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Acute on chronic exposure to endotoxin is associated with enhanced chemoreflex responses in preterm fetal sheep

Lindsea C. Booth, Paul P. Drury, Cameron Muir, Ellen C. Jensen, Alistair J. Gunn, and Laura Bennet
Fetal Physiology and Neuroscience Group, Department of Physiology, University of Auckland, Auckland, New Zealand

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Booth LC, Drury PP, Muir C, Jensen EC, Gunn AJ, Bennet L. Acute on chronic exposure to endotoxin is associated with enhanced chemoreflex responses in preterm fetal sheep. Am J Physiol Regul Integr Comp Physiol 304: R799–R803, 2013. First published March 13, 2013; doi:10.1152/ajpregu.00005.2013.—There is increasing evidence that exposure to infection can sensitize the fetus to subsequent hypoxic injury. However, it is unclear whether this involves compromise of the fetal cardiovascular adaptation to acute asphyxia. Chronically instrumented 103-day-old (0.7 gestational age, term is 147 days) fetal sheep in utero were randomized to receive either gram-negative lipopolysaccharide (LPS) as a continuous low-dose infusion for 120 h plus boluses of 1 μg LPS at 48, 72, and 96 h with asphyxia at 102 h (i.e., 6 h after the final LPS bolus) induced by umbilical cord occlusion for 15 min (LPS treated, n = 8), or the same volume of saline plus occlusion (saline treated, n = 7). Fetuses were killed 5 days after occlusion. LPS was associated with a more rapid fall in fetal heart rate at the onset of occlusion (P < 0.05) and with minimally lower values during occlusion (P < 0.05). The LPS-treated fetuses had lower fetal mean arterial blood pressure (BP) and greater carotid artery blood flow (CaBF) before occlusion (P < 0.05) but showed an increase in BP and fall in CaBF to similar values as saline controls during occlusion. There were no differences between the groups in femoral blood flow before or during occlusion. Contrary to our initial hypothesis, acute on chronic exposure to LPS was associated with more rapid cardiovascular adaptation to umbilical cord occlusion.

fetal sheep; lipopolysaccharide; asphyxia; chemoreflex

EXPOSURE TO INFECTION/INFLAMMATION from a variety of bacteria before and after birth is highly associated with mortality and neurodevelopmenal sequelae (18, 24). As previously reviewed there is compelling evidence that depending on the timing of administration, preexisting infection can either sensitize or protect the brain from subsequent moderate hypoxic-ischemic events, such as can occur around the time of birth (20). It is unclear whether this association may be partly due to compromise of cardiovascular adaptation to hypoxia (13). For example, in young adult rats, hypoxia induced 90 min after gram-negative lipopolysaccharide (LPS) infusion synergistically reduced blood pressure and superior mesenteric blood flow (5). Similarly, in preterm fetal sheep, LPS administration 1 h before 2 min of asphyxia, induced by occlusion of the maternal aorta, was associated with impaired peripheral vasoconstriction during asphyxia and a high rate of fetal death compared with saline pretreatment (12).

These data are difficult to interpret given that high-dose LPS by itself is associated with severe hypotension and a high risk of death over 4 to 7 h (7, 8, 10, 22, 25). Supporting a direct effect, there is evidence of impaired chemoreflex responses in adult patients with sepsis and multiple organ failure (29, 30) and after acute LPS injection in anesthetized adult cats and neonatal piglets (11, 23). In contrast, in adult rats there was marked RNSA activation during acute LPS exposure, with no evidence of inhibition of either the baroreflex or chemoreflex (31).

Although acute preexposure to LPS 3 to 6 h before hypoxia-ischemia in neonatal rodents can increase brain injury (9, 32, 37) and similarly when LPS was given before hypoxia in chicken embryos (33), intriguingly, exposure to LPS 72 h or more before hypoxia-ischemia also markedly increases injury in rodents (9, 14, 21), while an intermediate time of 24 h can be protective (9). These data all involve exposure to single doses of LPS. In clinical practice, although acute severe perinatal sepsis is well recognized and can trigger injury, the great majority of adverse outcomes are associated with subclinical infection, in some cases with acute exacerbations (17, 36). The impact of such subacute infection on the ability to adapt to later asphyxia is unknown.

