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Maternal Nutrient Restriction Reduces Carotid Artery Constriction Without Increasing Nitric Oxide Synthesis in the Late Gestation Rat Fetus

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ABSTRACT

Chronic reduction in substrate delivery to the fetus may induce redistribution of fetal cardiac output to maintain nutrient delivery to vital organs, including the brain. Reduced vasoconstriction, in conjunction with increased local synthesis of nitric oxide may contribute to "brain sparing." The authors hypothesized that maternal undernutrition would reduce vasoconstrictor responses in fetal carotid arteries due to increased nitric oxide. Timed pregnant Sprague-Dawley rats were randomized on day 0 of pregnancy to control (C) or nutrient restricted (NR) diet. Dams were killed on day 20 of pregnancy. Fetal carotid artery responses were assessed using a pressurized myograph system. Fetal body weight was reduced by NR diet. In NR fetuses, liver, lung, kidney, and heart weights were lower, whereas proportional brain weight was greater. Carotid artery constriction to endothelin-1 was similar in both groups; however, phenylephrine-induced constriction was decreased in NR arteries. Arteries from control fetuses constricted in response to increasing concentrations of L-NAME, whereas arteries from NR did not. There was also no effect of L-NAME on constriction to phenylephrine in arteries from NR fetuses. Our study indicates that the reduced carotid artery vasoconstriction to phenylephrine in NR fetuses, which is consistent with the maintenance of fetal brain blood flow, was not mediated by enhanced nitric oxide. Reduced phenylephrine but not endothelin-1–induced constriction suggests specific effects on adrenergic carotid artery function, which may implicate this pathway in the vascular adaptation to fetal undernutrition. **(*Pediatr Res* 58: 840–844, 2005)**

Abbreviations

eNOS, endothelial nitric oxide synthase
ET-1, Endothelin-1
IUGR, intrauterine growth restriction
L-NAME, N^G-nitro-L-arginine methyl ester
NOS, nitric oxide synthase
NR, nutrient restriction
PE, Phenylephrine

Intrauterine growth restriction (IUGR) involves a failure of the fetus to achieve its genetic potential for growth. Fetal growth restriction has significant impact on pregnancy outcome and neonatal health status where IUGR fetuses demonstrate increased neonatal mortality, morbidity, and prematurity rates (1,2). The pathophysiological processes that result in fetal growth restriction are multiple and diverse; globally one cause of growth restriction is thought to be malnutrition. Human populations studied after acute maternal malnutrition during pregnancy have demonstrated both impaired fetal growth and long-term consequences for adult health (3–7).

Compromised fetuses undergo cardiovascular adaptation to preserve blood flow to vital organs, such as the heart, brain, and adrenal glands, at the expense of peripheral tissues (8,9). This adaptation may involve changes in the regional vascular response to vasoconstrictor, and dilator factors, which serves to redistribute cardiac output. It has previously been demonstrated in fetal sheep that maternal nutrient restriction impaired endothelial-dependent relaxation, and vascular sensitivity to nitric oxide in small arteries from the femoral vascular bed (10,11); however, the effects of undernutrition on the function of arteries supplying blood flow to the brain are not known.

During acute hypoxia, the fetal redistribution of cardiac output, and maintenance of brain perfusion has been demonstrated to be caused in part by nitric oxide (12–14). In both the sheep fetus and newborn pig, inhibition of nitric oxide synthase

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with N^G-nitro-L-arginine methyl ester (L-NAME) during hypoxia has been demonstrated to attenuate changes in blood flow within the cerebral microcirculation (12,13). The circulatory adaptations that occur in the cerebral vascular beds of growth-restricted fetuses may involve specific compensatory adaptations in the nitric oxide synthase pathway. It is not known, however, whether nitric oxide also contributes to maintaining fetal brain blood flow in response to chronic undernutrition.

Although vascular dysfunction in peripheral arteries has been demonstrated in rodent and ovine neonatal offspring after manipulation of maternal diet during pregnancy (10,15,16), the effect of maternal nutrient restriction on fetal carotid vascular responses are not known. A comprehensive understanding of the regional changes in vascular function that may arise after reduced fetal growth is critical to understanding the abnormalities of regional blood flow profiles observed in human IUGR pregnancies (17–19).

We have examined the effect of 50% maternal nutrient restriction throughout pregnancy on isolated carotid artery function in the late gestation rat fetus, focusing specifically on the effect of IUGR on vasoconstrictor responses and nitric oxide modulation of arterial tone. We hypothesized that after maternal nutrient restriction, carotid artery responses to vasoconstrictors would be reduced and nitric oxide modulation of basal tone would be enhanced in the late gestation rat fetus.

