The cerebral critical oxygen threshold of ventilated preterm lambs and the influence of antenatal inflammation


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Andersen CC, Pillow JJ, Gill AW, Allison BJ, Moss TJ, Hooper SB, Nitsos I, Kluckow M, Polglase GR. The cerebral critical oxygen threshold of ventilated preterm lambs and the influence of antenatal inflammation. J Appl Physiol 111: 775–781, 2011. First published June 30, 2011; doi:10.1152/japplphysiol.00214.2011.—Perinatal inflammation is associated with adverse neurodevelopmental outcomes, which may be partly due to changes in the cerebral oxygen delivery/consumption relationship. We aimed to determine the critical oxygen delivery threshold of the brain of preterm, ventilated lambs and to determine whether the critical threshold is affected by exposure to inflammation in utero. Pregnant ewes received intra-amniotic injection of lipopolysaccharide or saline at 125 or 127 days of gestation. Pulmonary and systemic flow probes and catheters were surgically positioned in the fetus immediately before delivery at 129 days of gestation. After delivery, lambs were ventilated for 90 min using a positive end-expiratory pressure recruitment strategy. Cardio-respiratory variables and blood gases were measured regularly. Systemic and cerebral oxygen delivery, consumption (Fick), and extraction were calculated, and the relationship between cerebral delivery and consumption analyzed. Linear regression was used to define the transition or “critical” oxygen threshold as the point at which the slope of the oxygen delivery/consumption curve changed to be >10°. Four subgroups were defined according to the calculated critical threshold. A total of 150 measurements were recorded in 18 lambs. Fetal cerebral oxygen consumption was increased by antenatal lipopolysaccharide (P < 0.05). The postnatal critical oxygen threshold was 3.6 ml-kg⁻¹·min⁻¹, corresponding to cerebral oxygen consumption of 0.73 ml-kg⁻¹·min⁻¹. High oxygen delivery and consumption were associated with increased pulmonary and carotid blood flow and systemic extraction compared with low oxygen delivery and consumption. No postnatal effect of antenatal inflammation was observed. Inflammation in utero increases fetal, but not postnatal, cerebral oxygen consumption. Adverse alterations to pulmonary blood flow can result in reduced cerebral blood flow, oxygen delivery, and consumption. Regardless of exposure to inflammation, there is a consistent postnatal relationship between cerebral oxygen delivery and consumption.

organ oxygen delivery (Do2) is primarily determined by regional vascular resistance, cardiac output, and blood oxygen content, which is a function of hemoglobin concentration and the oxygen saturation of hemoglobin (33). An imbalance between Do2 and consumption (Vo2) that results in inadequate Do2 leads to hypoxic ischemia. Subsequent end-organ injury depends on both the depth of hypoxemia and the duration of hypoxic ischemia.

Increased blood flow and oxygen extraction are important compensatory mechanisms for reduced Do2. Failure of compensation is evident from the steepness of the slope of the Do2/Vo2 curve and/or elevation of lactate, a by-product of anaerobic metabolism (39). The critical oxygen threshold can be defined as the point at which Vo2 becomes dependent on Do2. This is the point at which compensation mechanisms, such as oxygen extraction in particular, are overwhelmed and unable to meet demand for oxygen. Previous work in preterm lambs has linked neuronal cell damage to a reduction in oxygen supply (4), supporting the pathophysiological concept of hypoxic ischemic end organ injury.

In normoxic premature newborns, blood flow to deep cerebral white matter is low (5). The combination of low cerebral blood flow and a shallow oxygen gradient from high oxygen extraction may render the deep cerebral white matter particularly vulnerable to hypoxic ischemia (22). Furthermore, long periods of ischemia created by low systemic blood flow states may increase the risk of reperfusion injury and, therefore, the risk of brain injury or death in premature newborns (25, 41). Cerebral oxygen extraction decreases as cardiac output increases in newborns, which is consistent with the concept that oxygen extraction balances Do2 and Vo2 (21). Preterm newborns with the highest cerebral oxygen extraction are at greatest risk of hemorrhagic parenchymal infarction (23), the most severe form of intraventricular hemorrhage in preterm infants. This suggests a link between an overwhelmed compensatory response, suboptimal Do2, and cerebral injury.

