Maternal nutrient restriction during pregnancy impairs an endothelium-derived hyperpolarizing factor-like pathway in sheep fetal coronary arteries

Praveen Shukla, Srivinvas Ghatta, Nidhi Dubey, Caleb O. Lemley, Mary Lynn Johnson, Amit Modgil, Kimberly Vonnahme, Joel S. Caton, Lawrence P. Reynolds, Chengwen Sun, and Stephen T. O’Rourke

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Maternal nutrient restriction during pregnancy impairs an endothelium-derived hyperpolarizing factor-like pathway in sheep fetal coronary arteries. Am J Physiol Heart Circ Physiol 307: H134–H142, 2014. First published May 9, 2014; doi:10.1152/ajpheart.00595.2013.—The mechanisms underlying developmentally programmed vascular function and reactivity are poorly understood. The effects of maternal undernutrition on fetal coronary vascular function were investigated in the midgestation sheep model of maternal undernutrition that is well established. In coronary artery rings isolated from control and nutrient-restricted (60% restriction) ewes, bradykinin-induced relaxation was impaired, and S-nitro-L-arginine (NLA) abolished the NLA-resistant response to bradykinin. Iberiotoxin or contraction with KCl abolished the NLA-resistant response to bradykinin. The BKCa openers, BMS 191011, and NS1619, and 14,15-epoxyeicosatrienoic acid (EET) activated BKCa-channel subunits but did not differ in fetal coronary arteries from control or undernourished ewes. The BKCa openers, BMS 191011 and NS1619, and 14,15-epoxyeicosatrienoic acid [a putative endothelium-derived hyperpolarizing factor (EDHF)] each caused fetal coronary artery relaxation and BKCa current activation that was unaffected by maternal nutrient restriction. Expression of BKCa-channel subunits did not differ in fetal coronary arteries from control or undernourished ewes. The results indicate that maternal undernutrition during pregnancy results in loss of the EDHF-like pathway in fetal coronary arteries in response to bradykinin, an effect that cannot be explained by a decreased number or activity of BKCa channels or by decreased sensitivity to mediators that activate BKCa channels in vascular smooth muscle cells. Under these conditions, bradykinin-induced relaxation is completely dependent on nitric oxide, which may represent an adaptive response to compensate for the absence of the EDHF-like pathway.

maternal nutrient restriction; fetal coronary artery; nitric oxide; endothelium-derived hyperpolarizing factor; BKCa channels

HUMAN EPIDEMIOLOGICAL AND experimental animal studies provide convincing support for the concept of developmental programming, which suggests that developmental insults or stressors [i.e., maternal undernutrition and exposure to stress-related hormones (e.g., cortisol)] during pregnancy result in reprogramming or adaptation of the fetal physiology in a manner that increases the risk of developing disease in subse-
quent years of life (42). Developmentally compromised infants have an increased risk of health complications, not just as infants but throughout their lifespan, including a range of metabolic, neurological, behavioral, reproductive, and cardiovascular disorders (1, 4, 30, 41).

Historically, the concept of developmental programming was based on epidemiological studies that focused exclusively on individuals with low birth weight. Subsequent studies in humans and animals now increasingly suggest that developmental programming can occur independently of birth weight (4, 41), such that individual organ systems or physiologic processes are adversely affected by one or more developmental insults or stressors, resulting in offspring phenotypes similar to those associated with low birth weight. These phenotypes include poor growth, increased adiposity, poor glucose tolerance, and dislipidemia (4, 16, 18, 50).

Endothelial cells lining the arterial lumen produce numerous vasoactive mediators that regulate the tone of the underlying vascular smooth muscle (13), thereby adjusting blood supply to the target organ to meet changes in metabolic needs. These mediators include both vasodilators [e.g., nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF)] and vasoconstrictors (e.g., endothelin). Several studies using a variety of animal species and experimental paradigms demonstrate that maternal undernutrition during pregnancy leads to endothelial dysfunction in the offspring and that these impairments contribute to the development of cardiovascular disease (3, 29, 41). Mechanisms underlying this endothelial dysfunction and the programming of cardiovascular disease are poorly understood, but emerging evidence suggests that a poor nutrient supply at critical periods of early development leads to permanent alterations in vascular structure or function. Impairment in both vasoconstriction and vasorelaxation responses has been reported. For example, in humans, low birth weight is associated with impaired endothelium-dependent relaxation in infants (32), children (33), and young adults (27). Similarly, maternal undernutrition leads to impaired endothelium-dependent and -independent relaxation of sheep femoral arteries (38) and rat mesenteric arteries (6, 48) and aortae (40) in the offspring. Moreover, in rats, maternal undernutrition causes alterations in vasoconstrictor responses in femoral and carotid arteries of offspring (39, 53).

