Effects of acute acidemia on the fetal cardiovascular defense to acute hypoxemia

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Thakor AS, Giussani DA. Effects of acute acidemia on the fetal cardiovascular defense to acute hypoxemia. Am J Physiol Regul Integr Comp Physiol 295: R90–R99, 2009. First published October 15, 2008; doi:10.1152/ajpregu.90689.2008.—In complicated pregnancy, fetal hypoxemia rarely occurs in isolation but is often accompanied by fetal acidemia. There is growing clinical concern about the combined effects of fetal hypoxemia and fetal acidemia on neonatal outcome. However, the effects on the fetal defense responses to acute hypoxemia during fetal acidemia are not well understood. This study tested the hypothesis that fetal acidemia affects the fetal defense responses to acute hypoxemia. The hypothesis was tested by investigating, in the late-gestation sheep fetus surgically prepared for long-term recording, the in vivo effects of acute fetal acidemia on 1) the fetal cardiovascular responses to acute hypoxemia and 2) the neural and endocrine mechanisms mediating these responses. Under general anesthesia, five sheep fetuses at 0.8 gestation were instrumented with catheters and Transonic flow probes around the femoral and umbilical arteries. After 5 days, animals were subjected to an acute hypoxemia protocol during intravenous infusion of saline or treatment with acidified saline. Treatment with acidified saline reduced fetal basal pH from 7.35 ± 0.01 to 7.29 ± 0.01 but did not alter basal cardiovascular variables, blood glucose, or plasma concentrations of catecholamines, ACTH, and cortisol. During hypoxemia, treatment with acidified saline increased the magnitude of the fetal bradycardia and femoral vasoconstriction and concomitantly increased chemoreflex function and enhanced the increments in plasma concentrations of catecholamines, ACTH, and cortisol. Acidemia also reversed the increase in umbilical vascular conductance during hypoxemia to vasoconstriction. In conclusion, the data support our hypothesis and show that acute acidemia markedly alters fetal hemodynamic, metabolic, and endocrine responses to acute hypoxemia.

ACTH: cortisol; catecholamines; umbilical vascular conductance; femoral vascular resistance

ONE OF THE MOST COMMON CHALLENGES to the fetus during late gestation is an episode of O2 deprivation. Acute hypoxemia, i.e., ~50% reduction of fetal arterial PO2 (PaO2), elicits integrated cardiovascular, metabolic, and endocrine responses that facilitate fetal survival during the period of reduced O2 availability (24, 43). In late gestation, the fetal cardiovascular defense to acute hypoxemia is triggered via a carotid chemoreflex, resulting in transient bradycardia and redistribution of the cardiac output toward essential circulations, such as those perfusing the fetal brain, heart, and adrenal glands, at the expense of peripheral vascular beds. Once initiated, the peripheral vasoconstriction is maintained by the release of vasoactive agents into the fetal circulation (6, 7, 23, 24). In addition, umbilical blood flow is maintained during acute hypoxemia or elevated when the severity of the hypoxemic challenge increases as a result of the combined effects of increased perfusion pressure and activity of nitric oxide across the umbilical vascular bed (48). The metabolic response to acute hypoxemia involves increased circulating concentrations of glucose and lactate (27, 29). The hyperglycemia during acute hypoxemia arises from the combined effects of reduced glucose consumption and increased glucose production by the fetus (27, 29); lactic acidemia results from the anaerobic metabolism of glucose in fetal peripheral tissues (9). Acute hypoxemia can also activate the pituitary-adrenal and sympathoadrenomedullary axes, peripheral effector pathways of the stress system (11). If the fall in basal PaO2 in the late-gestation fetus is greater than ~6 mmHg, activation of the pituitary-adrenal axis leads to increased release of ACTH from the anterior pituitary and glucocorticoids from the adrenal gland (2, 10). Similarly, hypoxemia leading to activation of the sympathoadrenomedullary axis promotes secretion of catecholamines in the fetal circulation (46). The increase in circulating catecholamine concentrations in the fetus contributes to the mechanisms mediating the peripheral vasoconstrictor (32) and glucogenic responses during hypoxemia (3).

Although it is accepted that the late-gestation fetus can adapt successfully to acute hypoxemia, episodes of reduced fetal oxygenation in human pregnancy rarely occur in isolation; i.e., they are often accompanied by fetal acidemia (36). Hence, there is a growing clinical concern about the effects of the combination of fetal hypoxemia and fetal acidemia compared with the effects of fetal hypoxemia alone on neonatal outcome (14, 17, 44, 47). However, the effects on the fetal defense responses to acute hypoxemia during fetal acidemia are not well understood. The present study tested the hypothesis that fetal acidemia affects the fetal defense responses to acute hypoxemia. The hypothesis was tested by investigating, in the late-gestation sheep fetus surgically prepared for long-term recording, the in vivo effects of acute fetal acidosis on 1) the fetal cardiovascular responses to acute hypoxemia and 2) the neural and endocrine mechanisms mediating these responses. The effects of fetal acidemia on the fetal hemodynamic responses to acute hypoxemia concentrated on essential and peripheral vascular beds via continuous measurement of umbilical and femoral blood flow, respectively, via perivascular Transonic flow probes.

