The ontogeny of hemodynamic responses to prolonged umbilical cord occlusion in fetal sheep

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Wassink G, Bennet L, Booth LC, Jensen EC, Wibbens B, Dean JM, Gunn AJ. The ontogeny of hemodynamic responses to prolonged umbilical cord occlusion in fetal sheep. J Appl Physiol 103: 1311–1317, 2007.—There is evidence that preterm fetuses have blunted chemoreflex-mediated responses to hypoxia. However, the preterm fetus has much lower aerobic requirements than at term, and so moderate hypoxia may not be sufficient to elicit maximal chemoreflex responses; there are only limited quantitative data on the ontogeny of chemoreflex and hemodynamic responses to severe asphyxia. Chronically instrumented fetal sheep at 0.6 (n = 12), 0.7 (n = 12), and 0.85 (n = 8) of gestational age (GA; term = 147 days) were exposed to 30, 25, or 15 min of complete umbilical cord occlusion, respectively. At all ages, occlusion was associated with early onset of bradycardia, profoundly reduced femoral blood flow and conductance, and hypertension. The 0.6-GA fetuses showed a significantly slower and lesser fall in femoral blood flow and conductance compared with the 0.85-GA group, with a correspondingly reduced relative rise in mean arterial blood pressure. As occlusion continued, the initial adaptation was followed by loss of peripheral vasoconstrictive and progressive development of hypotension in all groups. The 0.85-GA fetuses showed significantly more sustained reduction in femoral conductance but also more rapid onset of hypotension than either of the younger groups. Electroencephalographic (EEG) activity was suppressed during occlusion in all groups, but the degree of suppression was less at 0.6 GA than at term. In conclusion, the near-midgestation fetus shows attenuated initial (chemoreflex) peripheral vasomotor responses to severe asphyxia compared with more mature fetuses but more sustained hemodynamic adaptation and reduced suppression of EEG activity during continued occlusion of the umbilical cord.

chemoreflex; immaturity; preterm

THE INITIAL, RAPID FALL in fetal heart rate (FHR) and redistribution of blood flow away from peripheral organs during hypoxia or asphyxia (profound hypoxia with metabolic and respiratory acidosis) are key fetal adaptations that are generally believed to help maintain perfusion of vital organs and reduce myocardial work (15). The initial bradycardia is vagally mediated, while the peripheral vasoconstriction is mediated through α-adrenergic efferents (17). These reflex responses are primarily mediated by the carotid chemoreceptors (4, 17, 26) and are closely correlated with the degree of reduction in fetal arterial oxygen saturation and utero-placental flow (1, 25, 38).

Chemoreflex responses have been demonstrated from very early in gestation (31). However, preterm fetal sheep at 0.6 or 0.7 gestation show very different and apparently blunted or “immature” responses to moderate inhalational hypoxia, hemorrhagic hypotension, and partial umbilical cord occlusion compared with term (11, 20, 27, 28, 43). For example, 0.6-gestation fetal sheep did not show the initial bradycardia or hypertension that develops during moderate hypoxia in late gestation (11, 20, 27). Even from late gestation to full term there is a further developmental increase in the magnitude and persistence of fetal bradycardia and in the magnitude of the femoral constrictor response to moderate hypoxia in fetal sheep (15). These data have been suggested to indicate attenuated chemoreflex responses to oxygen deprivation that may contribute to the high incidence of hypoxic-ischemic injury in premature infants (2).

However, these studies have typically used relatively moderate levels of hypoxic stress. An important methodological consideration for studies of hypoxic stress is that the preterm fetus is known to have much greater anaerobic reserves and lower overall aerobic requirements compared with term (20, 29, 34, 40, 42). This suggests the hypothesis that mild or brief hypoxic insults may not be sufficient to elicit maximal chemoreflex responses in the preterm fetus, and thus a relatively severe degree of hypoxemia is required to robustly assess the maturation of the chemoreflex. Consistent with this hypothesis, several studies have demonstrated functional chemoreflex responses to severe asphyxia near-midgestation (9, 16, 30, 32), that are qualitatively similar to the full-term fetal sheep (6, 23, 32). However, there are only limited quantitative data on the ontogeny of the responses to such insults. Although severe asphyxia at 0.6 gestation elicited a similar immediate fall in FHR to that seen at term (9), the younger fetuses showed either no increase in blood pressure after 2 min or a small increase compared with marked hypertension near term (9, 29). The mechanisms of this difference remain unclear.

