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Differential effects of maternal hypoxia or nutrient restriction on carotid and femoral vascular function in neonatal rats

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Williams, Sarah J., Morag E. Campbell, I. Caroline McMillen, and Sandra T. Davidge. Differential effects of maternal hypoxia or nutrient restriction on carotid and femoral vascular function in neonatal rats. Am J Physiol Regul Integr Comp Physiol 288: R360–R367, 2005. First published November 4, 2004; doi:10.1152/ajpregu.00178.2004.—In response to reduced oxygen or nutrient supply, the fetus may redistribute cardiac output to conserve brain and heart growth, at the expense of the peripheral tissues; however, it is not known whether alterations in vascular function are maintained after birth or whether reduced fetal oxygen versus nutrient supply produces distinct effects. Using a pressure myograph, we examined isolated carotid and femoral artery responses to phenylephrine and endothelin-1 in neonatal rats, after either reduced maternal oxygen or global nutrient restriction during late gestation. Timed-pregnant Sprague-Dawley rats were randomly assigned to control (n = 10), hypoxia (12% O2, n = 9), or nutrient restriction (NR, 40% of control diet, n = 7) protocol and treated from day 15–21 of pregnancy. Pups were collected 3–12 h after birth. Neonatal weights (P < 0.001) and relative liver weights (P < 0.001) were lower in hypoxia and nutrient restriction treatments compared with control, while relative heart weights were greater in the hypoxia than in the control or nutrient restriction groups (P < 0.01). Constriction to phenylephrine was reduced in carotid arteries from the hypoxia and nutrient restriction groups compared with control (P < 0.001), while the femoral artery response was greater in hypoxia-treated neonates compared with control or nutrient-restricted neonates (P < 0.01). Only the hypoxia reduced carotid responses to endothelin-1, while no differences were observed in the endothelin-1 responses in femoral arteries. Maternal hypoxia and maternal nutrient restriction produced distinct effects on heart growth and neonatal vascular function, suggesting that regional changes in cardiovascular function after poor fetal growth are dependent on the nature of the insult in utero.

fetal growth; oxygen; artery; endothelin-1; phenylephrine

INTRAUTERINE GROWTH RESTRICTION (IUGR) is associated with increased morbidity and mortality during the perinatal period (13). A series of epidemiological studies has also demonstrated that poor growth in utero is associated with increased risk of developing cardiovascular disease and hypertension in later life (2, 3, 23). It has been suggested that the mechanisms by which the fetus adapts to a suboptimal intrauterine environment may allow its continued growth in utero but also increase the subsequent risk of developing cardiovascular disease (3, 31). It has now been demonstrated that infants (16, 29, 33), children (30), and young adults (18, 24) who were of low birth weight have impaired peripheral vascular function compared with contemporaries of appropriate birth weight. This may suggest that when growth in utero is restricted, early alterations in local vascular function result in a persistent impairment in peripheral vascular function after birth. While such changes may contribute to the increased risk of developing hypertension and cardiovascular disease in adult life, it is not yet clear how specific deficits in the in utero environment may alter vascular function after birth.

Placental insufficiency, which limits the supply of both oxygen and nutrients to the fetus, is a major clinical cause of IUGR (10, 20). There is now substantial evidence that in animal models, poor maternal nutrition during pregnancy can impair fetal (32, 35) and adult vascular function (4, 8, 9, 21). Similarly, acute hypoxia in vitro and chronic maternal hypoxia for 4 days has been demonstrated to impair endothelial function in the fetal guinea pig carotid artery; however, this impairment was not observed following 7 days of chronic hypoxia (44). Although it has been clearly demonstrated that one of the important fetal adaptations to either acute or sustained hypoxia is a redistribution of fetal cardiac output, to ensure a preferential supply of nutrients to the brain and the heart and maintain their growth, at the expense of the periphery (5, 31, 42), it is not yet known whether changes in regional vascular function after fetal hypoxia may persist after birth.