We recently reported that in 0.7 gestation fetal sheep, broadly equivalent to 28–32 wk of human development (3), the acute hypotension and death associated with high-dose, acute exposure to LPS is markedly attenuated by previous low-dose infusion of LPS (22). We used this approach, which helps to reduce the confounding effects of acute LPS-induced hypotension, to test the hypothesis that acute on chronic exposure to LPS would impair fetal chemoreflex responses and hemodynamic adaptation to acute severe asphyxia induced by complete umbilical cord occlusion for 15 min.

METHODS

Experimental preparation. All procedures were approved by the Animal Ethics Committee of The University of Auckland. Fifteen time-mated singleton Romney/Suffolk fetal sheep [98 ± 0 days gestation (ga); term = 147 days] were instrumented as previously described (22). Food, but not water, was withdrawn 18 h before surgery. Ewes were given 5 ml of Streptocin [procaine penicillin (250,000 UI/ml) and dihydrostreptomycin (250 mg/ml, Stockguard Labs, Hamilton, New Zealand)] intramuscularly for prophylaxis 30 min before the start of surgery. Anesthesia was induced by intravenous injection of Alfaxan (Alphaxalone, 3 mg/kg, Jurox, Rutherford, New South Wales, Australia), and general anesthesia was maintained...
using 2–3% isoflurane in O₂. The depth of anesthesia, maternal heart rate, and respiration were constantly monitored by trained anesthetic staff. Ewes received a constant infusion isotonic saline drip (at an infusion rate of ~250 ml/h) to maintain fluid balance.

Briefly, fetal hindlimbs and abdomen were exposed through a midline incision and a small incision in the uterus. The left femoral artery and vein were isolated and catheterized with polyvinyl catheters (inner diameter of 1.0 mm and 0.8 mm) to measure mean arterial blood pressure (BP) and mean venous pressure (VP), respectively. A 2 R-type ultrasonic blood flow probe (Transonic Systems, Ithaca, NY) was placed around the right femoral artery for measurement of femoral blood flow (FFB). The uterus was then closed in layers and a second incision made to expose the fetal head and upper chest. Polyvinyl catheters were placed in the fetal right brachial artery, for withdrawal of preductal arterial blood samples, right brachial vein, for LPS infusion, and the amniotic sac. Electrocardiogram (ECG) electrodes (Cooner Wire, Chatsworth, CA) were sewn around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA). The left carotid artery was then exposed and a 3S-type ultrasonic blood flow probe (Transonic Systems) secured around it to measure carotid blood flow (CaBF). The uterus was then closed and antibiotics (800 mg gentamicin, Pharmacia and Upjohn, Rydalmere, New South Wales, Australia) were administered into the amniotic sac. The maternal laparotomy skin incision was infiltrated with a local analgesic, 10 ml 0.5% bupivacaine plus epinephrine (AstraZeneca, Auckland, New Zealand). All fetal catheters and leads were exteriorized through the maternal flank. The maternal long saphenous vein was catheterized to provide access for postoperative maternal care and euthanasia.

Postoperatively, sheep were housed together in separate metabolic cages with access to water and food ad libitum. They were kept in a temperature-controlled room (16 °C, 10% humidity, 50 ± 10% relative humidity) for the duration of the study. Gentamicin (800 mg, Pharmacia and Upjohn) and benzylpenicillin sodium (600 mg, Novartis, Auckland, New Zealand) were administered intravenously to the ewe daily for 2 and 4 days, respectively. Fetal catheters were maintained patent by continuous infusion of heparinized saline (20 U/ml at 0.15 ml/h), and the maternal catheter was maintained by daily flushing.