Acquired cerebral white matter injury remains a significant burden for surviving premature newborns (20). Exposure to intrauterine inflammation independently increases the incidence and severity of peri/intraventricular hemorrhage and periventricular leukomalacia in very preterm infants (11, 13) and is a major risk factor for long-term adverse neurological outcomes (43). Recent studies showed that cerebral blood flow is influenced by antenatal exposure to inflammation (1, 38). Lipopolysaccharide (LPS), a component of most gram-negative bacteria, is used experimentally to induce fetal inflammation through activation of the toll-like receptor-4. Intra-amniotic administration of LPS results in inflammatory signaling in both maternal and fetal blood and tissue (17, 28) compared with controls and reduced middle cerebral artery blood flow velocities have been observed in newborn rat pups from dams.
exposed to intrauterine LPS. Intrauterine inflammation lowers the fetal partial pressure of arterial oxygen (P_{aO_2}) and leads to a compensatory increase in cerebral blood flow that is blunted if additional hemodynamic stressors, such as hypoxic hypotension, are superimposed (38).

Our laboratory has shown previously that intrauterine inflammation decreases neonatal pulmonary blood flow (PBF) and left ventricular output (LVO) in preterm lambs (35). Furthermore, the cardiopulmonary hemodynamic response to increases in positive end-expiratory pressure (PEEP) was altered in LPS-exposed lambs, potentially decreasing global D\dot{O}_2. However, the effect of intrauterine inflammation on the critical threshold for D\dot{O}_2 to the brain of premature newborns is not known. This study aimed to: 1) determine the critical oxygen threshold of the brain of neonatal preterm ventilated lambs; and 2) determine whether exposure to inflammation in utero alters this threshold.

We hypothesized that the presence of inflammation would impose a metabolic load that would result in an altered critical threshold for D\dot{O}_2 in the brain, making it more susceptible to hypoxic/ischemic injury.

**METHODS**

*Animal handling.* The study was approved by the animal ethics committees of the University of Western Australia, University of Sydney and Monash University. Ultrasound-guided intra-amniotic injection of LPS (E. coli 055:B5, 10 mg; Sigma Aldrich) was performed 2 (n = 7) or 4 (n = 7) days before preterm delivery at 129 ± 1 days of gestation (term = 150 days) in twin-bearing pregnant ewes (Merino), using an established technique (31). Controls received intra-amniotic injection of saline (2 ml; n = 4).

At delivery, surgery and instrumentation were performed under general anesthesia (Atrane Isoflurane, Bovac Animal Health) for insertion of polyvinyl catheters into the fetal carotid artery, jugular vein, and main pulmonary artery. Ultrasonic flow transducers (3–4 mm; Transonic Systems, Ithaca, NY) were placed around the left pulmonary artery and carotid artery, as described previously (34).

Real-time pulmonary and carotid arterial pressures (DTX, Viggospectramed) and flow through the left pulmonary and carotid arteries were recorded digitally, and mean flows were calculated (1 kHz: Powerlab, ADI, Castle Hill, Australia). Total PBF was calculated by assuming that flow in the left pulmonary artery equaled 40% of total PBF, based on the weight difference between the right and left lungs (18, 30). Thus PBF is a close approximation of LVO following the neonatal transition. Carotid arterial flow was used to estimate cerebral blood flow due to its established correlation with brain blood flow in newborn lambs and piglets (29, 40). The fetal chest was closed, the fetal trachea intubated orally, and lung liquid was drained passively.

*Experimental protocol.* Lambs were delivered, dried, weighed, and placed on positive-pressure ventilation for 30 min to allow birth-related changes in pulmonary hemodynamics to stabilize. An umbilical catheter was inserted, with ultrasound guidance, into the left atrium for measurement of left atrial pressure. An umbilical venous line was used for maintenance of anesthesia with Remifentanil (Dornitor 0.1 mg·kg\(^{-1}·\)min\(^{-1}\), Pfizer Animal Health) and Propofol (Repose, 0.05 mg·kg\(^{-1}·\)min\(^{-1}\), Norbrook Laboratories, Victoria, Australia). Lambs were ventilated with warmed, humidified gas (Dräger Babylog 8000+, Dräger Medizintechnik, Lübeck, Germany). Peak inspiratory pressure was set initially at 40 cmH\(_2\)O, and PEEP was 4 cmH\(_2\)O. Peak inspiratory pressure was subsequently altered to maintain a tidal volume of 5–7 ml/kg body wt to a maximum of 45 cmH\(_2\)O. Exogenous surfactant was not given. A rectal thermometer was used to ensure that lambs were kept in a neutral thermal environment.

