Adverse Intrauterine Conditions Diminish the Fetal Defense Against Acute Hypoxia by Increasing Nitric Oxide Activity

David S. Gardner, PhD; Abigail L. Fowden, PhD, ScD; Dino A. Giussani, PhD

Background—The incidence of perinatal morbidity arising from birth hypoxia or asphyxia has not changed significantly in recent years despite marked improvements in labor management. Perinatal mortality in these circumstances may therefore reflect antenatal compromise and subsequent alteration of the fetal capacity to respond to episodes of hypoxia that may occur during labor. Hence, we have investigated the effects of fetal pre-exposure to a period of adverse intrauterine conditions on the mechanisms mediating the fetal defense response to a subsequent episode of acute hypoxia in sheep.

Methods and Results—Sixteen fetal sheep were chronically instrumented at 118±2 days for recording of blood pressure, heart rate, and femoral and umbilical blood flows. In 8 of these fetuses, umbilical blood flow was reduced by 30% for 3 days (between days 125 and 128). The remaining 8 fetuses acted as sham-operated controls. Between 2 and 7 days after umbilical cord/sham compression, all fetuses were exposed to 2 episodes of acute hypoxemia on separate days during infusion with either saline or treatment with a combination of N\textsuperscript{G}-nitro-L-arginine methyl ester and sodium nitroprusside. We show that previous fetal exposure to a period of adverse intrauterine conditions, such as that induced by compression of the umbilical cord, elevates nitric oxide activity and results in a markedly diminished cardiovascular defense response to subsequent acute hypoxia.

Conclusions—The data imply that pre-exposure to adverse antenatal conditions may render the fetus more susceptible to the acute hypoxia or asphyxia that can accompany relatively uncomplicated labor and delivery. (Circulation. 2002;106:2278-2283.)

Key Words: fetus ■ pregnancy ■ hypoxia ■ nitric oxide

The fetus is able to withstand short periods of relatively mild adverse conditions in utero, such as the acute hypoxia or asphyxia that can accompany uncomplicated labor and delivery.¹ The fetus copes with this challenge by mounting a coordinated defense that involves cardiovascular, endocrine, and local components, which, in turn, facilitate physiological adaptation to the period of reduced oxygen availability.² Cardiovascular responses to acute hypoxia include hypertension, bradycardia, and peripheral vasoconstriction. The latter contributes to a redistribution of the combined fetal cardiac output away from peripheral circulations toward vital organs such as the heart, brain, and adrenal glands.³ Fetal bradycardia and peripheral vasoconstriction are triggered by a carotid chemoreflex.⁴ Although heart rate may return toward basal levels during acute hypoxia, the peripheral vasoconstriction is maintained by a balance between increased activity of vasoconstrictor and vasodilator factors in peripheral vascular beds that ultimately favors vasoconstriction.²

Clinical evidence suggests that the incidence of perinatal morbidity arising from birth hypoxia or asphyxia has remained high despite the marked improvements in obstetric practice and the management of labor in recent years.⁵ Perinatal mortality in these circumstances may therefore reflect antenatal compromise rather than the conditions encountered during labor per se.⁶ One possibility is that fetal exposure to adverse intrauterine conditions before labor may result in an attenuation of cardiovascular defense mechanisms, rendering the fetus more susceptible to the relatively mild or short-term hypoxia/asphyxia of uncomplicated labor and delivery.

To date, however, little is known about the extent to which fetal cardiovascular defense mechanisms against acute hypoxia are modified by prevailing and irreversible adverse intrauterine conditions,⁷ and no study has investigated how these mechanisms may be modified by a preceding yet reversible period of adverse intrauterine conditions. This is important because episodes of acute hypoxia are just as likely to occur after as during periods of adverse intrauterine conditions. Hence, this study investigated in sheep the cardiovascular defense responses of fetuses pre-exposed to a reversible period of adverse intrauterine conditions induced by a controlled but partial compression of the umbilical cord.