The effects of maternal undernutrition during pregnancy on fetal coronary arteries are poorly understood at present. The aim of the present study was to determine the effect of maternal nutrient restriction on fetal coronary arterial function using a well-established mid- to late-gestational sheep model of maternal undernutrition. Since the endogenous endothelium-de-
ependent vasodilator, bradykinin, is capable of activating several endothelial vasorelaxant signaling pathways (i.e., NO, prostacyclin, and EDHF) in arteries (14, 37, 47), bradykinin was used as a pharmacological tool to assess the effects of maternal undernutrition on endothelial function. We specifically tested the hypothesis that maternal undernutrition during the last two-thirds of pregnancy impairs endothelium-dependent vasorelaxation and large-conductance, calcium-activated potassium channel (BKCa) function in fetal coronary artery smooth muscle cells in late pregnancy.

MATERIALS AND METHODS

Materials

The following drugs or chemicals were used: acetylcholine, bradykinin, DL-DTT, glyburide, indomethacin, nitro-l-arginine (NLA), NS1619, serotonin, sodium nitroprusside, and soybean trypsin inhibitor (Sigma Chemical, St Louis, MO); BMS 191011 and ibetorixin (Tocris, Ellisville, MO); collagenase, elastase, and papain (Worthington Biochemical, Lakewood, NJ); and 14,15-epoxyeicosatetraenoic acid (EET) and 9,11-dideoxy-11α,9e-epoxyhexamethylo-PGF2α (U46619; Cayman Chemical, Ann Arbor, MI). All other chemicals were purchased from Sigma Chemical, unless stated otherwise. Drug solutions were prepared daily, kept on ice, and protected from light until used. All drugs were dissolved initially in double-distilled water, with the exception of aprikalim (70% ethanol), indomethacin (0.1 mM; sodium carbonate solution), glyburide (0.1 N; NaOH), BMS 191011, and NS1619 (DMSO), before further dilution in distilled water. Drugs were added to the organ chambers in volumes not greater than 0.2 ml. Drug concentrations are reported as the final molar concentration in the organ chamber. The composition of the physiological salt solution was as follows (in mM): NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, and glucose 11.1.

Methods

Animals and experimental design. This study was approved by the North Dakota State University (NDSU) Animal Care and Use Committee and was designed as described previously (11, 28). Briefly, nulliparous Western whiteface ewes, carrying singleton pregnancies, were transported to the NDSU Animal Nutrition and Physiology Committee and was designed as described previously (11, 28). Briefly, maternal undernutrition during early gestation. At

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day 28 of gestation, ewes were moved from the pasture into a temperature-controlled (14°C) facility with a 12:12 light:dark cycle with lights on at 0700 and off at 1900 for the remainder of the study. On day 45 of gestation, ewes were acclimated to a pelleted diet in a stepwise manner and provided trace mineral salt blocks and ad libitum access to water. Dietary treatments began on day 50 of gestation and consisted of a control [100% of NRC (2007) recommendations] or nutrient-restricted [60% of NRC (2007) recommendations] diet until day 130 of gestation (term = 145 days). Nutritional requirements were based on NRC (37a) recommendations for a 60-kg body wt pregnant ewe lamb during mid to late gestation. Feed offered to individual ewes was adjusted based on weekly body weight and body-weight change to achieve the desired average daily gain (11, 28).

Tissue collection. Ewes were killed on day 130 of gestation. The uterus was removed, and the gravid uterine horn was dissected. Fetuses of either sex (n = 7 control and 9 nutrient-restricted males; n = 7 control and 6 nutrient-restricted females) were removed, weighed, and exsanguinated. Fetal hearts were removed at dissection, weighed, and further processed. The fetal heart left- and right-ventricle thickness at top, mid, and base positions and overall average thickness of ventricles were recorded using digital calipers. Subsequently, the left-circumflex coronary artery and the anterior interven-

tricular coronary artery were isolated and placed into cold physiological salt solution.