Following the 30-min stabilization, all lambs underwent a PEEP recruitment challenge to assess the cardiopulmonary hemodynamic response to pulmonary vascularity stress (36): PEEP was incremented in 2-cmH\(_2\)O steps at 10-min intervals to 10 cmH\(_2\)O, followed by subsequent similar decrements back to baseline. Blood-gas analysis was performed on arterial blood samples collected at 10-min intervals (Rapidlab 865, Bayer Diagnostics, Siemens, Bayswater, Australia) with values corrected to the lamb’s core body temperature. Doppler ultrasound was used to assess the proportion of ducal shunting, as described previously (35).

Blood flow, cardiorespiratory variables (including heart rate, invasive blood pressure, inspired oxygen tension, mean airway pressure), and blood gases were measured at 10-min intervals for the calculations listed below. Systemic and cerebral D\dot{O}_2, \dot{V}O_2 (Fick), and extraction were calculated, and the relation between cerebral D\dot{O}_2 and \dot{V}O_2 was analyzed. The critical cerebral oxygen threshold was defined as the point on the D\dot{O}_2/\dot{V}O_2 curve (both in ml·kg\(^{-1}·\)min\(^{-1}\)) at which the slope changed to be >10° (39).

*Postmortem.* Bronchoalveolar lavage (BAL) was performed on the left lung for assessment of differential cell counts performed on cytospin samples of the BAL fluid stained with Diff-Quik (Fronine Lab Supplies) (15).

*Calculations.* Oxygenation index = (P_{aO_2} × mean arterial pressure)/P_{aO_2}, where P_{aO_2} is the inspired oxygen concentration.

Arterial oxygen content (C_{aO_2}) = (1.39·Hb·SaO_2/100) + (0.003·PaCO_2) (33), where Hb is the hemoglobin concentration (g/dl), and SaO_2 is the arterial oxyhemoglobin saturation.

Cerebral D\dot{O}_2 = (CBF·C_{aO_2}) (33), where CBF is cerebral blood flow. Cerebral \dot{V}O_2 = [CBF·(C_{a-vO_2})] (33), where [C_{a-vO_2}] is the difference in carotid arterial and jugular venous oxygen content.

Cerebral oxygen extraction = C_{a-vO_2}/C_{aO_2}.

Global calculations were determined as above, with the exception that PBF was used in place of carotid blood flow as a measure of systemic cardiac output (LVO). Venous oxygen content was similarly calculated using oxyhemoglobin saturation of blood in the pulmonary artery.

*Statistical analysis.* Results are expressed as mean ± SE. Statistical analysis was performed using SAS software (SAS Institute, Cary, NC). Differences between control and LPS lambs were determined using two-way repeated-measures ANOVA. Post hoc comparisons were performed using Holm-Sidak method.

Segmented linear regression was used to estimate the critical threshold. Cerebral D\dot{O}_2 and \dot{V}O_2 were modeled as a two-segment

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**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2-day LPS</th>
<th>4-day LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>3.5 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Sex, %male</td>
<td>50</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>Cord pH</td>
<td>7.15 ± 0.03</td>
<td>7.27 ± 0.01</td>
<td>7.21 ± 0.02</td>
</tr>
<tr>
<td>Cord PaCO_2, Torr</td>
<td>72.7 ± 4.7</td>
<td>58.2 ± 2.1</td>
<td>64.1 ± 3.5</td>
</tr>
<tr>
<td>Cerebral \dot{V}O_2, ml·kg(^{-1}·)min(^{-1})</td>
<td>0.55 ± 0.03 (n = 3)</td>
<td>0.67 ± 0.52* (n = 6)</td>
<td>0.74 ± 0.38* (n = 5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lambs. PaCO_2, arterial PCO_2; \dot{V}O_2, oxygen consumption. *P < 0.05, LPS vs. control.

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relation. Each segment was assumed to be linear, and the change point or critical threshold between the segments was estimated with the restriction that the entire relation be continuous. The model was estimated with PROC NLIN in SAS 9.2.

A mixed-model accounting for the repeated measures within lamb was used to test the difference between subgroups. Significance was accepted when \( P < 0.05 \).