The redistribution of fetal cardiac output during hypoxia may be partially dependent on the reported increased circulating levels of several vasoactive factors, including norepinephrine (14, 43) and endothelin-1 (1, 19), but may also involve changes in the local vascular responses to these circulating factors. The effects of chronic hypoxia in utero on the regional vascular responses to these vasoconstrictors after birth are not yet known.

To determine whether reduced oxygen supply in utero influences regional vasoconstrictor responses at birth, before the additional influences of various determinants of postnatal growth, we have investigated the effects of chronic maternal hypoxia on the vascular responses of two distinct arteries from neonatal offspring: the carotid artery, which supplies blood...
flow to the brain, and the femoral artery, which supplies the periphery. We have tested the hypothesis that chronic maternal hypoxia in rats reduces neonatal carotid artery responses to the vasoconstrictors phenylephrine and endothelin-1, while increasing the responses of neonatal femoral arteries to these factors. As it has been previously demonstrated that chronic hypoxia reduces food intake in pregnant rats (6, 15, 46), we also determined the effects of maternal nutritional restriction during late gestation on the vascular responses of arteries from the neonatal offspring.

MATERIALS AND METHODS

Animals. All procedures in this study were approved by the University of Alberta Animal Welfare Committee and were in accordance with the guidelines of the Canadian Council on Animal Care. Female Sprague-Dawley rats were obtained at 3 mo of age (Charles River, Quebec, Canada) and were mated within the animal facility after a minimum 1-wk acclimatization. Pregnancy was confirmed by the presence of sperm in a vaginal smear examined microscopically the following morning, and this was considered day 0 of pregnancy. All rats received food (standard lab rat chow) ad libitum from day 0–15 of pregnancy. On day 15, rats were randomized to control, maternal hypoxia, or maternal nutrient restriction protocols. Throughout pregnancy, rats were housed individually in standard rat cages, which were maintained in a clean conventional facility, with 60% humidity, a 12:12-h light-dark cycle, and ad libitum access to water. Control group rats (n = 10) were housed in room air on a rack in the same room as the chambers that hypoxic and nutrient-restricted dams were housed in, and were fed ad libitum throughout pregnancy. Food intake and weight gain were measured daily in all pregnant rats.

Maternal hypoxia. To reduce maternal oxygen supply during late gestation, nine pregnant rats were placed inside a Plexiglas chamber on day 15 of pregnancy, which was maintained at 12% oxygen by continuous infusion of a mixture of nitrogen and compressed air, without additional infusion of carbon dioxide. Previous investigations have demonstrated that maternal hypoxia, ranging between 9.0% and 14.0% oxygen for varying lengths of time, reduced fetal growth and altered organ proportions, validating the use of this technique to induce asymmetric fetal growth restriction (6, 46). The oxygen concentration of the chamber was monitored throughout treatment using a portable oxygen analyzer, which was calibrated daily (Hudson RCI, Temecula, CA). The chamber, which housed a maximum of three pregnant rats, maintained individually in separate standard rat cages at any time, was opened briefly once per day to weigh rats and feed as well as to clean the cages. Rats were removed from the chamber on the morning of day 22 of pregnancy, and housed in room air until delivery.

Maternal nutrient restriction. A group of pregnant rats (n = 7) randomized to a maternal nutrient restriction protocol were placed inside a second Plexiglas chamber on day 15 of pregnancy, which was continuously infused with compressed air. Oxygen concentration was checked periodically to ensure rats were exposed to 21% oxygen. The chamber was opened briefly once per day to allow rats to be weighed and cages cleaned. From day 15, rats were administered 11.5 ± 1 g standard rat chow/day, which was equivalent to the lowest food intake recorded in rats exposed to maternal hypoxia, and represented 40% of control food intake during this time. Although it was intended that the rats were to remain in the chamber until day 22 of pregnancy, in six of the seven rats, delivery occurred on day 21, and thus rats were removed from the chamber at this time. All rats were returned to normal diet after delivery.