**Experimental design.** LPS was administered as previously described (22). Briefly, at 102 ± 9 days gestation fetuses received a continuous infusion of either LPS (derived from *Escherichia coli*, serotype 055:B5, Sigma Aldrich, dissolved in normal saline; LPS-treated group, n = 8), or the same volume of normal saline (saline-treated group, n = 7) for 5 days. For the first 24 h LPS was infused at 100 ng/24 h (day 1) and then increased to 250 ng/day from day 2 to day 5 inclusive. On days 3, 4, and 5, at 10 AM, LPS-treated fetuses received a 1-μg bolus dose of LPS; saline-treated fetuses received the same volume of saline. Six hours after the bolus on day 5 (i.e., 102 h from the start of the infusions) all fetuses underwent complete umbilical cord occlusion induced by inflation of the occluder with normal saline over 2 s for 15 min (at 106 ± 0 g/cm²). Occlusion was performed by the same investigator at the same time of day in all cases. Fetal arterial blood samples were collected before occlusion, at 5 and 12 min during occlusion, and 10 min after umbilical cord occlusion. Blood was analyzed for pH, blood gases, hemoglobin (845 Blood Gas Analyzer and Co-oximeter, Ciba-Corning Diagnostics), and glucose and lactate content (model 2300, YSI, Yellow Springs, OH). After the end of occlusion, fetuses were allowed to recover for 5 days and then killed with an overdose of pentobarbital sodium (9 g/p 40 ml/kg) to the ewe; Pentobarbital 300, Chevnock International, Christchurch, New Zealand).

**Data analysis.** All physiological variables were recorded and saved continuously to disk for offline analysis using custom data acquisition programs (LabView for Windows, National Instruments, Austin, TX). Data recording was begun 24 h before the start of the experiment. Off-line physiological data analysis was performed using LabView-based customized programs (National Instruments). Pressure signals (Novatrans II, MX860, Medex, Hilliard, OH) were recorded using a fifth-order low-pass Butterworth filter with the cut-off frequency at 50 Hz. The signal was digitized at a sampling rate of 512 Hz, then filtered in software with a digital finite impulse response low-pass filter with a cutoff frequency of 30 Hz, sampled at 64 Hz, and stored to disk. BP was calculated as the continuous average of the recording and corrected by subtraction of intra-amniotic pressure. The fetal ECG was analog filtered using a first-order, high-pass filter at 0.05 Hz and a low-pass, eighth-order, low-pass Bessel filter at 100 Hz and digitized at 512 Hz. Carotid (CVC) and femoral (FVC) vascular conductance were calculated using the following formula: flow/(BP – VP). Data were averaged in 1-min and 5-s intervals. For 5-s data analysis, data were expressed as percentage baseline, taken as 15 min before occlusion.

**Statistics.** Statistical analysis was performed using SPSS (SPSS, Chicago, IL). Normality was confirmed with the Kolmogorov-Smirnov test. Differences in baseline physiological variables (10 min before occlusion) were tested using repeated measures ANOVA. Repeated measures ANOVA were also used to test biochemical measures and physiological variables during occlusion. Where appropriate post hoc analysis was performed using one-way ANOVAs. Statistical significance was accepted when P < 0.05. Data are means ± SE.

**RESULTS**

**Biochemical measurements.** All biochemical measurements were within our normal laboratory range for this stage of gestation (Table 1). There were no differences between saline-treated and LPS-treated fetuses before occlusion. Occlusion was associated with mixed respiratory and metabolic acidosis and hypoxia (P < 0.001). There was a significant interaction between time and group for pH and BE (P < 0.05), such that on post hoc analysis pH was significantly lower in the LPS-treated group compared with the saline-treated group during occlusion.