We therefore assessed the chemoreflex and hemodynamic responses to severe asphyxia induced by complete umbilical cord occlusion in fetal sheep at 0.6, 0.7, and 0.85 gestation. We examined relative suppression of EEG activity during occlusion at each age as a measure of anaerobic tolerance. These ages are broadly equivalent to the neural maturation of the human fetus at 26- to 28-wk, 28- to 32-wk, and 40-wk gestation, respectively (33). The incidence of neural injury is greatest in infants born before 28-wk gestation and falls with advancing maturity (2).

MATERIALS AND METHODS

Surgical preparation and postoperative care. All procedures were approved by the Animal Ethics Committee of The University of Auckland, New Zealand (e-mail: aj.gunn@auckland.ac.nz).

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Auckland, New Zealand. Groups of Romney ewes were time mated with Suffolk rams. Under general anesthesia, singleton fetal sheep were instrumented at either 86–89 days, 98–99, or 117–122 days gestational age (GA, term = 147 days).

Food but not water was withdrawn 18 h before surgery. Ewes were given 5 ml of Streptococci penicillin (250,000 IU/ml) and dihydrostreptomycin (250 mg/ml), Pitman-Moore, Wellington, NZ) intramuscularly for prophylaxis 30 min before the start of surgery. Anesthesia was induced by intravenous injection of Alphalaxone and Alphadalone (3 mg/kg, Schering-Plough Animal Health, Wellington, NZ), and general anesthesia was maintained using 2–3% halothane in O₂. The depth of maternal anesthesia and heart rate and respiration were monitored constantly by trained anesthetic staff. A 20-gauge catheter was placed in a maternal front leg vein, and the ewes were placed on a constant isotonic saline drip to maintain fluid balance.

Using sterile techniques as previously described (8, 9, 46), catheters were placed in the left fetal femoral artery and right brachial artery, and the amniotic sac. An ultrasound blood flow probe (type 2R at 0.6 and 0.7 GA and 3R at 0.85 GA, Transonic Systems, Ithaca, NY) was placed around the right femoral artery to measure femoral blood flow (FBF). A stainless steel electrode (Cooner Wire, Chatsworth, CA) was placed across the fetal chest to measure the fetal electrocardiogram (ECG). Two pairs of EEG electrodes (Cooner Wire) were placed on the dura over the anterior parasagittal parietal cortex and secured with cyanoacrylate glue. A reference electrode was sewn over the occiput. An inflatable silicone occluder was placed around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA) was placed across the fetal chest to measure the fetal electrocardiogram (ECG). Two pairs of EEG electrodes (Cooner Wire) were placed on the dura over the anterior parasagittal parietal cortex and secured with cyanoacrylate glue. A reference electrode was sewn over the occiput. An inflatable silicone occluder was placed around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA). All fetal leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized to provide access for postoperative care and euthanasia. Antibiotics (80 mg gentamicin, Pharmacia and Upjohn, Rydalmere, NSW, Australia) were administered into the amniotic sac before closure of the uterus. Amniotic fluid flow lost during surgery was replaced with normal saline warmed to 37°C. The maternal skin incision was infiltrated with a local anesthetic, 10 ml 0.5% bupivacaine plus epinephrine (AstraZeneca, Auckland, NZ) before closure.