Tissue collection. Within 3–12 h after birth, all pups were weighed, and litter size was reduced to eight pups, which were returned to the dam for use in other studies. The remaining neonatal rats were collected at this time, and were euthanized by decapitation. Brain, heart, lung, liver, and kidneys were dissected from one to four pups per litter for the determination of relative organ proportions. Vascular function experiments were performed in arteries isolated from one neonate per litter from a total of seven control, seven maternal hypoxia, and 5 maternal nutrient restriction litters. In the remainder of animals, the timing of birth prevented vascular experiments being performed; however, maternal and neonatal weight/organ weight data pertinent to the animal model have been included. Carotid and femoral arteries were gently dissected free of connective tissue in ice-cold DMEM buffer (27) (DMEM; 1 mmol/l sodium pyruvate, 25 mmol/l sodium bicarbonate, 5 mmol/l HEPES, 5 mmol/l D-glucose, containing 1.8 mmol/l calcium, 5.4 mmol/l potassium, and 137.2 mmol/l sodium, pH 7.4; Sigma, St. Louis, MO) for the assessment of vascular function. The HEPES-buffered medium is a closed system that maintains extracellular and intracellular pH, the latter through the intrinsic mechanisms of the cell such as Na+/H+ exchange and phosphates (17).

Vessel preparation and equipment. For each vessel, the dissection was extended to provide the longest segment of artery that is practical to dissect from a rat at this developmental stage, and the full segment was mounted for study, thus avoiding any regional differences in vessel reactivity. After dissection the sections of carotid and femoral arteries were mounted on a pressurized myograph system (Living Systems, Burlington, VT) in a 2.5-ml organ bath filled with DMEM. This technique was chosen as it applies minimal mechanical stress to the artery being studied, making it ideal for the study of delicate neonatal arteries. It should be noted, however, that endothelial function was not directly assessed in this protocol. Briefly, one end of the artery was gently opened and slipped over the tip of a glass cannula (60–80-μm diameter), which was then secured with a short section of synthetic thread. Blood was then flushed from the preparation by gently flowing medium through the lumen of the artery. A second glass cannula was positioned inside the open end of the artery and secured by thread. Flow through one cannula was blocked by means of a stop-cock, while flow through the second cannula enabled the artery preparation to be pressurized with 12 mmHg, using a pressure-servo-regulated peristaltic pump (Living Systems). The preparation was allowed to stabilize for 30 min at 37°C. This pressure was chosen on the basis of preliminary studies, which indicated that 12 mmHg was sufficient to maintain viable carotid and femoral arteries, with adequate lumen visibility to allow accurate measurement. Vessel viability was reduced at higher pressures. After stabilization, concentration-response curves were performed to determine the vascular response to increasing concentrations of the vasoconstrictor phenylephrine (1 nmol/l to 50 μmol/l; Sigma). After 4-min exposure to each concentration, artery lumen diameter and wall thickness were measured in two places using an inverted microscope (Nikon, TS100-F) and video camera (Sony, charge-coupled device monochrome XC-ST30) in concert with a video dimension analyzer (Living Systems; measurement precision 1–2 μm), and subsequently averaged. In a subset of arteries, after completion of the phenylephrine concentration-response curve, vessels were washed in DMEM for 1 h, before the vascular responses to endothelin-1 were determined by the same method (0.1 nmol/l to 0.1 μmol/l, Sigma). Fresh medium was supplied at 10-min intervals throughout the experiment.