Organ chamber studies. Left-circumflex coronary arteries were cleaned of adherent fat and connective tissue and cut into rings (3–4 mm in length), with care being taken to avoid damaging the vascular endothelium. Four to eight arterial rings were prepared from each heart. In some rings, the endothelium was removed by gently rubbing the intimal surface with fine forceps. Coronary arterial rings were suspended in water-jacketed organ chambers filled with 25 ml physiological salt solution, as described previously (46, 49, 51). The organ chamber solution was aerated with a mixture of 95% O2/5% CO2, and the temperature was maintained at 37°C throughout the experiment. Each ring was suspended by means of two fine stainless-steel wire clips passed through the lumen; one clip was anchored inside of the organ chamber and the other connected to a force transducer (Model FT03; Grass Instrument, Warwick, RI). Isometric tension was measured and recorded on a Grass polygraph. The tissues were stretched progressively to the optimal point of their length-tension relationship, using KCl (20 mM) to generate a standard contractile response. Optimal resting tension was held at ~1.5 g in rings from both control and nutrient-restricted animals (P > 0.05). After this procedure, the preparations were equilibrated at their optimal length for at least 30 min before further exposure to any vasoactive substances. Removal of the endothelium was confirmed in endothelium-denuded arterial rings by the absence of relaxation to the endothelium-dependent vasodilator, bradykinin (10−7 M).

Relaxation of fetal coronary arteries was studied in rings contracted with the thromboxane A2 mimetic, U46619 (3 × 10−6 M). After the U46619-induced contraction had reached a stable plateau, relaxation responses to increasing concentrations of bradykinin (10−10−10−6 M), sodium nitroprusside (10−10−10−5 M), aprikalim (10−10−10−5 M), NS1619 (10−5−10−5 M), BMS 191011 (10−9−10−5 M), or 14,15-EET (10−6 M) were obtained. A single concentration of 14,15-EET was used, since in preliminary studies, this concentration produced the most consistent and reproducible relaxations in isolated fetal coronary arteries; cumulative addition of 14,15-EET (10−7−10−5 M) failed to produce reproducible responses on a consistent basis in these preparations. Relaxation responses to bradykinin (10−10−10−6 M) were also obtained in rings contracted with KCl (60 mM). In some experiments, nutrient-restricted animals were incubated with indomethacin (10−5 M), NLA (3 × 10−5 M), ibetorixin (10−7 M), or glyburide (10−6 M) for 30 min before contracting the tissue with U46619. These inhibitors remained in contact with the tissues throughout the remainder of the experiment. Contractile responses to increasing concentrations of acetylcholine (10−9−10−5 M) and serotonin (10−9−10−5 M) were also determined.

Vascular smooth muscle cell isolation. Anterior interventricular fetal coronary arteries were cut into small pieces in low-calcium Tyrodes’s solution of the following composition (in mM): 145 NaCl, 4 KCl, 0.05 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose (pH = 7.4, adjusted with NaOH) at 4°C. Tissue pieces were incubated for 15 min with gentle shaking at 37°C in 1 ml of the low-calcium Tyrodes’s solution containing 1.5 mg/ml papain (14 U/mg) and 1 mg/ml DTT, followed by incubation for 15 min at 37°C in 2 ml of the low-calcium Tyrodes’s solution containing 2 mg/ml collagenase (196 U/ml), 0.5 mg/ml elastase (90 U/ml), and 1 mg/ml soybean trypsin inhibitor (10,000 U/ml). The enzyme solutions were removed by centrifugation at 500 g for 1–2 min, followed by removal of the supernatant with a pipette. Fresh low-calcium Tyrodes’s solution (5 ml) was then added to the tissue pieces. Single vascular smooth muscle cells were released by gently triturating with a 5-ml glass pipette and collected in the supernatant. The cells in the supernatant were centrifuged at 500 g for 5 min, resuspended in fresh low-calcium Tyrodes’s solution, and stored at 4°C. Cells were used within 5–8 h of isolation.