RESULTS

Baseline characteristics. Baseline characteristics, including cerebral \( \dot{V}O_2 \) before commencement of the experimental protocol, are shown in Table 1. There were no differences in birth weight, gestation cord pH, or cord arterial \( P\text{CO}_2 \). Fetal cerebral \( \dot{V}O_2 \) at baseline (Table 1) was significantly higher in LPS lambs compared with controls.

Inflammation. The presence of intrauterine inflammation was confirmed visually by experienced personnel in all LPS lambs by the presence of thickened and edematous fetal membranes characteristic of this experimental intervention (35). The total number of inflammatory cells in the BAL fluid was significantly higher in 2-day and 4-day LPS lambs compared with controls (controls: \( 1.4 \pm 0.4 \times 10^5/\text{kg}, 2\text{-day LPS: } 5.9 \pm 2.5 \times 10^5/\text{kg}, 4\text{-day LPS: } 4.3 \pm 1.7 \times 10^5/\text{kg}; P < 0.05 \)).

Experimental measurements. One hundred fifty measurements were recorded in 18 lambs. One (control) lamb died from a pneumothorax during the experiment. There was substantial biological variability in oxygen kinetics among the measurements. There was no antenatal treatment effect from administration of LPS on global \( \dot{D}\text{O}_2 \) (\( P = 0.654 \)), global \( \dot{V}O_2 \) (\( P = 0.369 \)), cerebral \( \dot{D}\text{O}_2 \) (\( P = 0.281 \)), or cerebral \( \dot{V}O_2 \) (\( P = 0.745 \); Fig. 1). Due to the PEEP recruitment strategy, there was a significant effect of time on global and cerebral \( \dot{D}\text{O}_2 \) (\( P < 0.001 \) for both; Fig. 1). PBF (used as a surrogate measure of LVO) was not different between groups (\( P = 0.977 \)), but decreased with increasing PEEP (\( P < 0.001 \)). There was no difference in the proportion of right-to-left compared with left-to-right shunting across the ductus arteriosus between groups (\( P = 0.834 \)). The proportion of right-to-left shunting increased in all groups throughout the ventilation procedure (\( P < 0.001 \)), suggesting a relative increase in pulmonary pressures over systemic pressure at high PEEP or as a direct result of raised intrathoracic pressure on LVO.

The slope of the relation between cerebral \( \dot{D}\text{O}_2 \) and \( \dot{V}O_2 \) in all lambs was not different between groups and thus was not affected by antenatal inflammation (Fig. 2). Accordingly, the segmented regression model was applied to the combined dataset. The cerebral critical oxygen threshold or transition point was determined from the cerebral \( \dot{D}\text{O}_2 \) and \( \dot{V}O_2 \) curve to be \( 3.6 \text{ ml·kg}^{-1}·\text{min}^{-1} \). This corresponded with a cerebral \( \dot{V}O_2 \) point of \( 0.73 \text{ ml·kg}^{-1}·\text{min}^{-1} \). For the pooled dataset, there was an exponential relationship between cerebral \( \dot{D}\text{O}_2 \) and \( \dot{V}O_2 \) (Fig. 3A; \( R^2 = 0.35; P < 0.001 \)), and a logarithmic relation was observed between cerebral \( \dot{D}\text{O}_2 \) and cerebral fractional oxygen extraction (Fig. 3B; \( R^2 = 0.24; P < 0.001 \)).

Given the lack of difference between experimental groups, the “critical” thresholds for cerebral \( \dot{D}\text{O}_2 \) and \( \dot{V}O_2 \) were used to divide measurements into four separate subgroups (see Fig. 2): low \( \dot{D}\text{O}_2 \)/low \( \dot{V}O_2 \), low \( \dot{D}\text{O}_2 \)/high \( \dot{V}O_2 \), high \( \dot{D}\text{O}_2 \)/low \( \dot{V}O_2 \), and high \( \dot{D}\text{O}_2 \)/high \( \dot{V}O_2 \). The calculated variables related to these subgroups are listed in Table 2. PBF (LVO) was significantly higher in the high \( \dot{D}\text{O}_2 \)/high \( \dot{V}O_2 \) subgroup compared with the