Postoperatively, ewes were housed together in individual metabolic cages with access to water and concentrate feed (Country Harvest Stockfeed, Cambridge, NZ) ad libitum in a temperature-controlled housing facility (16 ± 1°C, humidity 50 ± 10%) with a 12:12-h light-dark cycle. During postoperative recovery, antibiotics were administered daily for 5 days intravenously to the ewe (600 mg benzylpenicillin sodium, Novartis, Auckland, NZ; 80 mg gentamicin). Fetal catheters were maintained patent by continuous infusion of heparinized saline (10 U/ml at 0.2 ml/h at 0.6 GA and 20 U/ml at 0.2 ml/h at 0.7 GA and 0.85 GA), and the maternal catheter was maintained by daily flushing.

**Data acquisition.** Fetal mean arterial blood pressure (MAP; Novatrans II, MX860, Medex, Hilliard, OH) corrected by subtraction of amniotic pressure, FBF, ECG, and EEG were recorded continuously. The blood pressure signal was collected at 64 Hz and low-pass filtered at 30 Hz. The fetal ECG was analog filtered between 0.05 and 80 Hz and digitized at 512 Hz. The analog EEG signal (recorded using amplifier filter modules kindly supplied by Brainz, Auckland, NZ) was processed with a first-order high-pass filter at 1.6 Hz and a sixth-order Butterworth low-pass filter with a cut-off frequency of 50 Hz, and then digitally stored at a sampling rate of 64 Hz. EEG intensity (power) was derived from the intensity spectrum signal between 1 and 20 Hz. For data presentation purposes, the total EEG intensity (power) was normalized by log transformation (dB, 20× log intensity), and data collected from left and right hemispheres were averaged to give mean EEG intensity. All data were stored to disk using custom software (Labview for Windows, National Instruments, Austin, TX) for off-line analysis.

**Experimental design.** Experiments were conducted 4–5 days after surgery, at either 91 ± 1 days GA (0.6 GA, n = 12), 104 ± 1 days (0.7 GA, n = 12), or 125 ± 1 days (0.85 GA, n = 8). Fetal asphyxia was induced by rapid inflation of the occluder with sterile saline of a predefined volume known to totally compress the umbilical cord, as determined in pilot experiments with a Transonic flow probe placed around an umbilical vein (9). The total durations of occlusion were chosen to represent an acute, near-terminal insult for each age, as follows: 30 min at 0.6 GA (9, 16, 36), 25 min at 0.7 GA (8, 45), and 15 min at 0.85 GA (44, 46). If bradycardia persisted for more than 60 s after release of occlusion epinephrine (0.1 ml of 1:10,000 per kg estimated fetal weight) was given intravenously to the fetus. We were not able to compare the postocclusion recovery period between the groups because nearly all the 0.85-GA fetuses but no preterm fetuses required epinephrine.

Fetal blood gas analysis (Ciba-Corning diagnostics 845 gas blood analyzer and co-oximeter) and measurements of glucose and lactate levels (YSI model 2300, Yellow Springs, OH) were performed 60 min before occlusion (baseline) and during the occlusion (0.6 GA at 5 and 25 min, 0.7 GA at 5 and 20 min, 0.85 GA at 2 and 12 min). On completion of the experimental protocol, ewes and fetuses were euthanized by an intravenous overdose of pentobarbital sodium to the ewe (9 g. Pentobarb 300; Chemstock International, Christchurch, NZ). Fetuses were then removed by hysterectomy and weighed.

**Data analysis and statistical procedures.** Off-line physiological data analysis was performed using Labview-based customized programs (National Instruments, Austin, TX). One-minute averages of FHR (derived from the fetal ECG), MAP, FBF, and EEG intensity were calculated for each fetus. Femoral vascular conductance (FVC) was calculated by dividing mean FBF by MAP. The rate of initial fall in FHR after the start of occlusion was calculated on 1-s averaged data extracted from the R-R interval as previously reported (10), from the start of occlusion to the break point of the initial rapid fall. Because of the large differences in absolute values for all variables with advancing gestation, analysis of other parameters during occlusion was based on percent changes from baseline. Between-group comparisons for physiological and metabolic variables were made by one-way ANOVA (SPSS for Windows v12, SPSS, Chicago, IL). When statistical significance was detected, post hoc comparisons were made with Fisher’s least significance difference test. Statistical significance was accepted when P < 0.05. Data are means ± SE.