MATERIALS AND METHODS

Surgical Preparation

All procedures were performed under the United Kingdom Animals (Scientific Procedures) Act 1986 and were approved by the

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Ethical Review Committee of the University of Cambridge. Five Welsh Mountain sheep fetuses were surgically instrumented for long-term recording at 125 ± 1 days of gestation (full term ~145 days) using strict aseptic conditions (22). Food, but not water, was withheld from the pregnant ewes for 24 h before surgery. After induction with thiopentone sodium (20 mg/kg iv; Intraval Sodium, Merial Animal Health, Rhone Mérieux, Dublin, Ireland), general anesthesia (1.5–2.0% halothane in 50:50 O₂-N₂O) was maintained using positive-pressure ventilation. Midline abdominal and uterine incisions were made, the fetal hindlimbs were exteriorized, and, on one side, femoral arterial (0.86 mm ID, 1.52 mm OD) and venous (0.56 mm ID, 0.96 mm OD) catheters (Critchly Electrical Products, NSW, Australia) were inserted. The catheter tips were advanced to the descending aorta and inferior vena cava, respectively. Another catheter was anchored onto the fetal hindlimb for recording of the reference amniotic pressure. Transonic flow transducers were also positioned around the contralateral femoral artery (2R or 3S) and around one of the umbilical arteries, inside the fetal abdomen, close to the common umbilical artery (4SB). The uterine incisions were closed in layers, the dead space of the catheters was filled with heparinized saline (80 IU heparin/ml in 0.9% NaCl), and the catheter ends were plugged with sterile brass pins. The catheters were then exteriorized via a keyhole incision in the maternal flank and kept inside a plastic pouch sewn onto the maternal skin.

**Postoperative Care**

During recovery, ewes were housed in individual pens in rooms with a 12:12-h light-dark cycle; they had free access to hay and water and were fed concentrates twice daily (100 g of sheep nuts no. 6, H & C Beart, Kings Lynn, UK). Antibiotics were administered daily: penicillin G procaine (0.20–0.25 mg/kg, Depocillin; Mycofarm, Cambridge, UK) intramuscularly to the ewe and ampicillin intravenously to the fetus and into the amniotic cavity (150 mg/kg, Penbritin; SmithKline Beecham Animal Health, Hertfordshire, UK). The ewes also received 2 days of postoperative analgesia with phenylbutazone (10–20 mg/kg po; Equipalazone paste, Arnolds Veterinary Products, Shropshire, UK). Generally, normal feeding patterns were restored within 48 h of recovery. After 72 h of postoperative recovery, ewes were transferred to metabolic crates, where they were housed for the remainder of the protocol. The arterial, venous, and amniotic catheters were connected to sterile pressure transducers (COBE, Argon Division, Maxim Medical, Athens, TX), and calibrated mean fetal arterial blood pressure (corrected for amniotic pressure) and fetal heart rate (triggered via a tachometer from the pulsatility in the arterial blood pressure. Transonic flow transducers were also positioned around the contralateral femoral artery (4SB). The uterine incisions were closed in layers, the dead space of the catheters was filled with heparinized saline (80 IU heparin/ml in 0.9% NaCl), and the catheter ends were plugged with sterile brass pins. The catheters were then exteriorized via a keyhole incision in the maternal flank and kept inside a plastic pouch sewn onto the maternal skin.

**Blood Sampling Regimen**

During the acute hypoxia protocol, ascending aortic blood samples (0.3 ml) were taken using sterile techniques from the fetus at set time intervals to determine arterial blood gas and metabolic status (Fig. 1). Arterial blood gas and acid-base measurements were determined using a blood gas analyzer (modelABL5, Radiometer, Copenhagen, Denmark; measurements corrected to 39.5°C), percent saturation of Hb with O₂ (Sat Hb) and blood Hb concentration ([Hb]) were determined using a hemoximeter (model OSM3, Radiometer), and blood glucose and lactate concentrations were measured by an automated analyzer (2300 Stat Plus Glucose/Lactate Analyser, Yellow Springs Instrument, Farnborough, UK). An additional 4 ml of arterial blood were withdrawn at set intervals for hormone analyses (Fig. 1). The samples were collected under sterile conditions into chilled acidified (pH 0.9) saline at a rate of 0.3 ± 0.1 ml·kg⁻¹·min⁻¹ led to a decrement from baseline in fetal pHₐₑ to ~0.07 and persistently maintained the pH at this level for up to 3 h. During these pilot experiments, there was no change in any of the hemodynamic variables measured in the fetus or in fetal blood gases during 3 h of acid infusion under baseline conditions.