METHODS

Animal model. Female Sprague-Dawley rats (aged 12 to 16 wk) were mated. The morning on which sperm were identified in a vaginal smear was denoted as day 0 of pregnancy. From this time point, rats were randomly placed on *ad libitum* (standard lab chow, $n = 7$) or nutrient restriction (12 g per day of standard rat chow which has previously been described to represent approximately 50% of the average daily maternal intake during pregnancy in rats (20), $n = 6$). Rats were weighed on day 0 and day 20 of pregnancy.

Tissue collection. On day 20 of pregnancy (term = 22 d) dams were killed under surgical plane anesthesia (intraperitoneal injection of sodium pentobarbital, [Somnotol, MTC Pharmaceuticals, Ontario, Canada, 42.25 mg/kg body weight]), the abdominal cavity was opened, and the fetuses were rapidly delivered by caesarean section and placed in cold Dulbecco's medium. Dulbecco's medium contained Dulbecco's Modified Eagle medium base (Sigma Chemical Co.), supplemented with 1 mmol/L sodium pyruvate, 25 mmol/L sodium bicarbonate, 5 mmol/L HEPES, 5 mmol/L glucose, and nutrient amino acids and vitamins that improved viability of vessels studied *in vitro* (21).

The first three fetuses delivered by cesarean section from the right uterine horn were weighed after removal of the placenta and surrounding membranes, and after decapitation organs were removed and weighed, the weights from these three fetuses were averaged and represented $n = 1$ for each litter. The first two fetuses from the left uterine horn were delivered, decapitated, and right carotid arteries dissected for assessment of vessel function, experiments were performed on one artery from each pup, and results were averaged. Whereas the carotid artery is a conduit vessel, it has previously been utilized, in species in which cerebral resistance vessels are inaccessible because of limitations of size, to investigate the fetal vascular adaptations to growth restriction (22). This study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Pressure myography. Fetal carotid arteries were dissected clean of connective tissue and fat and using the techniques described by Halpern *et al.* (23). The carotid arteries were cannulated by 60- to 80-μm-diameter glass micropipettes and tied securely at each end. Vessels were mounted within a 2.5-mL organ bath used in conjunction with a pressurized myograph system (Living Systems Instrumentation Inc., Burlington, VT), which maintained isobaric pressure within the artery throughout the experiment through a servo-controlled peristaltic pump. The myograph system bath was filled with Dulbecco's medium (pH 7.4), which was maintained at 37°C with the aid of an in-built microprocessor temperature controller. Medium within the bath was changed at 10-minute intervals throughout the experiment. Arteries were imaged using a video camera, and internal diameter was measured using a video dimension analyser at reference markers to ensure repeated measurements at fixed points.

Experimental protocols. After mounting, the carotid arteries were pressurized to a fixed intraluminal pressure of 10 mm Hg and allowed to equilibrate for a period of 30 minutes. The buffer medium in the arterial chambers was changed at 10-minute intervals. The fixed intraluminal pressure of 10 mm Hg was determined from a series of preliminary experiments where fetal carotid artery viability at fixed resting intraluminal pressures from 6 to 18 mm Hg were assessed by measurement of vessel diameter and constriction to phenylephrine (PE) and endothelin-1 (ET-1). After the equilibration process, dose-response curves were performed to a variety of vasoconstrictor agents.

Briefly after administration of each drug dose to the arterial chamber, the vessels were allowed to stabilize for a period of 4 minutes before measurement of the lumen diameter from two fixed points in the vessel. At completion of each dose-response curve the vessel was washed and allowed to re-equilibrate for 30 min. This protocol was used for assessing vessel responses to the α1 adrenergic receptor agonist PE (10^{-9} to 10^{-5}mol/L), ET-1 (10^{-10} to 10^{-7} mol/L), and the competitive nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-7} to 10^{-4} mol/L). The effect of nitric oxide inhibition on PE induced constriction was assessed only in carotid arteries from NR fetuses after incubation with L-NAME (10^{-4} mol/L) because in control fetuses, the significant constrictor responses to L-NAME alone prevented further investigation of constrictor responses.

Data analysis. Data are summarized as mean ± SEM. Differences between control and nutrient restricted groups in maternal weight gain, litter size, fetal body weight, and fetal organ weight were examined using the *t* test. Two-way analysis of variance (ANOVA) for repeated measures with post hoc testing (Holm-Sidak) for multiple comparisons was used to assess differences in constrictor responses at each vasoconstrictor dose between groups of arteries. Dose-response curves are presented as the percentage vessel constriction from baseline. Statistical significance was set at $p < 0.05$.