Electrophysiology studies. Freshly isolated vascular smooth muscle cells were placed in a small recording chamber and were perfused constantly with extracellular solution, as described previously (26). The extracellular solution for recording BKCa current amplitude

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MATERNAL NUTRIENT RESTRICTION ALTERS FETAL CORONARY FUNCTION

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Table 1. Fetal weight and heart development parameters at day 130 of gestation

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>Control Diet, 100%</th>
<th>Restricted Diet, 60%</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>3.383 ± 214</td>
<td>3.212 ± 122</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Heart weight, g/kg</td>
<td>23.9 ± 1.1</td>
<td>22.2 ± 0.8</td>
<td>0.23</td>
<td></td>
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<tr>
<td>Heart weight/fetal weight, g/kg</td>
<td>7.1 ± 0.3</td>
<td>6.9 ± 0.3</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Average left-ventricle thickness, mm</td>
<td>5.1 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Left-ventricle thickness, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>6.0 ± 0.4</td>
<td>5.2 ± 0.3</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>5.6 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>3.7 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Average right-ventricle thickness, mm</td>
<td>4.2 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Right-ventricle thickness, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>4.5 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>4.8 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>3.3 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Note: All values are means ± SE. n = 6 for each group. Percent difference tests were performed within each diet group.

Quantitative real-time RT-PCR. Quantitative real-time RT-PCR was performed as reported previously (24). Briefly, snap-frozen fetal coronary arteries were homogenized in TRI Reagent (Molecular Research Center, Cincinnati, OH), according to the manufacturer's specifications. The quality and quantity of total cellular RNA (tcRNA) generated from tcRNA, collected from pooled tissues on day 130 of pregnancy.

Data analysis. Relaxation responses are expressed as a percentage of the initial tension induced by U46619, and contractile responses are expressed as a percentage of the maximal response evoked by KCl (60 mM). For each vasodilator or vasoconstrictor, both the maximal percent response and the EC50 were determined. The EC50 values were converted to the negative logarithms and expressed as −log molar EC50. Membrane capacitance was calculated by integrating capacitive currents generated by a 10-mV hyperpolarizing pulse after electronic cancellation of the pipette-patch capacitance. Peak current amplitudes were measured as the mean current during the last 100 ms of voltage steps and expressed in picoamperes/picoFarad (pA/pF) to normalize for differences in the cell membrane area between isolated from Applied Biosystems, which is primer limited and contains a VIC-labeled probe. The predeveloped assay reagent was adjusted further using one-fourth of the normal amount so that it would not interfere with amplification of the FAM-labeled gene of interest. The multiplex reaction was also used to generate standard curves for quantification of 18S and the gene of interest based on dilutions of the cDNA generated from tcRNA, collected from pooled tissues on day 130 of pregnancy.

Fig. 1. A: isometric tension recordings demonstrating responsiveness of isolated rings of fetal coronary artery to bradykinin. Arterial rings were contracted with 9,11-dideoxy-11α,9ε-epoxymethano-PGF2α (U46619), followed by cumulative addition of bradykinin at the time points marked by arrowheads. B: log concentration-response curves for bradykinin in producing relaxations of isolated fetal coronary arteries from ewes fed a control (100%) or nutrient-restricted (60%) diet during the last 1/3 of pregnancy. Data are expressed as a percentage of the initial increase in tension induced by U46619 (3 × 10−7 M), which averaged 2.22 ± 0.3 and 2.38 ± 0.2 g in fetal coronary arteries from ewes fed a control and nutrient-restricted diet, respectively (P > 0.05). Each point represents the mean ± SE; n = 6.
vascular smooth muscle cells. Data were analyzed using pCLAMP software (Axon Instrument). Expression of the BKCa \( \alpha \) - and \( \beta \)-subunit mRNA was normalized to the expression of 18S mRNA. All results are expressed as mean ± SE, and \( n \) refers to the number of animals from which blood vessels were taken. Values were compared by Student’s \( t \)-test for paired or unpaired observations or by ANOVA to determine significance between groups as appropriate. Values were considered to be significantly different when \( P < 0.05 \).

RESULTS

Fetal Weight and Heart Development Parameters at Day 130 of Gestation

At day 130 of gestation, neither fetal body weight nor fetal heart weight differed significantly between the control and nutrient-restricted ewes (\( P > 0.05 \); Table 1). Similarly, maternal nutrient restriction had no effect on fetal left and right ventricular-wall thickness, with the exception of right-ventricle base thickness, which was increased in fetuses from the nutrient-restricted group compared with controls (\( P = 0.04 \); Table 1).