**Experimental Protocol**

After ≥5 days of postoperative recovery, all fetuses were subjected to two experimental protocols that were carried out on consecutive days in a randomized order (Fig. 1). Each protocol consisted of a 3-h period consisting of 1.5 h of normoxia, 0.5 h of hypoxemia, and 1 h of recovery, during a slow intravenous infusion of heparinized saline vehicle (80 IU heparin/ml in 0.9% NaCl) or treatment with heparinized saline acidified with HCl (pH 0.9, 0.3 ± 0.1 ml·kg⁻¹·min⁻¹) with infusion rates titrated to achieve a reduction in fetal arterial pH (pHa) to ~7.29. The doses of HCl were calculated retrospectively from the fetal weights (2.9 ± 0.2 kg) obtained postmortem. All treatments started 1 h before the onset of hypoxemia and ran continuously through the hypoxic challenge and 15 min into recovery (Fig. 1). Acute hypoxemia in the fetus was induced by maternal inhalational hypoxia. Briefly, a large transparent respiratory hood was placed over the ewe’s head, and air was passed into the hood at a rate of ~50 l/min for the 1.5-h period of normoxia. After this control period, acute fetal hypoxemia was induced for 30 min by changing the concentrations of gases breathed by the ewe to 6% O₂ in N₂ with small amounts of CO₂ (15 l/min air, 35 l/min N₂, and 1.5–2.5 l/min CO₂). This mixture was designed to reduce fetal PaO₂ to ~10 mmHg and maintain arterial PCO₂ (Paco₂). After 0.5 h of hypoxemia, the ewe was returned to breathing air for the 1-h recovery period. At the end of the experimental protocol, the ewes and fetuses were euthanized with a lethal dose of pentobarbitone sodium (200 mg/kg iv; Pentoject, Animal Ltd, York, UK). Autopsy was carried out at 128 ± 1 days of gestation. Each fetuses was weighed, and the positions of the implanted catheters and the flow probes were confirmed.

In pilot experiments, acidified saline was infused at different concentrations, infusion rates, and durations to obtain the most suitable infusion regimen to achieve a reduction in blood gas concentration and to the fetus. In the acute hypoxemia protocol, the pHa of the fetus was determined using a blood gas analyzer (model ABL5, Radiometer, Copenhagen, Denmark; measurements corrected to 39.5°C), percent saturation of Hb with O₂ (Sat Hb) and blood Hb concentration ([Hb]) were determined using a hemoximeter (model OSM3, Radiometer), and blood glucose and lactate concentrations were measured by an automated analyzer (2300 Stat Plus Glucose/Lactate Analyser, Yellow Springs Instrument, Farnborough, UK). An additional 4 ml of arterial blood were withdrawn at set intervals for hormone analyses (Fig. 1). The samples were collected under sterile conditions into chilled acidified (pH 0.9) saline at a rate of 0.3 ± 0.1 ml·kg⁻¹·min⁻¹ led to a decrement from baseline in fetal pHₐₑ to ~0.07 and persistently maintained the pH at this level for up to 3 h. During these pilot experiments, there was no change in any of the hemodynamic variables measured in the fetus or in fetal blood gases during 3 h of acid infusion under baseline conditions.

**Fig. 1.** Diagrammatic representation of the acute hypoxemia protocol. Experimental protocol consisted of a 3-h period; 1.5 h of normoxia (N0–N75), 0.5 h of hypoxemia (short horizontal bar; H5, H15, and H30), and 1 h of recovery (R30 and R60) during saline infusion (n = 5) or treatment with acidified (pH 0.9) saline (long horizontal bar; 0.3 ± 0.1 ml·kg⁻¹·min⁻¹, n = 5). Arrows represent times at which arterial blood samples were collected; dashed arrows indicate 2 extra samples for blood gases.
heparin tubes (2 ml Li+/heparin tubes; LIP, Shipley, West Yorkshire, UK) containing reduced glutathione (4 nmol per tube; catalog no. G-4251, Sigma Chemical) and EGTA (5 nmol per tube; catalog no. E-4378, Sigma Chemical) for catecholamine (epinephrine and norepi-
ephine) analysis or into chilled EDTA tubes (2 ml K+/EDTA; LIP) for ACTH and cortisol analysis. All samples were centrifuged at 4,000 rpm for 4 min at 4°C. The plasma was dispensed into prelabeled tubes, and the samples were stored at −80°C until analysis. All hormone measurements were performed within 2 mo of sample collection.

Hormone Analyses

Catecholamine assay. Fetal plasma epinephrine and norepinephrine concentrations were measured by HPLC using electrochemical detection, as previously described in detail (18). The samples were pre-
pared by absorption of 250 μl of plasma onto acid-washed alumina, and 20-μl aliquots of the 100-μl perchloric acid eluates were injected onto the column. Dihydroyxbenzylamine was added as the internal standard to each plasma sample before absorption. The limit of sensitivity for the assay was 20 pg/ml for epinephrine and norepi-
ephine. Recovery ranged from 63% to 97%, and all catecholamine values were corrected for their respective recovery. The interassay coefficients of variation (CV) for epinephrine and norepinephrine were 7.3% and 6.2%.