**Table 1. Arterial pH, blood gases, glucose and lactate values before, 5 and 12 min during, and 10 min after the end of umbilical cord occlusion in fetal sheep either treated with saline or LPS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>During Occlusion</th>
<th>Postocclusion</th>
<th>10 min Postocclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 min</td>
<td>12 min</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>7.36 ± 0.00</td>
<td>7.03 ± 0.01</td>
<td>6.92 ± 0.00</td>
<td>7.21 ± 0.01</td>
</tr>
<tr>
<td>L</td>
<td>7.36 ± 0.01</td>
<td>6.98 ± 0.01*</td>
<td>6.90 ± 0.01*</td>
<td>7.21 ± 0.01</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>48.4 ± 1.0</td>
<td>91.2 ± 3.0</td>
<td>111.3 ± 3.0</td>
<td>45.1 ± 1.0</td>
</tr>
<tr>
<td>L</td>
<td>48.2 ± 1.1</td>
<td>96.5 ± 4.6</td>
<td>110.5 ± 6.3</td>
<td>47.1 ± 1.5</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>24.9 ± 0.7</td>
<td>8.7 ± 1.0</td>
<td>8.5 ± 1.2</td>
<td>35.3 ± 1.2</td>
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<tr>
<td>L</td>
<td>24.8 ± 0.7</td>
<td>7.3 ± 0.4</td>
<td>9.7 ± 0.6</td>
<td>32.6 ± 1.1</td>
</tr>
<tr>
<td>BE, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.9 ± 0.7</td>
<td>−8.3 ± 0.9</td>
<td>−11.6 ± 0.6</td>
<td>−9.7 ± 0.4</td>
</tr>
<tr>
<td>L</td>
<td>1.0 ± 0.4</td>
<td>−9.8 ± 1.0</td>
<td>−12.7 ± 0.6</td>
<td>−8.6 ± 0.5</td>
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<tr>
<td>Hb, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>9.0 ± 0.3</td>
<td>9.8 ± 0.5</td>
<td>10.1 ± 0.4</td>
<td>9.4 ± 0.4</td>
</tr>
<tr>
<td>L</td>
<td>8.1 ± 0.5</td>
<td>9.2 ± 0.5</td>
<td>8.8 ± 0.5</td>
<td>8.7 ± 0.5</td>
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<tr>
<td>Lactate, mmol/l</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.9 ± 0.1</td>
<td>3.4 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>L</td>
<td>0.7 ± 0.1</td>
<td>3.8 ± 0.4</td>
<td>4.1 ± 0.7</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1.1 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>L</td>
<td>1.0 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE. PaCO₂, arterial partial pressure of CO₂; PaO₂, arterial partial pressure of O₂; BE, base excess; Hb, hemoglobin concentration; S, saline treated (n = 7); L, lipopolysaccharide treated (n = 8). *P < 0.05 compared with saline-treated fetuses.
occlusion, although there was no significant difference at any single time point for BE.

Cardiovascular changes with occlusion. Before occlusion BP was significantly lower in LPS-treated fetuses ($P < 0.05$; Fig. 1). At the onset of occlusion there was a significant increase in both groups to a maximum of $59 \pm 1$ mmHg and $58 \pm 3$ mmHg in the saline-treated and LPS-treated fetuses, respectively. Overall, occlusion was associated with a significant change over time ($P < 0.001$) and an interaction between time and group ($P < 0.001$), such that BP was significantly higher in the LPS-treated group for the first 2 min of occlusion compared with the saline-treated group ($P < 0.05$). Adjusting for the difference in baseline values, the groups showed a similar relative increase in the first 30 s of occlusion (Fig. 2).

During the baseline period HR tended to be higher in the LPS-treated group ($P = 0.055$; Fig. 1). Occlusion was associated with a rapid fall in HR in both groups (minimum $87 \pm 6$ beats/min saline treated vs. $91 \pm 8$ beats/min LPS treated, NS), with a faster fall in HR in the first 30 s of occlusion in LPS-treated fetuses ($P < 0.05$; Fig. 2). Overall, occlusion was associated with a significant change over time ($P < 0.001$) and interaction between time and group ($P < 0.005$) such that HR was lower in the LPS-treated group at 5 and 7 to 11 min after the start of asphyxia compared with saline-treated group ($P < 0.05$).