**RESULTS**

**Blood composition.** Before the experiment, all fetuses had normal blood gases, pH, glucose, and lactate values for age (Table 1) according to the standards of our laboratory. Occlusion resulted in profound hypoxia and progressive severe metabolic and respiratory acidosis. Metabolic acidosis was more severe in younger fetuses than at term, reflecting the longer duration of occlusions.

**Physiological parameters.** Baseline FHR, MAP, FBF, FVC, and EEG intensity are shown in Table 2. Baseline FHR was significantly lower at 0.85 GA than in the two younger age groups, while MAP, FBF, FVC, and fetal weight were increased (0.85 GA, P < 0.05 vs. 0.6 GA and 0.7 GA). The differences between 0.7 GA and 0.6 GA were of lesser magnitude, with higher FBF but only a borderline increase in MAP at 0.7 GA (P = 0.05) and no significant difference in FHR or FVC.

**Fetal heart rate and mean arterial blood pressure.** Occlusion elicited a rapid fall in FHR in all groups. There was no significant difference between groups in the initial slope of the FHR deceleration or the depth of this fall (Table 3). After a brief relative increase in FHR at 5–7 min of occlusion at 0.85 GA (P < 0.05, 0.85 GA vs. 0.6 GA and 0.7 GA), FHR then showed a consistent and linear fall at all ages. Fetal asphyxia...
at the end of occlusion was significantly lower in the younger groups than at 0.85 GA (P < 0.05; Table 3), because of the more prolonged duration of occlusion. Despite this greater fall, all preterm fetuses showed rapid recovery of FHR (and MAP) after release of occlusion, whereas the majority (7 of 9) of 0.85-GA fetuses required epinephrine for persistent bradycardia. Three of the seven fetuses developed terminal cardiac arrest despite epinephrine.

There was an initial rise in MAP after the start of occlusion that was greatest near term (0.6 GA 144 ± 3%, 0.7 GA 167 ± 3%, 0.85 GA 189 ± 2%, P < 0.05, 0.6 GA vs. 0.7 GA, 0.85 GA, Fig. 1). MAP then progressively fell, and hypotension developed in all groups. The rate of fall in MAP increased with gestation, such that the 0.85-GA fetuses showed proportionately lower MAP than 0.6-GA fetuses from 9 min to the end of occlusion and than 0.7-GA fetuses at 15 min (Fig. 1, P < 0.05). MAP was significantly lower in 0.7-GA fetuses compared with the 0.6-GA fetuses in the last 5 min of the occlusion period of the 0.7-GA group. The mean of nadir of absolute MAP for all groups at the end of occlusion was 9.5 ± 0.6 mmHg (not significant between groups).

**Femoral blood flow and vascular conductance.** Occlusion was associated with a rapid fall in FBF and FVC (Fig. 1) that reached a nadir at the third (0.7 GA and 0.85 GA) or fifth minute (0.6 GA) of occlusion. The magnitude of the fall in percent FBF was greatest at 0.85 GA (FBF and FVC, P < 0.05, 0.85 GA vs. 0.6 GA, 1st–4th minute of occlusion). 0.7-GA fetuses showed an intermediate pattern (Fig. 1).

From the third to fourth minute of occlusion onward, fetal FVC at 0.6 GA and 0.7 GA gradually returned to baseline values. In contrast, FVC at 0.85 GA rose but remained suppressed for the remainder of occlusion compared with the other groups (P < 0.05, 0.85 GA vs. 0.6 GA, 9th–15th minute of occlusion; 0.85 GA vs. 0.7 GA, 10th–11th minute of occlusion). This was associated with a brief proportionate increase in FBF that was less toward term (P < 0.05, 0.85 GA vs. 0.6 GA and 0.7 GA). FBF then fell progressively in association with hypotension (Fig. 1).