Pharmacologic Studies

The endothelium-dependent vasodilator, bradykinin (\( 10^{-10} \)–\( 10^{-6} \) M) (19, 34), produced concentration-dependent relaxations that were similar in fetal coronary artery rings from control and nutrient-restricted ewes (Fig. 1 and Table 2; \( P > 0.05 \)). In nutrient-restricted ewes, bradykinin-induced relaxation of fetal coronary arteries was fully suppressed by the NO synthase (NOS) inhibitor NLA (\( 3 \times 10^{-5} \) M) and converted to a contractile response (Fig. 2). In contrast, NLA had no effect on bradykinin-induced relaxation in control fetal coronary artery rings (Fig. 3A). The cyclooxygenase inhibitor, indomethacin (35), had no effect on bradykinin-induced relaxation of fetal coronary arteries from control or nutrient-restricted ewes treated with or without NLA (data not shown). The NLA-resistant response to bradykinin in control coronary artery rings was unaffected by the selective ATP-sensitive potassium (\( K_{\text{ATP}} \)) channel blocker, glyburide (44), but was nearly abolished in the presence of iberiotoxin (\( 10^{-7} \) M), a selective BKCa channel blocker (20) (Fig. 3B), or by contracting the rings with a maximal depolarizing concentration of KCl (60 mM) instead of U46619 (Fig. 3C).

Sodium nitroprusside (\( 10^{-9} \)–\( 10^{-5} \) M), an exogenous NO donor (25), also caused relaxation of fetal coronary arteries that did not differ between tissues taken from control and nutrient-restricted ewes (Table 2). Similarly, maternal nutrient restriction had no effect on relaxation responses to the KATP channel opener (2) aprikalim (\( 10^{-9} \)–\( 10^{-5} \) M; Table 2), the BKCa channel openers (22, 43) NS1619 and BMS 191011 (\( 10^{-8} \)–\( 10^{-5} \) M; Fig. 4A and Table 2), or 14,15-EET (\( 10^{-6} \) M; Fig. 4B), a putative EDHF in coronary arteries (5, 9, 10, 21). Vasocostrictor responses to acetylcholine and serotonin in fetal coronary arteries were unaltered by maternal nutrient status (Table 2; \( P > 0.05 \)).

Table 2. \( pD_2 \) and \( E_{\text{max}} \) values for various vasodilators and vasoconstrictors in fetal coronary arteries at day 130 of gestation

<table>
<thead>
<tr>
<th></th>
<th>( pD_2 )</th>
<th>( E_{\text{max}} ) % Relaxation*</th>
<th>( pD_2 )</th>
<th>( E_{\text{max}} ) % Contraction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasodilators</td>
<td>Control Diet, 100%</td>
<td>Restricted Diet, 60%</td>
<td>Control Diet, 100%</td>
<td>Restricted Diet, 60%</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>8.06 ± 0.3</td>
<td>8.22 ± 0.3</td>
<td>86 ± 10</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>7.89 ± 0.2</td>
<td>8.03 ± 0.1</td>
<td>99 ± 1</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Aprikalin</td>
<td>6.44 ± 0.2</td>
<td>6.35 ± 0.2</td>
<td>97 ± 3</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>NS1619</td>
<td>5.86 ± 0.5</td>
<td>5.36 ± 0.5</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>BMS 191011</td>
<td>5.76 ± 0.4</td>
<td>5.51 ± 0.3</td>
<td>94 ± 6</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vasoconstrictors</th>
<th>( pD_2 )</th>
<th>( E_{\text{max}} ) % Contraction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>5.92 ± 0.1</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5.74 ± 0.2</td>
<td>90 ± 2</td>
</tr>
</tbody>
</table>
Whole-cell BKCa currents were nearly identical in fetal coronary artery smooth muscle cells from control and nutrient-restricted ewes (Fig. 5). Similarly, the whole-cell BKCa currents obtained in the presence of NS1619 (10^{-5} M) or 14,15-EET (10^{-6} M) did not differ significantly between fetal coronary smooth muscle cells from control and nutrient-restricted ewes (Fig. 6; P > 0.05).

**Quantitative RT-PCR**

Quantitative RT-PCR identified the expression of BKCa α and β subunits in fetal coronary arteries, which did not differ between the control and nutrient-restricted ewes (Fig. 7; P > 0.05).

**DISCUSSION**

An increasing number of epidemiological studies in humans, corroborated by numerous controlled studies in animal models, provide compelling evidence for the concept of developmental programming. Such studies show a strong association between various stressors or insults to the developing fetus and the subsequent increased risk of developing a range of pathologies as adults, e.g., obesity, diabetes, and coronary artery disease (1, 4, 30, 41). With regard to the cardiovascular system, adverse maternal nutrition and/or low birth weight are associated with impaired endothelium-dependent relaxation and increased sensitivity to vasoconstrictors in several animal species, including...
humans, later in life (6, 7, 29, 32, 33, 38–40, 48, 53). Such adverse vascular changes are hallmark characteristics of endothelial dysfunction, which is a known risk factor for coronary artery disease (12, 17).