Before occlusion, CaBF and CVC were significantly higher in the LPS-treated fetuses ($P < 0.05$; Fig. 1). There was a substantially greater absolute initial fall in both CaBF and CVC ($P < 0.01$); however, adjusting for the baseline differences there was no significant relative difference between groups in the first 30 s of occlusion (Fig. 2). Over the remainder of occlusion CaBF fell ($P < 0.001$) with no significant differences between groups; after the first 3 min CVC progressively returned to baseline values in both groups.

Before occlusion there was no significant difference in either FBF or FVC between groups (Fig. 1). FBF and FVC fell similarly in the first 3 min of occlusion (Fig. 1 and 2) and then progressively increased back toward baseline values with continued occlusion in both groups ($P < 0.001$), with no significant difference between groups.

DISCUSSION

This study shows for the first time that relatively prolonged, acute on chronic, preexposure to LPS does not compromise the hemodynamic responses to acute severe asphyxia induced by umbilical cord occlusion in preterm fetal sheep. Indeed the initial fall in fetal HR at the start of occlusion was significantly greater than in controls, consistent with enhanced chemoreflex responses. Furthermore, although LPS pretreatment was asso-

![Fig. 1. Time sequence of changes in fetal heart rate (HR, bpm), mean arterial blood pressure (BP, mmHg), carotid blood flow (CaBF, ml/min), and vascular conductance (CVC, ml·min⁻¹·mmHg⁻¹), femoral blood flow (FBF, ml/min), and vascular conductance (FVC, ml·min⁻¹·mmHg⁻¹) in fetuses treated with either saline ($n = 7$) or lipopolysaccharide (LPS) ($n = 8$) before and during complete umbilical cord occlusion for 15 min. Data are 1-min means ± SE. The 15-min period of umbilical cord occlusion is indicated by the shaded area. *$P < 0.05$.](#)

![Fig. 2. Time sequence of changes for the first 60 s of complete umbilical cord occlusion for percent baseline fetal heart rate (%HR), mean arterial blood pressure (%BP), carotid blood flow (%CaBF), and vascular conductance (%CVC), femoral blood flow (%FBF), and vascular conductance (%FVC) in fetuses pretreated with either saline ($n = 7$) or LPS ($n = 8$). Data are 5-s means ± SE. The start of the period of umbilical cord occlusion is shown by the dashed line. *$P < 0.05$.](#)
associated with relative hypotension and vasodilation of the carotid but not femoral vascular bed, the LPS-treated group nevertheless showed a highly similar increase in blood pressure and pattern of vasoconstriction, with an essentially identical degree of hypotension and continuing peripheral vasoconstriction at the end of occlusion to saline controls.

Conceptually, the hemodynamic responses of the fetus to acute severe asphyxia include two key phases: an initial, rapid chemoreflex-mediated fall in HR and decrease in peripheral vascular conductance (2, 4, 15, 19) that help reduce workload on the heart and redirect blood flow to the essential organs, followed by a subsequent longer period of progressive hypoxic-decompensation ultimately terminated by profound systemic hypotension (34). In the present study preoclclusion exposure to LPS was not associated with any evidence of reduced chemosensor response. Indeed, HR fell more quickly in the LPS-treated group at the onset of occlusion, consistent with an enhanced reflex. This finding is in contrast with previous clinical studies of severe sepsis (29, 30) and acute LPS injection in animal models (11, 23). Similarly, we found no effect of acute on chronic LPS infusion on peripheral vasoconstriction as measured in the femoral vascular bed or on the initial increase in BP, relative to baseline values, in contrast with a previous report of blunted vasoconstruction during occlusion 1 h after LPS injection in the carcass and liver of preterm fetal sheep (12). These differences likely simply reflect that in the previous studies asphyxia was induced around the time of the maximal hemodynamic and biochemical compromise after LPS administration (6, 12) compared with exposure over more than 4 days in this study.