Fig. 1. Global oxygen delivery (using pulmonary blood flow and arterial oxygen content) (\( \dot{D}\text{O}_2 \); A), cerebral \( \dot{D}\text{O}_2 \) (using carotid artery blood flow and arterial oxygen content; B), and cerebral oxygen consumption (\( \dot{V}O_2 \)) (using carotid flow and arterial and jugular venous saturation; C) in control (●), or in lambs that received LPS 2 days (□) or 4 days (▴) before birth. The level of positive end-expiratory pressure (PEEP) is shaded. Values are means ± SE. No difference was observed between groups in any of the variables during ventilation; however, there was a significant effect of time on global and cerebral \( \dot{D}\text{O}_2 \) (\( P < 0.001 \)).
the clinical observation that the risk of hypoxic-ischemic brain damage is increased when there is inflammation/infection, even without a clear role for cytokines (12, 14). Previous studies in chronically instrumented fetal lambs found that alteration in cerebral blood flow is impaired if a hypoxic stress is combined with exposure to intrauterine inflammation (38). In our study, we did not find a difference between cerebral blood flow, \(D\bar{O}_2\), or \(V\bar{O}_2\) during ventilation between LPS and control lambs. Despite the apparent disparity in postnatal \(D\bar{O}_2\) between 4-day LPS lambs and the other groups (Fig. 1B), this did not reach statistical significance. However, our findings, and those of others (38), suggest that the uterine environment may be a more vulnerable time for cerebral hypoxic ischemic injury than the immediate postnatal period. The higher \(V\bar{O}_2\) in inflamed lambs may lead to an increased susceptibility to white matter injury postnatally.

The fetal inflammatory response to intra-amniotic LPS is well characterized (26), with inflammation maximal within 24 h, and changes in pulmonary function and structure evident after 4 days (2, 16). Although we have measured an increased

Fig. 2. Relation between cerebral \(D\bar{O}_2\) and \(V\bar{O}_2\) in control (○), or in lambs that received LPS 2 days (□) or 4 days (▼) before birth. Dashed lines indicate calculated cerebral “critical” \(D\bar{O}_2\) threshold (3.6 ml·kg\(^{-1}\)·min\(^{-1}\)) and the corresponding threshold for cerebral \(V\bar{O}_2\) (0.76 ml·kg\(^{-1}\)·min\(^{-1}\)). Solid line indicates predicted values based on regression analysis before and after the critical oxygen threshold was calculated on the independent values of all control and LPS lambs in subgroups low \(D\bar{O}_2\)/low \(V\bar{O}_2\) and high \(D\bar{O}_2\)/low \(V\bar{O}_2\). Regression analysis: subgroup low \(D\bar{O}_2\)/low \(V\bar{O}_2\), \(y = 0.22x + 0.14\); \(r^2 = 0.15\), \(P < 0.001\); subgroup high \(D\bar{O}_2\)/low \(V\bar{O}_2\), \(y = 0.64x + 0.03\); \(r^2 = 0.08\), \(P < 0.001\). Each point represents an individual measurement.

low \(D\bar{O}_2\) subgroups (\(P < 0.001\)). Carotid artery flow was higher (\(P < 0.05\)) and cerebral (\(P < 0.05\)) and systemic (\(P < 0.001\)) extraction was lower in the high \(D\bar{O}_2\) subgroups compared with the low \(D\bar{O}_2\) subgroups. Carotid arterial oxygen, arterial \(PCO_2\), oxygenation index, and core body temperature were not different between subgroups. Heart rate was higher in high \(V\bar{O}_2\) subgroups compared with the low \(D\bar{O}_2\)/low \(V\bar{O}_2\) subgroup. The mean time that low \(D\bar{O}_2\) occurred in all groups was 55 min, which corresponded to the time of highest PEEP (10 cmH\(_2\)O) in the protocol.

DISCUSSION

Acquired cerebral white matter injury from hypoxia/ischemia is a significant burden for surviving premature newborns. Exposure to inflammation in utero increases the risk and severity of white matter injury (20, 43). This study investigated the critical oxygen threshold of the brain in neonatal preterm ventilated lambs exposed to intrauterine inflammation. We observed biological variability in cerebral oxygen kinetics in premature lambs exposed to intrauterine inflammation; however, baseline values of cerebral \(V\bar{O}_2\) were higher in inflammation-exposed fetuses compared with controls. There was a consistent relationship between cerebral \(D\bar{O}_2\) and \(V\bar{O}_2\), regardless of antenatal exposure to inflammation. Lambs with both low cerebral \(D\bar{O}_2\) and \(V\bar{O}_2\) had significantly lower pulmonary, cerebral, and systemic blood flow and higher systemic oxygen extraction.