In the present study, we assessed the effect of maternal nutrient restriction during pregnancy on fetal coronary artery function. The major finding of this study is that although maternal undernutrition during pregnancy has no quantitative effect on endothelium-dependent relaxation of fetal coronary arteries in response to bradykinin, the underlying mechanisms mediating this response differ between arteries obtained from control and nutrient-restricted animals. In fetal coronary arteries from control animals, bradykinin-induced relaxation is resistant to inhibitors of endothelial NOS (eNOS) and cyclooxygenase, suggesting that the response is mediated primarily via a pathway that is independent of NO or prostacyclin. By contrast, bradykinin-induced relaxation of fetal coronary arteries from nutrient-restricted animals is abolished completely by inhibition of eNOS, suggesting that the response is mediated solely by the release of NO from the endothelium under these conditions. These data are consistent with the notion that qualitatively different mechanisms underlie endothelium-dependent relaxation of fetal coronary arteries, depending on maternal nutritional status during pregnancy.

Bradykinin evokes endothelium-dependent relaxation by activating multiple vasodilator pathways. Two such pathways include the release of NO and prostacyclin from endothelial cells (23, 35). A third pathway, which is resistant to inhibitors of eNOS and cyclooxygenase, is associated with endothelium-dependent hyperpolarization of vascular smooth muscle cells and is generally attributed to the release of an EDHF or in some blood vessels, the direct transfer of endothelial cell hyperpolarization...
Concentrations) are typical of the classic EDHF pathway (8, 13); endothelial cells do not express BKCa channels, and BKCa diminishes the likelihood of a role for gap junctions, since that iberiotoxin abolishes the response to bradykinin mechanism that is sensitive to changes in membrane potential.

K channel blockade, inhibition by elevated external K illustrates that the coronary smooth muscle to vascular smooth muscle via gap junctions (8, 9, 13).

The present findings indicate that at least two pathways are present in fetal coronary arteries; i.e., endothelium-derived NO mediates bradykinin-induced relaxation in fetal coronary arteries from nutrient-restricted ewes, whereas the response in control arteries is resistant to eNOS inhibition and thus unlikely to involve NO. The mechanism underlying this NLA-resistant response in coronary arteries from well-nourished animals is not clear but appears to involve activation of BKCa channels, since it was abolished by the potent and selective BKCa channel blocker, iberiotoxin (20), whereas the selective KATP channel blocker, glyburide, was without effect. Moreover, the NLA-resistant relaxation did not occur in arteries contracted by a high external concentration of K⁺, which markedly reduces or eliminates the driving force for efflux of K⁺ through membrane K channels and is consistent with a mechanism that is sensitive to changes in membrane potential (8). That iberiotoxin abolishes the response to bradykinin diminishes the likelihood of a role for gap junctions, since endothelial cells do not express BKCa channels, and BKCa channels do not typically play a role in the electrotonic transfer of endothelial hyperpolarization via gap junctions. These characteristics (i.e., NO and prostacyclin independent, inhibition by K channel blockade, inhibition by elevated external K⁺ concentrations) are typical of the classic EDHF pathway (8, 13); however, since membrane potential was not measured in the present study, we cannot state definitively that hyperpolarization of the coronary smooth muscle occurred. Given these similarities and limitations, the data are most consistent with an “EDHF-like” pathway mediating the response to bradykinin in control coronary arteries.

In coronary arteries of fully developed animals, bradykinin causes the release of NO and EDHF (14, 37, 52). The EDHF pathway is attributed to the release of endothelium-derived EETs (e.g., 14,15-EET) that activate BKCa channels in coronary vascular smooth muscle to cause relaxation (8–10). In the present study, we found that 14,15-EET also causes vasorelaxation and activates BKCa channels in smooth muscle cells from fetal coronary arteries. Maternal nutrient restriction had no effect on these responses to 14,15-EET nor did it affect fetal coronary artery relaxations or the increase in whole-cell BKCa currents induced by the BKCa channel openers, BMS 191011 (43) or NS1619 (22). Moreover, maternal nutrient restriction did not alter the expression of BKCa α and β subunits in fetal coronary arteries. Thus the absence of the EDHF-like response to bradykinin in undernourished animals cannot be explained by a decreased number or activity of BKCa channels or by a decreased sensitivity to mediators that activate BKCa channels in vascular smooth muscle cells. These results point to the possibility that the lack of an EDHF-like response to bradykinin is the result of a marked reduction in the synthesis or release of a putative EDHF, whether it is an EET or another yet-to-be-identified mediator. In addition, alterations in bradykinin signaling (e.g., receptor number or subtype, receptor coupling, intracellular signaling mechanisms) or enzymatic degradation could also play a role in the effect of maternal nutrient restriction.