In part, the mechanisms are likely to include induction of self-tolerance to LPS, consistent with our previous report (22), with no fetal hypotension or tachycardia before occlusion despite administration of high-dose LPS just 6 h earlier. Nevertheless, it is not obvious how greater tolerance alone could mediate an enhanced response. There is some evidence that preexisting mild hypoxia can enhance the fetal chemoreflex responses to a subsequent acute profound insult (35). However, in the present study there was no apparent metabolic effect of the LPS infusion before occlusion. Although pH was slightly but significantly lower in LPS-treated fetal sheep during occlusion compared with saline-treated animals, there were no significant differences in fetal blood gases. Alternatively, previous studies have highlighted acute induction of proinflammatory cytokines in the medulla oblongata of rat pups (1) and carotid bodies in adult cats (11). We have previously reported that acute on chronic exposure is associated with marked induction of serum levels of the anti-inflammatory cytokine IL-10 (22). Others have reported induction of protective cytokines in the brains of adult rodents (28). Speculatively, such a switch to protective cytokines may have enhanced carotid body and brainstem function.

Carotid vascular conductance was significantly increased in LPS-treated animals before occlusion consistent with our previous report (22) and previous studies that found increased carotid blood flow despite hypotension (10, 25). Despite this marked central vasodilation, occlusion was associated with a rapid reduction in conductance that was not significantly different to saline-treated fetuses. This finding is consistent with a previous report that isolated rat aortic rings have reduced contractile responses to phenylephrine after LPS due to the induction of nitric oxide production, but that hypoxia was associated with enhanced and sustained contraction, that was mediated by hypoxia-dependent inhibition of nitric oxide production (41).

The mild reduction on blood pressure in the LPS-treated group before occlusion in the present study is consistent with the finding of relatively lower blood pressure in preterm infants with chorioamnionitis (38, 39). Interestingly, these studies suggest that chorioamnionitis was associated with no change in middle cerebral artery Doppler blood flow velocities, or cerebral oxygenation, although variability was attenuated (40). Thus, similarly to the present study, these data suggest that cerebral autoregulation is broadly intact despite exposure to infection/inflammation. Furthermore, the finding of an enhanced chemoreflex response in the present study raises the possibility that infection could expose the brain to greater swings in blood pressure and flow. Further studies are needed to test this speculation; however, it is somewhat reassuring that although absolute BP did increase at a faster rate at the start of umbilical cord occlusion, the maximum BP was the same as the saline-treated group, with enhanced vasoconstriction of the carotid vasculature.

In the present study, we found that after the initial chemoreflex phase of umbilical cord occlusion, LPS pretreatment was associated with reduced HR but no difference in either the maximal increase in BP or the subsequent progressive development of hypotension. In the fetus stroke volume is relatively constrained (16), and thus, reduced HR compared with saline treatment suggests reduced cardiac output. Given similar BP this denotes a corresponding increase in total vascular resistance. Since femoral and carotid vascular conductance were similar between groups during occlusion, it is likely LPS treatment was associated with disproportionately greater constriction in other vascular beds. Previous studies have shown that the mesenteric vasculature, for example, is particularly important in sustaining blood pressure during shock in adult animals (27) and shows a distinctive reperfusion pattern after severe asphyxia in preterm fetal sheep (26).

**Perspectives and Significance**

Clinically, severe infection is associated with a high risk of death or neurodevelopmental handicap after preterm birth. Although large boluses of LPS can compromise adaptation to asphyxia, in practice subclinical infection followed by acute exacerbations is far more common. The present study suggests that the adverse effects of acute on chronic perinatal infection/inflammation are not mediated by greater hemodynamic compromise during subsequent acute hypoxia/asphyxia events. Indeed, we found that preterm fetal sheep show enhanced initial, chemoreflex responses and sustained vasoconstriction allowing arterial blood pressure to be maintained at saline-control values throughout profound asphyxia induced by complete umbilical cord occlusion.

**GRANTS**

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

REFERENCES