The finding of an increased baseline cerebral \(V\bar{O}_2\) of LPS lambs (Table 1) is consistent with similar findings in adults in the presence of inflammation and systemic sepsis (3, 27). These findings indicate that antenatal inflammation imposes a measurable increased metabolic load on the fetal brain that remains at preterm delivery. This finding may partially explain
systemic response at these time points (26), the fetus is not acutely unwell, as it would be if LPS were administered systemically. In this study, we showed a three- to fourfold increase in inflammatory cells in the BAL fluid after LPS exposure. It is, therefore, possible that the systemic inflammatory response elicited by intra-amniotic LPS was not robust enough to cause significant changes to cerebral oxygen kinetics. Indeed, chronic intra-amniotic administration of LPS in pregnant sheep (32) resulted in milder fetal brain injury than that caused by systemic fetal administration (10), suggesting that acute sepsis may have a more profound effect on oxygen kinetics. The apparent disparity in cerebral DO2 in the 4-day LPS groups raises the intriguing possibility that a longer exposure to LPS in utero may have detrimental consequences in preterm neonates, although this would require a larger sample size. Our laboratory showed previously that LVO was significantly reduced 7 days after LPS (35), which would likely reduce cerebral DO2. Given the dynamic temporal nature of the fetal response to intra-amniotic LPS, the timing of the exposure of inflammation before delivery is likely an important determinant of cerebral outcome.

This study showed an interaction between systemic and cerebral DO2 (but not VO2) and duration of the protocol, which may indicate the progression of respiratory distress syndrome/lung injury in these lambs. The experimental intervention was designed to stress the circulatory system, by using high airway pressures known to adversely affect cardiopulmonary hemodynamics (35, 37). This is consistent with the association in preterm newborns of low systemic blood flow (superior vena cava) and immaturity, high mean airway pressure, and an open ductus arteriosus (8, 24). Mostly, newborns operate with an excess of delivery over VO2, thereby permitting stable VO2.

The association between low systemic blood flow and the risk of death or brain injury in premature newborns is well established (25, 41). Compensation during hypoxemia requires increasing blood flow and/or oxygen extraction. Once oxygen extraction is maximized, end-organ injury is theoretically determined by blood flow. Our study highlights the importance of blood flow during low DO2 states.

It is of interest that global oxygen extraction was higher than cerebral oxygen extraction in the low DO2 and VO2 group. This may be explained by the relatively small brain size (therefore, smaller contribution to cerebral kinetics) of premature lambs compared with human newborns. Nonetheless, lambs with high delivery and consumption had much higher blood flow, permitting lower systemic and cerebral extraction, as would be expected.

We found a significant relationship between cerebral blood flow and DO2 with PBF. PBF was measured in the left main pulmonary artery, below the level of the ductus arteriosus. By adjusting for the size difference between the lung lobes (18, 30), we calculated the total blood flow entering the lung. This closely approximates LVO (or systemic blood flow), assuming closure of the foramen ovale. Our laboratory has shown previously that antenatal inflammation and postnatal ventilation, particularly with high airway pressures, significantly reduces PBF and, subsequently, LVO directly, and reestablishes a “fetal” circulation phenotype, including a right-to-left shunt through the ductus arteriosus (35, 37). Episodes of low DO2 occurred at a later time and were associated with higher PEEP, lower PBF and LVO, and greater right-to-left shunting through the ductus arteriosus, as shown in Fig. 1A (35, 37).

Table 2. Summary measures and calculated variables separated into 4 groups, according to the critical threshold for cerebral oxygen delivery and consumption