ACTH assay. Fetal plasma ACTH concentrations were measured using a commercially available double-antibody 125I-RIA kit (Dia-
sorin, Stillwater, MN), as previously described (42). The kit con-
tained a set of ACTH standards (porcine; range 20–500 pg/ml), 125I-labeled synthetic ACTH, and a second antibody-precipi-
tating complex (goat anti-rabbit serum,125I-labeled antibody, and 4.6% for deoxycortisol. Antiserum at 50% binding with other cortisol-related compounds were 0.5% for cortisone, 2.3% for corticosterone, 0.3% for progesterone, and 4.6% for deoxy cortisol.

Data and Statistical Analyses

Values are means ± SE. Fetal arterial blood pressure, heart rate, femoral blood flow, femoral vascular resistance (FVR), umbilical blood flow, and umbilical vascular conductance were recorded continuously at 1-s intervals using the computerized data acquisition system. The concentration of radioactive protons ([H+]) was calculated using the following equation: [H+] = 10−pH. The fetal arterial blood O2 content (mmol/l) was calculated as follows: ([Hb] × Sat Hb)/100 × 0.62, where [Hb] (g/dl) is the blood concentration of Hb, Sat Hb is the percent O2 saturation of Hb, and 1 mol of Hb (64,450 mol wt) binds 4 mol of O2. The contribution of O2 dissolved in plasma is regarded as negligible (21).

For the acute hypoxemia experiment, values for all arterial blood gas and metabolic variables are expressed at 0 (N0) and 75 (N75) min of normoxia, 5 (H5), 15 (H15), and 30 (H30) min of hypoxemia, and 30 (R30) and 60 (R60) min of recovery. Values for all endocrine variables are expressed at 0 (N0) and 75 (N75) min of normoxia, 5 (H15) and 30 (H30) min of hypoxemia, and 30 (R60) min of recovery. Summary measure analysis was applied to the serial cardiovascular data to focus the number of comparisons (34). The area under the curve was calculated at 30-min intervals (N1, N2, N3, H, R1, R2) for the absolute data and for the change from mean normoxic baseline data describing the hemodynamic responses. All measured variables were assessed statistically using a two-way ANOVA with repeated measures comparing the effect of time and treatment and interactions between time and treatment. Functional chemoreflex analysis was performed to assess the effects of acidemia on fetal cardiac and vasomotor chemoreflex responses. For this analysis, linear regression lines were plotted for the cardiovascular chemoreflex response of each fetus, and a comparison between the slopes was conducted according to Armitage and Berry (4). The relationship between parallel measure-
ments of plasma concentrations of ACTH and cortisol in all individual fetuses was assessed using the Pearson product moment correlation coefficient test. The mean change in adrenocortical respon-

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Hormone Analyses

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The lower limit of detection of the assay was 1.0–1.5 ng/ml. The intra-assay CV was 5.3% for a mean value of 13 ng/ml. The interassay CV for two plasma pools (mean concentration = 13 and 28 ng/ml) were 13.6 and 11.4%, respectively. The cross-reactivities of the antiserum at 50% binding with other cortisol-related compounds were 0.5% for cortisone, 2.3% for corticosterone, 0.3% for progesterone, and 4.6% for deoxy cortisol.

Data and Statistical Analyses

Values are means ± SE. Fetal arterial blood pressure, heart rate, umbilical blood flow, and umbilical vascular conductance were recorded continuously at 1-s intervals using the computerized data acquisition system. The concentration of radioactive protons ([H+]) was calculated using the following equation: [H+] = 10−pH. The fetal arterial blood O2 content (mmol/l) was calculated as follows: ([Hb] × Sat Hb)/100 × 0.62, where [Hb] (g/dl) is the blood concentration of Hb, Sat Hb is the percent O2 saturation of Hb, and 1 mol of Hb (64,450 mol wt) binds 4 mol of O2. The contribution of O2 dissolved in plasma is regarded as negligible (21).

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siveness to ACTH (gradient and intercept of the cortisol-ACTH relationship) was assessed statistically using Student’s t-test for paired data.

Where a significant effect of time or treatment was indicated using an ANOVA with repeated measures, Tukey’s post hoc test was used to isolate the statistical differences. For all comparisons, statistical significance was accepted when \( P < 0.05 \).