Data analysis. All data are presented as means ± SE. Maternal characteristics, neonatal weight, organ weights, and relative organ proportions were compared using one-way ANOVA and, where relevant, Tukey’s post hoc analysis. Maternal weight gain and food intake before and after the start of treatment were compared using two-way ANOVA for repeated measures, followed by Tukey’s post hoc test. Artery lumen diameter and wall thickness at baseline were compared among groups using one-way ANOVA and Tukey’s post hoc test. Concentration-response curves are presented graphically as the mean percent constriction ± SE at each point. Percent constriction was determined as the artery lumen diameter after drug administration expressed as a fraction of baseline lumen diameter. Vascular re-
sponses in this study were compared among groups using two-way ANOVA for repeated measures, as the nature of the concentration response curve generated by femoral arteries to phenylephrine made calculation of EC$_{50}$ values inappropriate. Wherever possible, comparisons of EC$_{50}$ values were also performed; however, for this purpose, EC$_{50}$s were calculated using Sigma Plot 8.0 pharmacology standard curves analysis. Statistical significance was defined as $P < 0.05$ and is indicated within all figures by the use of different letters.

**RESULTS**

**Maternal characteristics.** There was no difference in maternal age or weight at the start of pregnancy, or in the observed litter size among groups (Table 1). There were also no significant differences in the number of stillborn pups among the treatment groups (Table 1). Food intake before treatment was not different among groups. Maternal hypoxia significantly reduced food intake during the treatment period, compared with control, ($P < 0.001$), whereas food intake in the maternal nutrient restriction group was also lower ($P < 0.001$) than control, but not significantly different from the maternal hypoxia group (Fig. 1A). Similarly, weight gain before pregnancy was not different among groups, but weight gain during treatment was significantly reduced in both maternal hypoxia and maternal nutrient restriction groups ($P < 0.001$; Fig. 1B). Maximal weight gain on day 21 of pregnancy was greater in control (C) than in maternal hypoxia (H) or maternal nutrient restriction (NR), (C 149.8 ± 2.5 g vs. H 132.8 ± 2.2 g or NR 126.0 ± 1.7 g, $P < 0.001$). When the day of delivery was compared among groups, delivery occurred earlier in maternal nutrient restriction dams than in maternal hypoxia dams (H 22.4 ± 0.2 days vs. NR 21.2 ± 0.1 days, $P < 0.05$); however, neither group was significantly different from control (C 21.8 ± 0.1 day).

**Neonatal and organ weights.** Neonatal body weight was reduced by both maternal hypoxia and maternal nutrient restriction, compared with control (C: 6.5 ± 0.1 g vs. H: 5.9 ± 0.1 g or NR: 5.6 ± 0.1 g, $P < 0.001$, Fig. 2A). Neonatal heart and brain weights were significantly higher in offspring from maternal hypoxia than maternal nutrient restriction, while liver weights were lower in both groups compared with control ($P < 0.05$; Table 2). Lung weights were not different among groups; however, combined kidney weights were significantly lower in pups from maternal nutrient restriction dams than from either control or maternal hypoxia (Table 2). When organ weights were expressed as a proportion of body weight, it was observed that heart weights constituted a greater proportion of body weight in pups from maternal hypoxia than from either control or nutrient restriction. (Fig. 2B; $P < 0.05$) Proportional brain weights were also greater in maternal hypoxia offspring than control (Fig. 2C; $P < 0.05$). The relative brain weight in pups from maternal nutrient restriction tended to be greater than control (Fig. 2C; $P = 0.076$) but was not different from maternal hypoxia. Proportional liver weight was reduced in both maternal hypoxia and maternal nutrient restriction compared with control (Fig. 2D; $P < 0.01$), whereas there was no significant difference in the proportional weight of either lung or kidney between groups (data not shown).