**Electroencephalographic activity.** Occlusion resulted in a rapid reduction in EEG intensity (power) in all groups in the first few minutes, with a significant overall difference between gestations on suppression of EEG activity (P = 0.01, Fig. 2), such that after the first 3 min of occlusion there was greater residual EEG activity at 0.6 GA compared with 0.85 GA [at 14 min of occlusion (i.e., the penultimate minute at 0.85 GA), 8.7 ± 2.0 dB vs. 3.1 ± 1.6 dB, P < 0.05]. 0.7-GA fetuses showed an intermediate fall in EEG activity (4.2 ± 1.7 dB, not significant).

**DISCUSSION**

The present study has examined a controversial area in our understanding of the maturation of fetal cardiovascular responses to asphyxia: the hypothesis that the preterm fetus has attenuated or immature chemoreflex-mediated changes in fetal heart rate and peripheral vasoconstriction compared with term (11, 20, 27). Conceptually, two phases of the cardiovascular responses of the fetus to acute severe asphyxia can be distinguished: the initial, rapid chemoreflex-mediated adaptations (3, 4, 17, 26), and the subsequent longer period of progressive hypoxic-decompensation ultimately terminated by profound systemic hypotension. We demonstrate for the first time that preterm fetuses not only have a functional chemoreflex (8, 9, 16, 30, 32) but that the initial fall in FHR after the start of umbilical cord occlusion is of similar rate and magnitude to that seen near-term even in near-midgestation fetuses. In contrast, the initial fall in fetal conductance and in FBF were proportionately slower and reduced in magnitude compared with term.

Table 1. Blood gas and composition parameters

<table>
<thead>
<tr>
<th>Parameter/Gestation</th>
<th>Baseline</th>
<th>Occlusion Time 1</th>
<th>Occlusion Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 GA</td>
<td>7.39 ± 0.01</td>
<td>7.06 ± 0.08</td>
<td>6.76 ± 0.08</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>7.38 ± 0.01</td>
<td>7.05 ± 0.08</td>
<td>6.82 ± 0.08</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>7.36 ± 0.01</td>
<td>7.21 ± 0.08</td>
<td>6.95 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Po2, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 GA</td>
<td>25.1 ± 0.6</td>
<td>7.1 ± 1.0</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>22.3 ± 0.9</td>
<td>6.0 ± 0.7</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>19.6 ± 1.6</td>
<td>5.4 ± 0.9</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 GA</td>
<td>1.1 ± 0.7</td>
<td>−6.2 ± 0.6</td>
<td>−14.2 ± 0.6</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>2.5 ± 0.5</td>
<td>−4.4 ± 1.4</td>
<td>−11.2 ± 1.3</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>−0.3 ± 0.1</td>
<td>1.3 ± 0.8</td>
<td>−8.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 GA</td>
<td>1.0 ± 0.3</td>
<td>4.5 ± 0.4</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>0.7 ± 0.0</td>
<td>3.8 ± 0.2</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>1.0 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 GA</td>
<td>1.1 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>0.9 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>0.7 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SE. Blood samples were taken 60 min before the experiment (baseline) and during the occlusion period [0.6-gestation group (0.6 GA), 5 and 25 min; 0.7-gestation group (0.7 GA), 5 and 20 min; 0.85-gestation group (0.85 GA), 2 and 12 min]. BE, base excess. Between-group comparisons by one-way ANOVA and LSD test, aP < 0.05, 0.6 GA vs. 0.7 GA; bP < 0.05, 0.6 GA vs. 0.85 GA; cP < 0.05, 0.7 GA vs. 0.85 GA; dP < 0.05, eP < 0.01 vs. baseline.