RESULTS

Fetal Arterial Blood Gas and Metabolic Status

Basal values for fetal arterial blood gas status and fetal concentrations of blood glucose and lactate were similar in all fetuses and were within the normal range for the Welsh Mountain sheep fetus at \( \sim 125 \) days gestation (Table 1, Fig. 2). Infusion with saline had no effect on basal arterial blood gas or metabolic status, but it produced a significant decrease in fetal \( \text{pH}_a \) with a corresponding increase in \( [\text{H}^+] \left[ -0.06 \pm 0.01 \right. \) and \( 0.74 \pm 0.08 \times 10^{-8} \), respectively, \( P < 0.05 \); Table 1). In all fetuses, acute hypoxemia induced significant falls in \( \text{pH}_a \), \( \text{PaO}_2 \), Sat Hb, and \( \text{O}_2 \) content and a significant increment in fetal \( \text{Hb} \) concentration (Table 1). The magnitude of these responses was similar in all fetuses. The increment in fetal \( [\text{H}^+] \) by the end of hypoxemia relative to the corresponding basal value at N75 in fetuses infused with heparinized saline was not significantly different from that in fetuses treated with acidified saline \( (0.92 \pm 0.09 \times 10^{-8} \) and \( 1.21 \pm 0.11 \times 10^{-8} \), respectively; Table 1). Acute hypoxemia during saline infusion induced significant increments in blood glucose \( (0.76 \pm 0.25 \text{ mmol/l}) \) and lactate \( (4.74 \pm 0.40 \text{ mmol/l}) \) concentrations (Fig. 2). Treatment with acidified saline significantly enhanced the glycemic response \( (2.08 \pm 0.38 \text{ mmol/l}) \) but had no significant effect on the lactic acidemic response \( (5.95 \pm 0.95 \text{ mmol/l}) \) to acute hypoxemia compared with saline-infused controls (Fig. 2). During recovery, \( \text{pH}_a \) remained significantly depressed, and blood glucose and lactate concentrations were significantly elevated above basal values in all fetuses, whereas \( \text{PaO}_2 \), Sat Hb, and [Hb] returned toward basal values (Table 1, Fig. 2).

Table 1. Fetal arterial blood gas status

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Acute Hypoxemia</th>
<th>Recovery</th>
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<tr>
<td></td>
<td>N0</td>
<td>N75</td>
<td>H5</td>
</tr>
<tr>
<td>pHa</td>
<td></td>
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<tr>
<td>Saline</td>
<td>7.36±0.01</td>
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<td>Acidified saline</td>
<td>7.36±0.01</td>
<td>7.29±0.01*†</td>
<td>7.26±0.01</td>
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<td>[H+] ×10^-8</td>
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<tr>
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<td>4.37±0.05</td>
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<td>5.13±0.05*†</td>
<td>5.55±0.09†</td>
</tr>
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<td>Arteral PaCO2, mmHg</td>
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<tr>
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<td>55.0±0.8</td>
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<tr>
<td>Arteral PaO2, mmHg</td>
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<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>23.0±0.4</td>
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<td>20.8±0.3</td>
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<td>Sat Hb, %</td>
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<td>[Hb], g/dl</td>
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<tr>
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<td>O2 content, mmol/l</td>
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<tr>
<td>Acidified saline</td>
<td>3.10±0.16</td>
<td>2.93±0.19</td>
<td>1.57±0.12</td>
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</table>

Values are means ± SE (\( n = 5 \)) at 0 (N0) and 75 (N75) min of normoxia at 5 (H5), 15 (H15), and 30 (H30) min of hypoxia, and 30 (R30) and 60 (R60) min of recovery for fetuses exposed to 0.5 h of hypoxia during saline infusion (\( n = 5 \)) or treatment with acidified (\( \text{pH} = 0.9 \)) saline \( (0.3 ± 0.1 \text{ mg·kg}^{-1}·\text{h}^{-1}) \). \( \text{pH}_a \), arterial \( \text{pH} \); [\( \text{H}^+ \)], concentration of protons; Sat Hb, saturation of Hb with O2; [Hb], blood Hb concentration; \( \text{O}_2 \) content, arterial blood \( \text{O}_2 \) content. *\( P < 0.05 \) vs. N0; †\( P < 0.05 \) vs. saline infusion (2-way repeated measures ANOVA with Tukey’s post hoc test).

Fetal Cardiovascular Responses to Acute Hypoxemia

Basal values for fetal arterial blood pressure, heart rate, femoral blood flow, and FVR were similar in all fetuses (Fig. 3). Infusion with saline or treatment with acidified saline had no effect on basal cardiovascular function (Fig. 3). Acute hypoxemia during saline infusion induced significant increments in fetal arterial blood pressure \( (8.8 ± 0.8 \text{ mmHg}) \) and FVR \( (4.32 ± 0.91 \text{ mmHg·ml}^{-1}·\text{min}) \) and significant falls in fetal heart rate \( (−20 ± 3 \text{ beats/min}) \) and femoral blood flow \( (−26 ± 3 \text{ ml/min}) \); Fig. 3). Treatment with acidified saline had no effect on the pressor increment during acute hypoxemia \( (9.3 ± 2.1 \text{ mmHg}) \); however, it significantly enhanced the magnitude of the changes from baseline in the femoral vasoconstrictor \( (10.68 ± 1.60 \text{ mmHg·ml}^{-1}·\text{min}) \) and bradycardic \( (−57 ± 10 \text{ beats/min}) \) responses to acute hypoxemia (Fig. 3). Interestingly, there was a rebound hypertensive response in all fetuses treated with acidified saline during the first 15 min of recovery following the hypoxic challenge \( (P < 0.05) \); Fig. 3). During recovery, all cardiovascular variables returned toward basal values in saline-infused controls. In contrast, in fetuses treated with acidified saline, all cardiovascular variables remained significantly elevated above or reduced below both basal and control values by the end of the experimental protocol (Fig. 3).