**Carotid and femoral vascular responses.** After equilibration, and before the beginning of the experiment, the lumen diameters of carotid and femoral arteries were not significantly different among groups (carotid: C, 146.6 ± 17.9 μm; H, 137.4 ± 13.2 μm; NR, 144.4 ± 18.0 μm; femoral: C, 167.8 ±

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<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wks</th>
<th>Pregroup Weight, g</th>
<th>Litter Size</th>
<th>Total Number Stillborn/Live Pups</th>
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<td>Control ($n = 10$)</td>
<td>15.3±0.6</td>
<td>307±7</td>
<td>15.4±0.7</td>
<td>3/151</td>
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<td>Maternal hypoxia ($n = 9$)</td>
<td>16.0±0.7</td>
<td>312±8</td>
<td>13.6±0.6</td>
<td>8/114</td>
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<tr>
<td>Maternal nutrient restriction ($n = 7$)</td>
<td>15.3±0.4</td>
<td>318±5</td>
<td>15.4±0.7</td>
<td>1/108</td>
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</table>

Values are means ± SE. There were no significant differences among the groups.

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![Fig. 1. Maternal food intake (A) and weight gain (B) from day 15–22 of pregnancy were lower ($P < 0.001$) in the maternal hypoxia ($n = 9$) and maternal nutrient restriction ($n = 7$) groups than in control ($n = 10$). Different letters denote significant ($P < 0.05$) differences among groups.](http://ajpregu.physiology.org/DownloadedFrom)
Arterial wall thickness was also not different among groups (carotid: C, 41.2 ± 7.6 μm; H, 30.7 ± 4.3 μm; NR, 43.0 ± 9.6 μm; femoral: C, 24.5 ± 3.1 μm; H, 24.0 ± 3.6 μm; NR, 28.5 ± 6.6 μm). Similarly, the wall:lumen ratios of carotid and femoral arteries at baseline were not different among groups (carotid: C, 0.30 ± 0.06; H, 0.22 ± 0.02; NR, 0.32 ± 0.09; femoral: C, 0.16 ± 0.02; H, 0.13 ± 0.02; NR, 0.16 ± 0.04). Although carotid artery wall thickness tended to be greater (P = 0.09) than femoral artery wall thickness in the control group, no significant difference was observed in wall thickness between the two arteries in maternal hypoxia or maternal nutrient restriction groups.

Both arterial constriction in response to increasing concentrations of phenylephrine, and the maximal constriction attained were lower in the carotid artery of pups from either maternal hypoxia or maternal nutrient restriction dams compared with control (P = 0.002; Fig. 3A), while the sensitivity to phenylephrine was not different among groups (EC50: C: 45.1 ± 26 nmol/l, H: 138.0 ± 59.8 nmol/l, NR: 89.5 ± 25 nmol/l). In contrast, femoral artery constriction in response to increasing concentrations of phenylephrine was significantly enhanced in arteries of pups from maternal hypoxia compared with those from either control or maternal nutrient restriction dams (P = 0.003; Fig. 3B). Femoral arteries did not sustain constriction at the highest doses of phenylephrine, and thus the maximal constriction was compared at a concentration of 1 μmol/l. Maximal constriction was greatest in femoral arteries from pups of maternal hypoxia-treated dams, and it was significantly higher than those from control or maternal nutrient restriction dams (H: 55.2 ± 9.2% vs. C: 27.8 ± 5.4% or NR: 33.0 ± 7.2%, P < 0.01).

In a subset of experiments, the vascular response to endothelin-1 was also determined. The cumulative response to increasing endothelin-1 concentration was less in carotid arteries of neonates from maternal hypoxia, but not maternal nutrient restriction, compared with control (Fig. 4A, P < 0.05). There were no differences, however, in the maximal carotid