Table 2. Baseline data

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, g</th>
<th>FHR, beats/min</th>
<th>FBF, ml/min</th>
<th>FVC, ml/min</th>
<th>MAP, mmHg</th>
<th>EEG, dB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 GA</td>
<td>1,283 ± 155</td>
<td>189 ± 3</td>
<td>3.8 ± 0.7</td>
<td>0.1 ± 0.0</td>
<td>35.5 ± 0.7</td>
<td>16.5 ± 1.3</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>1,623 ± 126</td>
<td>191 ± 4</td>
<td>10.9 ± 0.8</td>
<td>0.3 ± 0.0</td>
<td>37.3 ± 0.5</td>
<td>17.7 ± 0.9</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>3,329 ± 170</td>
<td>172 ± 6</td>
<td>46.2 ± 5.4</td>
<td>1.1 ± 0.1</td>
<td>45.6 ± 0.6</td>
<td>19.2 ± 1.3</td>
</tr>
</tbody>
</table>

Data are means ± SE. FHR, fetal heart rate; FBF, femoral blood flow; FVC, femoral vascular conductance; MAP, mean arterial pressure; EEG, electroencephalogram activity. Comparisons by one-way ANOVA and LSD test, aP < 0.05, 0.6 GA vs. 0.7 GA; bP < 0.05, 0.6 GA vs. 0.85 GA; cP < 0.05, 0.7 GA vs. 0.85 GA.
The attenuation in initial peripheral vasoconstriction was associated with a correspondingly smaller relative increase in mean arterial blood pressure at 0.6 gestation that was maximal after 3 min of occlusion. This is broadly consistent with one previous report that 0.6-gestation fetuses did not show a significant increase in MAP after 2 min of maternal aortic occlusion (29). The mechanisms underpinning this apparent sensitization of the α-adrenergic component of the chemoreflex toward term gestation remain unclear. Booth et al. (12) have shown that cardiac entrained renal sympathetic nerve activity (SNA) discharges are present from as early as 99 days gestation in fetal sheep. There is some evidence that resting SNA increases with maturation (35), but fetal SNA responses to pathological conditions have not been quantified.

Although bradycardia was sustained throughout the period of occlusion, a brief increase in FHR was seen in the 0.85-gestation group during the early phase of occlusion, at a time when blood pressure had started to return to baseline values. A similar response was seen after partial occlusion in near-term fetal sheep (18) and is unlikely to be a baroreflex because blood pressure had not fallen below baseline values (41). At this age, sympathetic activity acts to limit the fall in FHR, at least in the first 5 min of umbilical cord occlusion or hypoxia (24, 37). It is likely that the transient relative increase in FHR in the present study reflects a more rapid rise in circulating catecholamines in the first few minutes of severe asphyxia near term compared with 0.6 or 0.7 gestation (13, 29), although we cannot rule out some degree of attenuation of parasympathetic drive (3).

As occlusion continued, the initial peripheral vasoconstriction was only sustained for 3–5 min in all fetuses and was followed not by overt vasodilatation, but rather a return toward control values. This loss of vasoconstriction was associated with increased FBF and consequent progressive hypotension. There were significant ontogenetic differences in this response. At 0.6 gestation, FVC returned to baseline values, whereas at 0.85 gestation, femoral conductance remained significantly suppressed compared with the two younger groups. Importantly, even at 0.6 gestation, FBF never returned to baseline values during the occlusion, reflecting the development of hypotension.

A similar pattern has been described previously in other peripheral vascular beds (7, 29, 39) but remains poorly understood. It is improbable that failure of peripheral vasoconstriction reflects a significant reduction in circulating catecholamines during continuing severe asphyxia (29). It is also unlikely to be mediated by local tissue acidosis since a similar biphasic pattern occurs during partial umbilical cord occlusion at term (18) but not during moderate inhalational hypoxia despite similar partial pressures of oxygen (17), suggesting that the combined influences of chemo- and mechanoreflexes are important during occlusion. There are preliminary data that renal vasodilatation during this phase of umbilical cord occlusion is temporally associated with marked attenuation in renal SNA in 0.7-gestation fetal sheep (5), but it is unknown whether this is the case in other peripheral vascular beds.