Basal values for umbilical blood flow and umbilical vascular conductance were similar in all fetuses: in saline-treated fetuses, umbilical blood flow was 196 ± 29 ml/min and umbilical vascular conductance was 4.02 ± 0.46 ml·min⁻¹·mmHg⁻¹; in
Acidified saline-treated fetuses, umbilical blood flow was 222 ± 30 ml/min and umbilical vascular conductance was 4.40 ± 0.40 ml·min⁻¹·mmHg⁻¹. Acute hypoxemia during saline infusion induced significant increments in fetal arterial blood pressure, umbilical blood flow (43 ± 9 ml/min), and umbilical vascular conductance (0.15 ± 0.22 ml·min⁻¹·mmHg⁻¹; Fig. 4). In marked contrast, treatment with acidified saline not only abolished the umbilical vasodilator response to acute hypoxemia, but it resulted in a significant decrement from baseline in umbilical vascular conductance during the challenge (−0.97 ± 0.28 ml·min⁻¹·mmHg⁻¹; Fig. 4). During recovery, umbilical blood flow and vascular conductance remained significantly elevated above basal values in saline-infused fetuses but returned toward basal values by the end of the experimental protocol in fetuses treated with acidified saline (Fig. 4).

**Functional Chemoreflex**

Acute hypoxemia reduced fetal PaO₂ to a similar level in all fetuses during infusion with saline or acidified saline (Table 1). Within the first 15 min of the onset of acute hypoxemia, the fall in PaO₂ was associated with a significant reduction in fetal heart rate and an insignificant increase in FVR in both groups. However, the nadir for fetal heart rate and the maximum increase in FVR during the first 15 min of hypoxemia were greater when fetuses were treated with acidified saline (−75 ± 7 beats/min and 12.48 ± 1.39 mmHg·ml⁻¹·min) than when they were infused with normal saline (−35 ± 7 beats/min and 3.97 ± 0.99 mmHg·ml⁻¹·min). Consequently, when individual values for heart rate and FVR were plotted against corresponding values for PaO₂ during normoxia and the first 15 min of acute hypoxemia (data not shown), the slopes for all individual data points representing the cardiac (−7.0× vs. −2.8x) and femoral vasoconstricor (1.2x vs. 0.3x) chemoreflex function were greater in fetuses during treatment with acidified saline than during infusion of normal saline (P < 0.05).

**Fetal Endocrine Responses to Acute Hypoxemia**

Basal values for fetal plasma concentrations of epinephrine, norepinephrine, ACTH, and cortisol were similar in all fetuses (Figs. 5 and 6). Infusion with saline or treatment with acidified saline had no effect on basal catecholamine concentrations or basal pituitary-adrenocortical function (Figs. 5 and 6). Acute hypoxemia during saline infusion induced significant increments from baseline in plasma concentrations of epinephrine (414 ± 89 pg/ml), norepinephrine (2,665 ± 353 pg/ml), ACTH (305 ± 70 pg/ml), and cortisol (11 ± 6 ng/ml; Figs. 5 and 6). Treatment with acidified saline resulted in a marked enhancement in the plasma epinephrine (10,412 ± 5,541 pg/ml), norepinephrine (9,474 ± 3,689 pg/ml), ACTH (572 ± 154 pg/ml), and cortisol (37 ± 2 ng/ml) responses to acute hypoxemia (Figs. 5 and 6). During recovery, plasma concentrations of all hormones returned toward basal values in all fetuses (Figs. 5 and 6).

Correlation analysis of paired plasma ACTH and cortisol values for all individual fetuses under basal and hypoxic conditions during saline infusion (r = 0.62, n = 20, P < 0.01) or treatment with acidified saline (r = 0.79, n = 20, P < 0.01) revealed significant linear relationships (Fig. 7). Treatment with acidified saline did not affect the intercept or the slope of the steroid-peptide relationship compared with saline infusion (Fig. 7; P > 0.05), thereby suggesting no change in the set point or the sensitivity of the adrenal cortex to ACTH stimulation.

**DISCUSSION**

This study tested the hypothesis that fetal acidemia affects the fetal defense responses to acute hypoxemia by investigating the in vivo effects of acute fetal acidemia on the fetal cardiovascular, metabolic, and endocrine responses to acute hypoxemia in the late-gestation ovine fetus. The data support the hypothesis tested and show that acute acidemia had no effect on basal cardiovascular, metabolic, or endocrine function but markedly enhanced the bradycardia, femoral vasoconstriction, and metabolic and endocrine responses to acute hypoxemia in the fetus. In marked contrast, acute acidemia not only prevented the dilator response of the umbilical vascular bed to acute hypoxemia, but it also reversed the response to constriction. Greater carotid chemoreflex function and an enhanced increase in plasma catecholamines may contribute to the physiological mechanisms underlying the altered cardiovascular and metabolic responses to acute hypoxemia in acutely acidic fetuses.