Table 2. Neonatal organ weights

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control (n = 17 pups/10 litters)</th>
<th>Maternal Hypoxia (n = 17 pups/9 litters)</th>
<th>Maternal Nutrient Restriction (n = 14 pups/7 litters)</th>
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</thead>
<tbody>
<tr>
<td>Brain, mg</td>
<td>276.6 ± 6.5±b</td>
<td>291.1 ± 5.1±a</td>
<td>261.2 ± 5.3b</td>
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<tr>
<td>Heart, mg</td>
<td>42.3 ± 1.5±b</td>
<td>46.6 ± 2.6±a</td>
<td>35.1 ± 1.7b</td>
</tr>
<tr>
<td>Liver, mg</td>
<td>338.2 ± 16.4±</td>
<td>270.2 ± 17.5±</td>
<td>237.2 ± 7.1b</td>
</tr>
<tr>
<td>Kidney, mg</td>
<td>62.9 ± 2.9±</td>
<td>58.0 ± 2.8±</td>
<td>48.8 ± 2.6±</td>
</tr>
<tr>
<td>Lung, mg</td>
<td>130.7 ± 6.7±</td>
<td>106.7 ± 7.5±</td>
<td>121.1 ± 6.2±</td>
</tr>
</tbody>
</table>

Values are means ± SE. Different letters denote significant differences among groups (P < 0.05).
artery constriction to endothelin-1 or in the sensitivity to endothelin-1 among groups (EC50: C, 7.11 ± 2.78 nmol/l, H, 30.3 ± 15.0 nmol/l, NR, 11.2 ± 10.5 nmol/l). No differences were apparent in the femoral artery response to increasing endothelin-1 concentrations, sensitivity (EC50: C, 26.0 ± 13.1 nmol/l; H, 27.1 ± 6.84 nmol/l; NR, 56.1 ± 18.4 nmol/l), or the maximal constriction at 0.1 μmol/l endothelin-1 among groups (Fig. 4B).

DISCUSSION

Reduction in either maternal oxygen or nutrient supply during the last week of gestation resulted in reduced neonatal size and perturbations of neonatal organ weight and proportion. These effects are consistent with previous reports of the impact of reduced oxygen or nutrient supply during pregnancy on fetal growth (46, 47). Conservation of brain and heart growth in offspring from maternal hypoxia dams and reduced liver growth in offspring from both hypoxia and nutrient restriction dams suggest that that the two treatments caused changes in peripheral resistance that redistributed cardiac output. Chronic maternal hypoxia, however, specifically increased relative neonatal heart weight, suggesting that fetal growth was differentially affected by the two maternal treatments. To our knowledge, this study is the first to determine the impact of reduced oxygen and/or nutrient supply in utero on regional vasoconstrictor responses in the neonatal rat. Although the changes in vascular function observed in offspring from maternal hypoxia supported our hypothesis, interestingly, maternal nutrient restriction produced distinct effects on vascular function, highlighting that the neonatal vascular consequences of fetal growth restriction are dependent on the specific nature of the in utero environment.

Fig. 3. A: carotid artery response to phenylephrine was significantly blunted (P < 0.01) in arteries from both maternal hypoxia (n = 7) and maternal nutrient restriction (n = 5) offspring compared with control (n = 6). B: femoral artery response to phenylephrine was significantly greater (P < 0.01) in only the offspring of maternal hypoxia (n = 6) compared with either maternal nutrient restriction (n = 4) or control (n = 7). Different letters denote significant differences among groups.

Fig. 4. A: carotid artery response to endothelin-1 was blunted (P < 0.05) in only offspring of maternal hypoxia (n = 3) compared with those from control (n = 3), or maternal nutrient restriction (n = 3). Different letters denote significant differences among groups. B: no differences (P > 0.05) were observed in the femoral artery response to endothelin-1 among groups (control, n = 5; maternal hypoxia, n = 2; maternal nutrient restriction, n = 4).
Several authors have used chronic maternal hypoxia to restrict fetal growth in rats previously and have demonstrated that along a range of oxygen reductions imposed at different times throughout gestation, this treatment results in reduced fetal or neonatal body weight and altered fetal organ growth (5, 15, 25, 28, 34, 36, 37, 46, 47). Decreased maternal food intake as a consequence of maternal hypoxia has also been reported previously (6, 15, 46). It is interesting to postulate that this may have effectively resulted in a greater cumulative substrate deficit and thus constituted a dual insult for the offspring exposed to maternal hypoxia. Although there was a decrease in food intake in the dams exposed to chronic hypoxia, it is likely that the available oxygen supply also limited metabolism, such that nutrient supply was appropriate to metabolic demand. The partial recovery of maternal appetite as hypoxia treatment continued indicates that the pregnant dam may be able to adapt to reduced oxygen. In contrast, the decreased available food in the maternal nutrient restriction group may have presented a greater fetal nutrient deficit. The similar effects of the two treatments on birth weight and organ growth do not suggest that the restriction of fetal growth was more severe in dams exposed to hypoxia.