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Methods

Surgery
Sixteen Welsh Mountain ewes carrying singleton pregnancies of known gestational age were used in the study. All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. All animals were fasted for 24 hours before surgery. Surgery was performed under aseptic conditions at 118 ± 2 days of gestation (term, ∼145 days of gestation). Anesthesia was induced with sodium thiopentone (20 mg/kg IV intraval sodium; Rhone Mérieux) and maintained with 1% to 2% halothane in 50:50 O2/N2O. In brief, fetal vascular and amniotic catheters were inserted, and transit-time flow transducers (Transonic) were placed around the contralateral femoral and an umbilical artery, close to the common umbilical artery, inside the fetal abdominal cavity. An inflatable occluder cuff (In Vivo Metrics) was positioned around the proximal end of the umbilical cord as described previously in detail.8

Experimental Protocol
No experiment was performed until at least 5 days after surgery. At 124 ± 1 days, baseline mean unilateral umbilical blood flow was recorded in all fetuses for 1 day. At 125 ± 1 days, the animals were divided randomly into 2 groups, and in 8 of the fetuses, the occluder cuff was inflated to reduce umbilical blood flow by 30% for 3 days (umbilical cord compressed, UCC).5 The duration of the challenge was chosen arbitrarily. The occluder cuff was then deflated, allowing the return of umbilical blood flow to baseline. In the remaining 8 fetuses, the occluder cuff was not inflated throughout the protocol (sham compressed). Between 2 and 7 days after the end of umbilical cord compression or sham-compression, 6 fetuses from each group were exposed to 2 episodes of acute hypoxia: (1) during fetal infusion with saline (0.9% NaCl at 0.25 mL/min IV) and (2) during fetal combined intravenous treatment with Nω-nitro-L-arginine methyl ester (L-NAME) and sodium nitroprusside (the nitric oxide “clamp”; 100 mg/kg L-NAME bolus dissolved in 1 mL saline and injected intravenously, followed by 5.1 ± 0.2 μg · kg−1 · min−1, mean ± SD, infusion of nitroprusside dissolved in saline; Sigma). The nitric oxide clamp combines fetal treatment with nitric oxide synthase inhibitor L-NAME with the nitric oxide donor sodium nitroprusside. Although fetal treatment with L-NAME alone leads to pronounced systemic vasoconstriction and hypertension,10 combined treatment of the fetus with both L-NAME and sodium nitroprusside compensates for the toxic production of the gas, maintains basal cardiovascular function, and blocks de novo synthesis of nitric oxide during acute hypoxia.9 The order in which acute hypoxia was induced either during saline infusion or during treatment with the nitric oxide clamp was randomized. Fetal cardiovascular variables (arterial blood pressure, heart rate, and femoral and umbilical blood flow) were recorded continuously during each episode of acute hypoxia. To assess vasoconstrictor mechanisms in the fetal defense response, plasma catecholamine concentrations were measured during acute hypoxia, and the femoral vasopressor responses to the α-adrenergic receptor agonist phenylephrine (12.5 to 50 μg/mL IV) were examined. The vasodilator contribution was determined by the nitric oxide clamp technique. At the end of all protocols, the ewes and fetuses were euthanized with a lethal dose of sodium pentobarbitone (200 mg/kg Pentoject; Animal Ltd), and the positions of implanted catheters, occluders, and flow probes were confirmed.

Blood Sampling
Fetal arterial blood samples (0.5 mL) were drawn into sterile syringes daily and at appropriate intervals during the acute hypoxia protocols (4 mL) for measurement of arterial blood gases, pH, and percent saturation of hemoglobin (SatHb) (ABL5 blood gas analyzer and OSM2 hemoximeter, Radiometer; fetal blood corrected to 39.5°C) and determination of plasma concentrations of catecholamines by high-performance liquid chromatography (HPLC) by electrochemical detection.11 The limit of sensitivity for the assay was 20 pg/mL for epinephrine and norepinephrine. The interassay coefficients of variation for epinephrine and norepinephrine were 7.3% and 6.2%, respectively.8

Measurements, Calculations, and Statistical Analysis
Fetal arterial blood pressure was corrected for amniotic pressure. Changes in femoral and umbilical vascular resistances were calculated by dividing arterial blood pressure by either femoral or umbilical blood flow. Values for all variables are expressed as mean ± SEM unless otherwise stated and were compared by 2-way ANOVA with repeated measures (Sigma-Stat; SPSS Inc) comparing the effect of time, group (control versus UCC), and interactions between group and time. Summary-measure analysis was conducted on cardiovascular data obtained during the acute hypoxia protocols to focus the number of comparisons.12 When a significant effect of time or group was indicated, the post hoc Student’s t test for unpaired data was used. For all comparisons, statistical significance was accepted at a value of P < 0.05.