The fetal bradycardia and peripheral constrictor response to acute hypoxemia are established carotid chemoreflexes, since bilateral section of the carotid sinus nerves abolishes the reduction in heart rate and the initial increase in FVR during acute hypoxemia (5, 25). As the hypoxic episode continues,
catecholamines are released into the fetal circulation and act to maintain the peripheral vasoconstriction (30, 32). The origin of the increase in epinephrine and norepinephrine in fetal plasma during acute hypoxemia has been shown to be primarily from the fetal adrenal gland, inasmuch as adrenal demedullation completely abolished the rise in plasma epinephrine concentration and reduced the norepinephrine response to 10% of normal (31). Since a large component of the increase in total

![Fig. 3. Fetal cardiovascular responses to acute hypoxemia.](image)

![Fig. 4. Fetal umbilical hemodynamic responses to acute hypoxemia.](image)
catecholamine output from the fetal adrenal gland during acute hypoxemia is mediated via activation of the splanchnic nerves (31), elevations in plasma concentrations of epinephrine and norepinephrine in the fetal sheep circulation are good indexes of increased sympathetic outflow during stimulated conditions.

Data in the present study show that acute acidemia enhanced the bradycardic and femoral vasoconstrictor response to hypoxemia, possibly by sensitizing the carotid chemoreflex. During hypoxemia, the glomus cells of the carotid body become uncoupled, leading to a decrease in their junctional conductance, which results in the release of transmitters toward the carotid sinus nerve sensory terminals (15). The junctional channel proteins between glomus cells are directly regulated by pH, with acidemia shown to increase their uncoupling (1). Hence, acute acidemia could sensititize the fetal carotid body, thereby promoting a greater increase in autonomic outflow for any given fall in PaO2. The enhanced bradycardic response to acute hypoxemia supports this notion, suggesting a greater vagal outflow in acidemic fetuses. Evidence of an enhanced increase in sympathetic outflow during acute hypoxemia in acidemic fetuses is derived from the marked augmentation of the plasma epinephrine and norepinephrine responses to acute hypoxemia. Similarly, Lewis and Sadeghi (33) demonstrated that acidemia significantly potentiated the magnitude of the plasma catecholamine response to acute hypoxemia in the ovine fetus in response to splanchnic nerve stimulation. In vitro studies using the rat adrenal medulla showed that acidemia can directly stimulate catecholamine secretion, in isolation (19) or as an additive effect with hypoxemia (41). The enhanced plasma catecholamine response to acute hypoxemia in acidemic fetuses may also act to maintain the greater overall peripheral vasoconstrictor response.

In marked contrast to the effects on the peripheral circulation, further data reported in the present study show that acute acidemia reversed the umbilical hemodynamic response to hypoxemia from dilatation to constriction. In a previous investigation of the effects of acute acidemia on basal cardiovascular and endocrine function in the ovine fetus, Wood and Chen (49) reported that infusion of acid into the fetal circulation increased fetal PaCO2. In the adult animal with spontaneous ventilation, arterial acidemia increases ventilation via reflex mechanisms, correcting any increase in PaCO2. However, in the fetus, gas exchange is controlled by alterations in umbilical-placental blood flow. Wood and Chen, therefore, concluded that the increase in fetal PaCO2 following acute acidemia in their study may represent failure of the umbilical-placental vascular bed to increase flow to buffer this effect, but they did not measure umbilical blood flow. The results of our study support this prediction and confirm that umbilical vascular conductance not only fails to increase, but it actually decreases, during acute acidemia. The reduction of fetal pH reported in the present study (an average of −0.05) was significantly less than that reported previously (−0.4) (49). The comparatively milder acidemia may not only explain the lack of an increase in fetal PaCO2 in the present study during basal conditions, but it also highlights how sensitive the umbilical vascular bed may be to
increases in fetal catecholamine levels, outweighing any vasodilator effects during stressful conditions. Several studies have shown that the umbilical vasculature does constrict in response to adrenergic receptor stimulation (8, 53). Taken together, past and present evidence, therefore, suggests that fetuses compromised by acidemia may be at greater risk during acute hypoxic episodes, whereby augmented stimulated plasma catecholamine responses in the fetus may compromise umbilical-placental perfusion, triggering a vicious cycle.