The increase in heart weight and proportion observed in offspring from maternal hypoxia-treated dams in this study is consistent with previous reports in fetal rats after chronic maternal hypoxia (10.5%) from day 19 to day 21 of gestation (47). Intermittent hypobaric hypoxia, either prenatally or postnatally, in rats also increased heart weight and proportion of body weight (34). It is interesting that several recent studies have demonstrated perturbations in heart development and function after chronic maternal hypoxia (25, 47).

Our results demonstrated that reductions in maternal oxygen or nutrient supply produced effects on vascular function, in the absence of significant structural differences, that were consistent with the redistribution of cardiac output and remained evident in the neonate at birth. Although both maternal hypoxia and maternal nutrient restriction reduced the carotid artery response to phenylephrine, only hypoxia increased the femoral artery response to this vasoconstrictor. Thus changes in vascular function, which serve to maintain brain blood flow in utero in fetuses exposed to either hypoxia or nutrient restriction, are still evident some 12 h after birth. Only exposure of the fetus to maternal hypoxia, however, resulted in an enhanced femoral vasoconstrictor response to phenylephrine, which highlights a differential effect of restriction of oxygen or nutrient supply on peripheral vascular function in the neonate. Furthermore, although there was a marked reduction in the carotid artery response to endothelin-1 after exposure to maternal hypoxia, maternal nutrient restriction had no effect on the carotid artery vasoconstrictor response to this agonist. There were also no differences observed in the femoral artery response to endothelin-1 between neonates exposed to maternal hypoxia or nutrient restriction.

The regional changes in vascular function, which were observed after each maternal insult, may reflect specific fetal vascular adaptations to either reduced oxygen or nutrient supply. Although fetal plasma norepinephrine levels have consistently been reported as increased in response to hypoxia (12, 38), maternal fasting also results in increased fetal plasma norepinephrine concentrations (7). Spontaneously hypoglycemic fetal sheep do not have increased basal norepinephrine concentrations, but there is a greater increase in plasma norepinephrine in these fetuses in response to acute hypoxia (12). These findings may suggest that catecholamines are involved in the fetal adaptation to both hypoxia and undernutrition. In vascular tissue, expression of the precursor protein for endothelin-1 is increased by hypoxia-inducible factor-1 signaling (22), and the reported increase in fetal plasma endothelin-1 levels in response to hypoxia (1, 16) may represent a specific adaptation to a reduced oxygen supply.

Although this study has demonstrated that either reduced oxygen or nutrient supply during gestation may alter neonatal vascular responses, the mechanisms involved in these alterations are not yet clear. Neonatal carotid artery constriction in response to increasing concentrations of both phenylephrine and endothelin-1 was lower after chronic maternal hypoxia, while sensitivity to each agonist was unchanged. However, the maximal constriction to endothelin-1 was not significantly different among groups, which may suggest that differences in contractile capacity of carotid arteries did not account for the observed responses. The effect of hypoxia on fetal vascular smooth muscle development is currently unclear. Tension generated to depolarizing KCl was lower in coronary arteries from fetal sheep exposed to long-term, high-altitude hypoxia (11). Consistent with the results of the current study, however, in which no significant effect on carotid wall or lumen size was observed, this treatment appears to have little effect on cerebral artery size or structure in the fetal sheep (26). Acute hypoxia in vitro also slightly decreased the response to depolarizing KCl in chick embryo femoral arteries (40); however, it has previously been demonstrated in chick embryos that chronic hypoxia increased the aortic wall: lumen ratio as a consequence of medial hypertrophic growth (39), which would suggest that conduit artery smooth muscle mass may be increased, at least in some arteries, by chronic hypoxia. However, in our study there was no difference in the wall thickness or wall:lumen ratio in either the carotid or femoral arteries among the groups, suggesting that specific structural changes of the arteries do not account for the observed differences in vasoconstriction.