Results

Computerized Model for Induction of Adverse Intrauterine Conditions by Partial Cord Compression
Baseline unilateral umbilical blood flows were not different in control (187 ± 23 mL/min) and UCC (207 ± 27 mL/min) fetuses. In control fetuses, umbilical blood flow remained unchanged from baseline for the duration of the experiment (Figure 1A). Inflation of the occluder cuff in UCC fetuses reduced umbilical blood flow by 29 ± 3% from baseline (to 146 ± 18 mL/min) over the period of umbilical cord compression (Figure 1B). For the 24-hour period after deflation of the cord occluder, umbilical blood flow returned to a level that was significantly greater than baseline (Figure 1B). During partial umbilical cord compression, fetuses developed mild asphyxia, which was characterized by significant falls in pH, PaO2, and SatHb and an increase in arterial PaCO2 (Table 1). After 1 day of recovery from cord compression, values for all these variables had returned to basal conditions.

Measurements During Acute Hypoxia in the Fetus
Baseline arterial blood gas status during acute hypoxia with a background of saline infusion or during treatment with the nitric oxide clamp was similar in control and UCC fetuses (Table 2). The reductions in PaO2 and SatHb were similar during acute hypoxia with saline infusion or during treatment with the nitric oxide clamp and to a similar level in each of the 2 groups of fetuses (Table 2). Although acute hypoxia occurred without changes to PaCO2 in either group, there were significant reductions in pH, by 45 minutes of hypoxia in both control and UCC fetuses (Table 2). After each episode of acute hypoxia, values for PaO2, PaCO2, and SatHb, but not pH, returned to baseline by 45 minutes of recovery.

Fetal Cardiovascular Responses to Acute Hypoxia During Saline Infusion
Baseline fetal cardiovascular variables were similar in sham control and UCC fetuses during saline infusion [arterial blood pressure, 50.6 ± 4.6 versus 55.9 ± 4.7 mm Hg; heart rate, 155 ± 5 versus 161 ± 4 bpm; femoral vascular resistance, 1.50 ± 0.21 versus 1.64 ± 0.15 mm Hg · (mL/min)−1]. In all fetuses, acute hypoxia during saline infusion led to significant
Fetal Vasoconstrictor Activity During Acute Hypoxia With Saline Infusion
Baseline fetal plasma norepinephrine, but not epinephrine (Figure 3A), and total plasma catecholamine (epinephrine plus norepinephrine; 710±82 versus 445±57 pg/mL) concentrations were significantly greater in UCC than control fetuses. Acute hypoxia led to significant increases in the plasma concentration of norepinephrine (by 15 and 45 minutes of hypoxia), epinephrine (by 45 minutes of hypoxia) (Figure 3, A and B), and total catecholamines (to 1041±200 versus 2208±466 pg/mL by 15 minutes of hypoxia) in control and UCC fetuses, respectively. The increase in the plasma concentrations of norepinephrine and of total catecholamines after 15 minutes of hypoxia was significantly greater in UCC than control fetuses (Figure 3A). During recovery from acute hypoxia, plasma norepinephrine and total catecholamines (1152±162 versus 624±128 pg/mL) remained at a significantly elevated level in UCC relative to control fetuses (Figure 3A).

Fetal Responses to Phenylephrine Injection
Arterial blood pressure increased significantly at 12.5 μg (16±1 versus 17±1 mm Hg), 25 μg (19±1 versus 19±1 mm Hg), and 50 μg (23±1 versus 27±1 mm Hg) phenylephrine, and heart rate decreased significantly at 12.5 μg (−40±6 versus −54±6 bpm), 25 μg (−53±5 versus 54±5 bpm), and 50 μg (−60±8 versus 74±8 bpm) phenylephrine in control and UCC fetuses, respectively. There were no significant differences between groups. However, the calculated increase in femoral vascular resistance after 12.5 and 50 μg of phenylephrine was significantly greater in UCC than control fetuses (Figure 3C).

