left shunting through the DA, with subsequent cardiovascular sequelae, including decreased LVO. This study indicates that the antenatal environment may predispose the newborn preterm infant to cardiopulmonary diseases such as PPHN.

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DISCLOSURES

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

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