### Table 3. Rate and magnitude of initial fall in FHR, and final nadir of FHR during umbilical cord occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Fall in FHR, beats/min</th>
<th>Initial Slope, beats·min⁻¹·s⁻¹</th>
<th>Maximal Fall in FHR, beats/min</th>
<th>FHR Nadir, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 GA</td>
<td>78.4±4.7</td>
<td>8.0±0.8</td>
<td>106.7±4.2</td>
<td>51.6±2.7*</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>65.9±6.7</td>
<td>6.2±1.0</td>
<td>99.9±5.6</td>
<td>61.0±4.0*</td>
</tr>
<tr>
<td>0.85 GA</td>
<td>64.8±9.4</td>
<td>7.1±1.2</td>
<td>93.3±6.3</td>
<td>74.0±5.5</td>
</tr>
</tbody>
</table>

Data are means ± SE. Between-group comparisons by one-way ANOVA and LSD test, *P < 0.05, 0.6 GA vs. 0.85 GA, †P < 0.05, 0.7 GA vs. 0.85 GA.
Loss of initial vasoconstriction was associated with a further, progressive fall in heart rate, and, after ~10 min in all groups, with overt hypotension. The rate at which percent FHR fell in this phase was not different between groups. However, the final absolute and relative nadir of FHR was lower in younger fetuses because of the longer duration of occlusion. Likely contributors to impaired cardiac function mediating the continuing fall in FHR include severe hypoxia, acidosis, depletion of myocardial glycogen, and cardiomyocyte injury (22). There is no evidence for continuing reflex mechanisms at this time (3). Despite the similar rate of fall of heart rate between groups in this late phase, hypotension developed more rapidly toward term gestation (32). A potential limitation of the present study is that blood gases were taken at different times. Nevertheless, all fetuses received the same insult, complete occlusion of the umbilical cord, and the ultimate severity of the oxygen debt as reflected by metabolic acidosis was least near term.

Fetal MAP is a function of combined ventricular output and vascular resistance, and combined ventricular output is the product of heart rate and stroke volume. Since stroke volume is relatively constrained in the fetus compared with afterbirth (21), and given similar relative falls in FHR and greater residual vasoconstriction in the second half of the occlusion in the 0.85-gestation fetuses, the greater hypotension in older fetuses must reflect reduced stroke volumes, presumptively due to greater impairment of cardiac contractility. The remarkable ability of the preterm fetus to survive during prolonged periods of asphyxia has been closely linked to levels of cardiac glycogen, which are highest near-midgestation, and fall toward term and postnatally (40). Thus the rapid onset of hypotension near-term most likely reflects more rapid depletion of cardiac glycogen stores.

Occlusion was associated with suppression of cerebral EEG activity at all gestational ages. These data are consistent with previous studies that report that EEG suppression is a key, actively mediated response to hypoxia or asphyxia in the fetal sheep (8, 23) and other species (14). Critically, 0.6-gestation fetuses showed significantly greater residual activity after the first 3 min of occlusion. This is consistent with reduced dependency of the 0.6-gestation fetal sheep on oxidative metabolism, with significantly lower cerebral blood flow per 100 g weight (19, 29) and lower basal oxygen consumption compared with near term (19).

In conclusion, the present study demonstrates that the preterm fetal sheep adapts to episodes of severe asphyxia by...
mounting chemoreflex responses that are comparable to those observed in mature fetuses. Although the rate and magnitude of initial fall in heart rate were identical from near-midgestation to late gestation, the rapidity and intensity of peripheral vasoconstriction were moderately attenuated near-midgestation, strongly suggesting a reduced gain of α-adrenergic control. The younger fetuses also exhibited both greater ability to survive complete occlusion of the umbilical cord and reduced suppression of EEG activity, supporting the hypothesis that this change may reflect much greater overall anaerobic tolerance in the preterm fetus.

GRANTS

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