Interestingly, although acute acidemia had no effect on the magnitude of the arterial blood pressure response to acute hypoxemia, there was a “rebound” hypertension at the beginning of the recovery period. During hypoxemia, the effects of increasing plasma catecholamine levels on the fetal heart are opposed by increased vagal discharge. The sudden increase in fetal arterial blood pressure during recovery in acidemic fetuses may result from the augmented catecholamine drive, in the absence of any vagal outflow, on fetal heart rate and cardiac output. Greater circulating levels of catecholamines will also require a longer duration for their metabolic disposition, thereby prolonging the duration of their effects, as demonstrated by the maintained elevation of fetal arterial blood pressure and femoral vasoconstriction throughout recovery in the acidemic fetus.

It is well established that activation of the sympathetic nervous system and an increase in circulating concentration of catecholamines are responsible for the fetal glycemic response to acute hypoxemia (3, 28). Data from the present study support this theory and show that although acute fetal acidemia had no effect on basal metabolic function and basal plasma catecholamine concentrations, it enhanced the glycemic and catecholaminergic responses to acute hypoxemia in the late-gestation fetus. However, since acidemia has been shown to also enhance glucose-stimulated insulin release (39), the overall effect of acidemia on fetal circulating glucose concentrations is likely to be partially offset by increased insulin release. In contrast to the glycemic response, acute fetal acidemia did not significantly affect the lactic acidemic response to acute hypoxemia, despite the elevated fetal peripheral vasoconstrictor response. This may be attributed to acidemia-enhanced uptake of lactate by various organs, as has been shown for the liver (45). In addition, the placenta can act as a major site of lactate clearance from the fetal circulation, especially during periods of adverse intrauterine conditions (26). This effect may be enhanced by acidemia, inasmuch as the reduction in umbilical arterial perfusion would slow the transit time of circulating lactate through the umbilicoplacental vascular bed, thereby favoring placental lactate uptake.

Previous studies have shown that acute acidemia in the late-gestation fetus stimulates the hypothalamus-pituitary-adrenal (HPA) axis under basal conditions, resulting in an increase in ACTH release from the pituitary and an increase in cortisol release from the adrenal cortex (49, 51). In contrast, results from the present study show that acute fetal acidemia had no effect on basal concentrations of plasma ACTH and cortisol. This difference may again be due to failure to attain the threshold of acidemia required to stimulate ACTH and cortisol secretion, since the degree of acidemia induced in fetuses in the present study was significantly less than that reported previously (49, 51). During acute hypoxemia, fetal acidemia markedly increased the magnitude of the plasma ACTH and cortisol responses, but it had no effect on the intercept or the slope of the steroid-peptide relationship, thereby demonstrating that there was no change in the set point or sensitivity of the adrenal cortical response to ACTH. This is in agreement with previous studies that reported no effect of acidemia on ACTH-stimulated cortisol secretion (38). The sensitizing effects of acute acidemia on the fetal HPA axis may therefore be at the level of the hypothalamus and/or the pituitary. At the level of the hypothalamus, acidemia may increase the release of corticotropin-releasing hormone (CRH) and/or AVP, since both hormones have been shown to stimulate the release of ACTH from the fetal anterior pituitary (16, 52). Although there is no available evidence for an effect of acidemia on the release of hypothalamic CRH, Wood and Chen (49) reported that acidemia directly stimulates the release of AVP in fetal sheep, whereas Gardner and colleagues (20) showed a markedly enhanced AVP response to acute hypoxemia in spontaneously acidemic fetuses. Furthermore, since catecholaminergic neurons in the hypothalamus have been shown to be functional in the sheep fetus during late gestation under conditions of acute hypoxic stress (35), it is possible that acidemia could increase the release of CRH from hypothalamic neurons by enhancing sympathetic activity (33). At the level of the pituitary, acidemia can directly stimulate the secretion of ACTH in the sheep fetus (51), but whether this is
a direct effect on pituitary corticotroph cells or occurs higher up the axis remains unknown, and the mechanism mediating this effect in the adult appears different from that in the fetus. For instance, in the adult, but not the fetus, acidemia stimulates the release of ACTH via thromboxane (12, 13, 50).

Perspectives and Significance

The data in the present study show that acute acidemia markedly affects the fetal hemodynamic, metabolic, and endocrine responses to an episode of acute hypoxemia in the late-gestation ovine fetus. Components of these findings are of clinical relevance. For instance, spinal anesthesia is widely used in clinical practice, inasmuch as it is commonly considered to be more practical and safer than other techniques for the mother and unborn child (37). A recent meta-analysis concluded that spinal anesthesia induces fetal metabolic acidosis, an effect that is exacerbated by ephedrine when used to manage maternal blood pressure (40). Although acidemia enhances the fetal cardiovascular, metabolic, and endocrine defense to acute hypoxic stress, the data in the present study show that acidemia and increased concentrations of catecholamines are associated with important constrictor effects on the umbilico-placental circulations, particularly during fetal distress. These findings should be considered when planning the management of maternal anesthesia and when assessing cardiovascular indices of fetal health, for instance, via Doppler blood flow velocimetry or fetal heart rate monitoring, in pregnancies with suspected fetal hypoxia and acidemia.

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GRANTS

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