Fetal Cardiovascular Responses to Acute Hypoxia During the Nitric Oxide Clamp
Prevention of de novo NO production using the nitric oxide clamp had no effect on baseline femoral vascular resistance [control, 1.87±0.28 versus UCC, 2.12±0.25 mm Hg · (mL/min)−1] but increased the magnitude of the increase in femoral vascular resistance after acute hypoxia (Figure 3C).

### Table 1. Fetal Carotid Arterial Blood Gas Status During Umbilical Cord/Sham Compression

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Umbilical Cord Compression</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−1 day</td>
<td>−1 hour</td>
<td>+1 hour +8 hours +1 day +2 days +3 days +4 days</td>
</tr>
<tr>
<td>pHa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.36±0.01</td>
<td>7.35±0.01</td>
<td>7.34±0.01</td>
</tr>
<tr>
<td>UCC</td>
<td>7.34±0.01</td>
<td>7.34±0.01</td>
<td>7.31±0.01†</td>
</tr>
<tr>
<td>Paco2, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>48.4±1.7</td>
<td>50.2±0.8</td>
<td>50.1±1.7</td>
</tr>
<tr>
<td>UCC</td>
<td>52.2±1.6</td>
<td>52.1±1.6</td>
<td>54.7±1.7</td>
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<tr>
<td>PacO2, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.5±0.8</td>
<td>24.0±1.5</td>
<td>24.3±1.1</td>
</tr>
<tr>
<td>UCC</td>
<td>23.6±1.4</td>
<td>23.1±1.0</td>
<td>20.2±1.3†</td>
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<tr>
<td>Sattb, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68.1±2.3</td>
<td>67.7±2.8</td>
<td>67.9±3.9</td>
</tr>
<tr>
<td>UCC</td>
<td>66.8±2.3</td>
<td>65.6±2.3</td>
<td>58.7±3.1*</td>
</tr>
</tbody>
</table>

*P<0.05, baseline vs umbilical cord compression or recovery; †P<0.05, control vs UCC.
vascular resistance during acute hypoxia in both control (Figure 4, A versus B) and UCC (Figure 4, C versus D) fetuses. Indeed, UCC fetuses treated here with the nitric oxide...response to acute hypoxia (Figure 4, C versus D).

**Discussion**

This is the first study to demonstrate that fetal exposure to a relatively short period of reversible adverse intrauterine conditions during late gestation diminishes the fetal vasoconstrictor response to a subsequent episode of acute hypoxia. The findings have important clinical implications, because the fetus that is less able to redistribute its blood flow during a subsequent acute challenge may be more susceptible to intrapartum complications. This finding may explain, at least in part, why perinatal morbidity arising from birth hypoxia or asphyxia has not been affected by the marked improvements in obstetric practice and the management of labor in recent years.

The present study also has implications for the physiological mechanisms involved in the fetal defense response to an episode of acute hypoxia and the adaptation of this response to a period of antenatal compromise. In uncompromised fetuses, the carotid bodies increase their afferent discharge in response to acute hypoxia and trigger both a vagally mediated bradycardia and a sympathetically mediated vasoconstriction in peripheral vascular beds, which can be abolished by fetal treatment with α-adrenergic receptor antagonists. With continuing hypoxia, many endocrine pathways are recruited, resulting in an increased plasma concentration of catecholamines, vasopressin, cortisol, angiotensin II, and neu-ropetide Y in the fetal circulation. Each of these humoral factors coordinates maintained peripheral vasoconstriction and, together with local vasodilatation in the brain, heart, and adrenal glands, aids redistribution of blood flow in the fetus during hypoxic stress. The increase in fetal plasma catecholamines during hypoxia also opposes the vagally mediated bradycardia, returning the fetal heart rate toward baseline values and promoting tachycardia after the end of the acute hypoxic episode.