Several studies have investigated the effect of different developmental insults or stressors on fetal vascular reactivity. In these studies, there appeared to be specific alterations in fetal vascular reactivity dependent on animal species, stressor, and/or vascular bed. For example, in a sheep placental embolization model, sodium nitroprusside-induced relaxation of fetal coronary arteries was unaltered (7), which is consistent with the findings from the present study and suggests that maternal nutrient restriction has no adverse effect on the smooth muscle guanylyl cyclase/cGMP signaling pathway. In contrast, sodium nitroprusside-induced relaxation was blunted in the femoral artery of nutrient-restricted fetal sheep (38), and NO-mediated relaxation of fetal coronary arteries was potentiated by exposure to betamethasone during gestation (19). In humans, low birth weight is linked to impaired endothelium-dependent relaxation of brachial arteries later in life (27, 32). In the present study, there was no quantitative difference in bradykinin-induced, endothelium-dependent relaxation of fetal coronary arteries from control and nutrient-restricted ewes, which is in agreement with previous findings in sheep fetal coronary arteries from the placental embolization model (7); however, the underlying mechanism(s) involved in endothelium-dependent relaxation of fetal sheep coronary arteries under these experimental conditions were not elucidated. In this regard, the present study clearly demonstrates that different mechanisms mediate bradykinin-induced relaxation of fetal coronary arteries from animals fed a normal vs. nutrient-restricted diet during mid to late gestation. It is also worth noting that the observed functional differences occurred, inde-

![Fig. 7. Expression of mRNA for BKCa α (A) and β (B) subunits in fetal coronary arteries from ewes fed a control (100%) or nutrient-restricted (60%) diet during the last 3/5 of pregnancy. The mRNA expression is normalized with respect to the total 18S mRNA expression in pooled tissues. Each point represents the mean ± SE; n = 7–8.](http://ajpheart.physiology.org/)
pendent of a reduction in fetal weight, inasmuch as the day 130 fetal weight did not differ between the control and nutrient-restricted animals. These observations are in agreement with the increasing number of studies suggesting that developmental programming can occur, independent of low birth weight (4, 41). For example, in humans and in animal models, offspring of mothers who experience nutrient restriction early in pregnancy but receive adequate nutrition later in pregnancy, resulting in normal birth weights, still exhibit many of the same phenotypes (e.g., poor growth, increased adiposity, poor glucose tolerance, and dyslipidemia) as offspring from mothers that are undernourished for the entire pregnancy (4, 16, 18, 50).

Redundancy in cellular signaling pathways is an evolutionarily conserved mechanism to ensure optimum physiologic performance with changing micro- and macroenvironments. Within the vascular wall, multiple vasodilatory signaling systems are present to maintain adequate tissue perfusion under stressful or pathologic conditions. For example, endothelium-dependent relaxations appear to be unaffected in arteries from animals with hypercholesterolemia, heart failure, and knockout of eNOS, but further investigation into the underlying mechanism of relaxation reveals that the NO-dependent component is markedly inhibited or abolished and that an EDHF pathway is upregulated, such that the relaxation response is not compromised (31, 36, 45). The results of the present study suggest that a similar type of compensatory response can also occur in fetal coronary arteries and add to our knowledge of how the developing fetus adapts to changes in its nutritional environment. As our understanding of developmental programming of the cardiovascular system continues to evolve, the present findings suggest several lines of inquiry for future investigation, including, for example, studies designed to determine the effects of undernutrition at different stages of pregnancy, whether the effects are observed in offspring, and what happens to the responses in adulthood. Such information may provide new insights into potential mechanisms linking exposure to developmental insults during pregnancy and the increased risk of cardiovascular disease later in life.

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DISCLOSURES
No conflicts of interest are declared by the authors.

AUTHOR CONTRIBUTIONS

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