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low O2 Delivery &lt; 3.6 ml·kg⁻¹·min⁻¹</th>
<th>High O2 Delivery &gt; 3.6 ml·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Consumption &lt; 0.76 ml·kg⁻¹·min⁻¹</td>
<td>Consumption &gt; 0.76 ml·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td></td>
<td>Consumption &lt; 0.76 ml·kg⁻¹·min⁻¹</td>
<td>Consumption &gt; 0.76 ml·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Carotid blood flow, ml·kg⁻¹·min⁻¹</td>
<td>20.3 ± 7a</td>
<td>30 ± 9a</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>Pulmonary blood flow (LVO), ml·kg⁻¹·min⁻¹</td>
<td>121 ± 74a</td>
<td>103 ± 73a</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>Systemic VO2, ml·kg⁻¹·min⁻¹</td>
<td>2.2 ± 3.7</td>
<td>1.4 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>Cerebral VO2, ml·kg⁻¹·min⁻¹</td>
<td>0.46 ± 0.17</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>68</td>
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<tr>
<td>Cerebral O2 extraction, %</td>
<td>0.21 ± 0.1a</td>
<td>0.35 ± 0.13b</td>
</tr>
<tr>
<td></td>
<td>69</td>
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<tr>
<td>Systemic O2 extraction, %</td>
<td>0.28 ± 0.15a</td>
<td>0.27 ± 0.15a</td>
</tr>
<tr>
<td></td>
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<td>CAO2, vol%</td>
<td>12.2 ± 4.1</td>
<td>10.4 ± 3.1</td>
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<tr>
<td></td>
<td>70</td>
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<tr>
<td>Jugular Hb, g/dl</td>
<td>118 ± 11</td>
<td>121 ± 9</td>
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<td>Carotid PaCO2, Torr</td>
<td>47.4 ± 30.6</td>
<td>32.5 ± 7</td>
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<tr>
<td></td>
<td>70</td>
<td>19</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>163 ± 24</td>
<td>192 ± 33</td>
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<td>73</td>
<td>19</td>
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<tr>
<td>MAP, cmH2O</td>
<td>13 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>19</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>33 ± 24</td>
<td>44 ± 16</td>
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<tr>
<td></td>
<td>69</td>
<td>19</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n, no. of measurements. LVO, left ventricular output; MAP, mean airway pressure; Hb, hemoglobin; CAO2, arterial oxygen content. a,b Letters that are different indicate statistical significance (P < 0.05).
Cardiopulmonary factors, including resuscitation, mechanical ventilation, rapid volume expansion, and a patent ductus arteriosus, are closely associated with cerebral pathology in immature newborns (9). Our findings highlight the importance of adequate PBF. The relationship between poor pulmonary and cerebral blood flow and \( \text{DO}_2 \) was apparent in both the presence and absence of inflammation, highlighting that early respiratory care may be a critical factor in determining white matter injury, irrespective of the antenatal environment.

It is important to note that lambs in this study were not given antenatal corticosteroids or exogenous surfactant at birth. Antenatal corticosteroids improve the pulmonary hemodynamic transition in preterm neonatal lambs (6), which may mitigate the observed hemodynamic and oxygenation-related consequences of low-PBF states. Administration of exogenous surfactant improves oxygenation, but does not alter pulmonary hemodynamics or the influence of PEEP on PBF (7). Thus it is unlikely that surfactant would improve \( \text{DO}_2 \) in lambs with low pulmonary, systemic, and cerebral blood flow.

Although this study uses measurements similar to those used in premature babies, these measurements differ from those used previously in lambs, making direct comparisons difficult. Carotid flow and jugular oxygen saturation include blood to and from nonbrain sources (more so in lambs than human newborns), with the result that cerebral blood flow may be overestimated and oxygen extraction may be underestimated. There are well-established mathematical concerns about Fick methodology (19) (substrate difference multiplied by flow). The difficulty in using shared variables has long been debated, but is unlikely to modify the shape of the \( \text{DO}_2 \) and \( \text{VO}_2 \) relationship (39). Furthermore, the Fick method may underestimate pulmonary \( \text{VO}_2 \), which may have been significant in some of the animals in our study.

With regard to translating our findings to human infants, it is important to recognize that the fetal sheep brain at 129 days of gestation is similar to that of a late-gestation (~34 wk) human brain. From our understanding of cerebral autoregulation, a more mature brain is likely to be less susceptible to brain injury after birth. While studies of acute fetal insults, such as brief umbilical cord occlusion, have demonstrated significant disruption to cerebral autoregulation in lambs at this gestation (42), it is likely that younger gestation lambs may have an increased susceptibility.

In summary, there is biological variability in brain oxygen kinetics in preterm lambs. Despite increased fetal cerebral \( \text{VO}_2 \) values in lambs exposed to intrauterine inflammation, there is a consistent relationship between cerebral \( \text{DO}_2 \) and \( \text{VO}_2 \) postnatally, irrespective of inflammation. Lambs with cerebral \( \text{DO}_2 \) and \( \text{VO}_2 \) lower than the critical threshold had significantly lower pulmonary, cerebral, and systemic flow and higher systemic extraction, highlighting the importance of blood flow in particular. Studies of the relation between cerebral \( \text{DO}_2 \) and \( \text{VO}_2 \) may permit development of physiological therapeutic goals.

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