Maternal nutrient restriction did not reduce carotid artery responses to endothelin-1 in this study, which may indicate that the decreased response was specific to phenylephrine, rather than related to changes in endothelial or vascular smooth muscle function. Although previous authors have investigated the effects of maternal undernutrition on isolated vascular function in resistance-sized arteries from peripheral vascular beds in fetal sheep (32, 35), and adult rat (4, 8, 21), to our knowledge, the effects on carotid artery function have not previously been examined. The increase in femoral artery vasoconstriction after maternal hypoxia treatment was specific to phenylephrine, although the limited data available for endothelin-1 in this vascular bed should be interpreted cautiously.

The sympathetic nervous system has been clearly demonstrated to be involved in the redistribution of cardiac output in response to acute hypoxia in the fetus (14), and it has also been demonstrated that circulating norepinephrine levels are higher in low- than in high-birth weight piglets at 3 mo of age (38). Two recent studies have investigated the effects of chronic hypoxia during incubation on the functioning of peripheral arteries in the chick embryo, and they have demonstrated increased femoral periarterial innervation (41) and increased basal sympathetic tone in mesenteric arteries (39), respectively.

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Our results would suggest an increased responsiveness of peripheral arteries to α1-adrenergic receptor activation after chronic hypoxia in the fetal rat, which is still evident at birth. Although there were no differences in the responses of femoral arteries from offspring of nutrient-restricted dams relative to control in this study, it has previously been demonstrated that nutrient restriction impaired endothelium-dependent and -independent relaxation of resistance-sized arteries from the femoral vascular bed in fetal sheep (32, 35). It is possible that any reductions in femoral blood flow after maternal nutritional restriction affect the functioning of resistance-sized arterioles, but not conduit arteries. Interestingly, it has previously been demonstrated that reduced maternal protein intake produces alterations in the function of small mesenteric arteries, without affecting responses in the thoracic aorta of adult male offspring (45). The differences we observed between the responses of femoral arteries from the offspring of hypoxia and nutrient-restricted dams may reflect underlying differences in the mechanisms mediating redistribution of cardiac output in response to these two treatments. Several possible mechanisms may contribute to the altered regional vascular responses to phenylephrine and endothelin-1 observed in this study, including regional changes in receptor expression, calcium sensitivity, or vascular smooth muscle maturation.

In conclusion, we have demonstrated that both reduced maternal oxygen and nutrient supply during late gestation restricted fetal growth and led to perturbations of neonatal vascular responses to vasoconstrictor agents in the absence of changes in artery wall thickness or wall:lumen diameter ratio. As regional vascular responses were differentially altered by reduced oxygen versus nutrient supply, this study further demonstrates that changes in vascular function at birth are dependent on the specific nature of the insult during development. The regional changes in vascular function observed in this study may be the reason that cardiac output is redistributed during fetal life and thus may have contributed to conserving brain growth during development. Persistent alterations in vascular function in the IUGR neonate may impact cardiovascular regulation in these infants, however, and thus contribute to the development of adverse postnatal outcomes associated with impaired fetal growth. These data also highlight the heterogeneity of vascular effects after impaired fetal growth that may be expected within human populations, in which the underlying causes of fetal growth restriction are diverse.

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