In the present study, fetal exposure to a reversible period of adverse intrauterine conditions almost abolished the periph-
eral vasoconstrictor response to a subsequent episode of acute hypoxia. This could have resulted from either a decreased vasoconstrictor response, an enhanced vasodilator response, or a combination of the two. There was a greater increase in plasma catecholamines during acute hypoxia in UCC than control fetuses; furthermore, fetal treatment with increasing bolus doses of the $\alpha$-adrenergic agonist phenylephrine led to greater femoral vasopressor responses in UCC than in control fetuses. These data therefore suggest that attenuation of the peripheral vasoconstrictor response to acute hypoxia in UCC fetuses is not caused by either reduced release of catecholamines into the fetal circulation or reduced sensitivity of the peripheral vasculature to sympathomimetic agents. Treatment of control fetuses with the nitric oxide clamp revealed a greater increase in femoral vascular resistance during acute hypoxia, confirming previous reports that increased nitric oxide activity results in the near abolition of peripheral vasoconstriction. However, in fetuses previously compromised by a reversible period of umbilical cord compression, the balance between vasodilator and vasoconstrictor influences on the peripheral circulations is shifted, resulting in an upregulation of vasodilator activity, in particular nitric oxide.

Both elevated catecholaminergic and nitrergic activity may have differential regional circulatory effects in the UCC fetus. For example, in peripheral circulations, represented in the present article by the femoral vascular bed, an upregulation of nitric oxide activity results in increased blood flow. Indeed, preliminary evidence from our laboratory suggests this may be true, because UCC fetuses show a greater increase in umbilical vascular conductance than sham controls during subsequent acute hypoxia (D.S.G., D.A.G., unpublished observations). Blunted peripheral vasoconstriction with enhanced vasodilatation in other circulations is suggestive of enhanced cardiac output during subsequent acute hypoxia in UCC fetuses. The trend toward a faster return of heart rate to basal values during subsequent acute hypoxia in UCC fetuses may reflect greater $\beta$-adrenergic activity on the fetal heart, which may facilitate an increase in cardiac output.

vascular resistance during acute hypoxia reflects a balance between enhanced vasodilator and vasoconstrictor influences on the peripheral circulations that ultimately favors vasodilatation. However, in fetuses previously compromised by a reversible period of umbilical cord compression, the balance between vasodilator and vasoconstrictor influences on the peripheral circulations is shifted, resulting in an upregulation of vasodilator activity, in particular nitric oxide.

Figure 3. Fetal plasma norepinephrine (A) and epinephrine (B) during acute hypoxia and femoral vascular resistance response to phenylephrine injection (C). Values are mean±SEM for control (n=8) and UCC (n=8) fetuses. Fetal arterial blood samples were collected for measurement of catecholamines by HPLC at 15 and 45 minutes of normoxia, after 15 and 45 minutes of hypoxia, and after 45 minutes of recovery. Doses of phenylephrine (12.5 to 50 $\mu$g/mL IV) were administered to all fetuses in random order. Statistical differences are *P<0.05, normoxia vs hypoxia or recovery; †P<0.05, sham control vs UCC fetuses.

Figure 4. Fetal vasoconstrictor response to acute hypoxia. Values are mean±SEM for change from baseline in femoral vascular resistance in control fetuses during saline infusion (A, n=8) and during treatment with nitric oxide clamp (B, n=6) and in UCC fetuses during saline infusion (C, n=8) and during treatment with nitric oxide clamp (D, n=6). Statistical differences are *P<0.05, baseline vs hypoxia or recovery; †P<0.05, saline vs nitric oxide clamp; ‡P<0.05, sham control vs UCC fetuses.
drive to the heart. The extent to which increased nitric oxide activity reflects local upregulation in the endothelium of peripheral circulations and/or enhanced production of nitric oxide–dependent vasodilator factors in the placental and/or fetal circulations remains to be determined. In this regard, the compensation in umbilical blood flow in the first 20 hours after relief of umbilical cord compression in the present study is interesting and may indicate enhanced activity of nitric oxide–dependent vasodilators in the fetoplacental circulations, because the compensation appears resistance- rather than pressure-dependent.

In conclusion, we have shown that fetal pre-exposure to a reversible period of adverse intrauterine conditions upregulated nitric oxide–dependent vasodilator mechanisms and markedly reduced the peripheral vasoconstrictor response to a subsequent episode of acute hypoxia, even when induced up to a week after the end of the period of adversity. We suggest that antecedent fetal compromise may diminish the cardiovascular responses to acute oxygen deprivation. This may increase fetal susceptibility to episodes of hypoxia or asphyxia, such as those that may accompany even relatively uncomplicated labor and delivery.

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References
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