RESULTS

At the start of pregnancy, maternal age and weight were similar in both groups. Weight gain during pregnancy was significantly lower in nutrient restricted (NR) dams *versus* control dams (C: 118.8 ± 9.8 g *versus* NR: 38.6 ± 5.01 g, $p < 0.001$). There were no differences observed in litter size between the two groups (C: 13.1 ± 4.4 pups per litter *versus* NR: 13.5 ± 2.1). Fetal body weight was reduced by maternal nutrient restriction (C: 3.93 ± 0.25g *versus* NR: 2.92 ± 0.08 g, $p < 0.001$).

Fetal liver and lung weights were significantly lower in fetuses from nutrient restricted pregnancies ($p < 0.01$), fetal kidney and

Table 1. *Fetal organ weights*

	Control	Nutrient Restriction	
Brain (mg)	178.9 ± 0.3	175.7 ± 0.73	NS
Heart (mg)	26.5 ± 1.3	21.1 ± 1.4	p=0.017
Liver (mg)	324.9 ± 14.8	224.4 ± 9.7	p=0.001
Kidney (mg)	28.0 ± 1.3	22.3 ± 1.8	p=0.022
Lung (mg)	132.8 ± 6.3	100.7 ± 4.6	p=0.002

heart weights were also lower in the NR group ($p < 0.05$, Table 1). No significant differences in brain weights were found between control and restricted fetuses. When fetal organ weights were expressed as a proportion of body weight, relative brain weight was higher in the NR group relative to control (Fig. 1, $p < 0.01$). Proportional liver weight was lower in the NR group (Fig. 1, $p < 0.05$), whereas there were no significant differences in proportional heart (Fig. 1), lung or kidney weights between the NR and control groups (data not shown).

Carotid vascular responses. After equilibration and before commencement of concentration response curves there was no significant difference in the pressurized diameter of carotid arteries from control or NR groups (C: 145.6 ± 9.1 μm *versus* NR: 147.5 ± 11.9 μm). Neither maximal carotid artery vasoconstriction nor sensitivity to ET-1 was significantly different between the two groups (Fig. 2). Vasoconstriction in response to PE, however, was significantly lower in the carotid arteries from NR fetuses compared with controls (Fig. 3, $p < 0.001$). There was no difference in the EC_{50} values for PE-induced vasoconstriction between the two groups (EC_{50}: C: 4.1 ± 1.6 × 10^{-8} mol/L verses NR: 1.4 ± 0.7 × 10^{-7} mol/L).

To determine whether PE-induced vasoconstriction was reduced in carotid arteries from NR fetuses because of enhanced nitric oxide synthesis, we assessed the effects of L-NAME (nitric oxide synthase inhibitor) on vascular function. In arteries from control fetuses, the addition of L-NAME resulted in significant vasoconstriction. We therefore assessed the contribution of nitric oxide to basal arterial tone by performing a concentration-response curve to L-NAME. Increasing L-NAME concentrations induced vasoconstriction in the control group but had little effect in the NR group. The effect of L-NAME in carotid arteries from the control group was significantly greater than NR fetuses (Fig. 4, $p < 0.001$), demonstrating that basal nitric oxide production is an important regulator of vascular tone in carotid arteries of control, but not NR fetuses.

To confirm that nitric oxide did not mediate the reduced constriction to PE in carotid arteries from NR fetuses, vasoconstriction to PE in carotid arteries from NR fetuses was assessed in the presence or absence of L-NAME (10^{-4} mol/L). L-NAME produced no significant change in the PE-induced vasoconstrictor response in this group (Fig. 5). PE-induced vasoconstriction was not assessed using this protocol in the control group as L-NAME induced marked vasoconstriction in carotid arteries from this group, (as demonstrated in Fig. 4), meaning that further constriction in response to PE was not measurable.

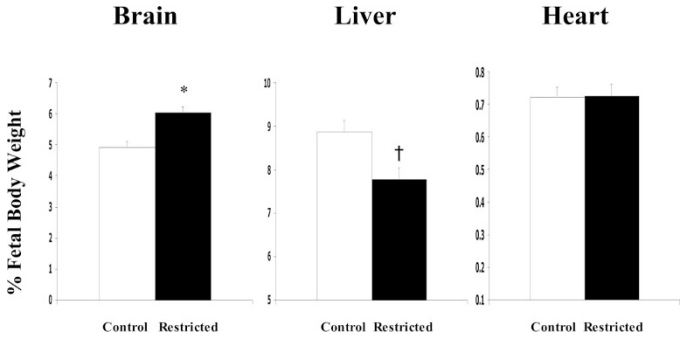

Figure 1. Proportional organ weights. Proportional brain weight was greater in nutrient restricted (black bars) compared with control (open bar) fetuses (*$p < 0.01$). Proportional liver weights were lower in nutrient restricted fetuses compared with controls (†$p < 0.05$). There were no differences between the groups in proportional heart weight.

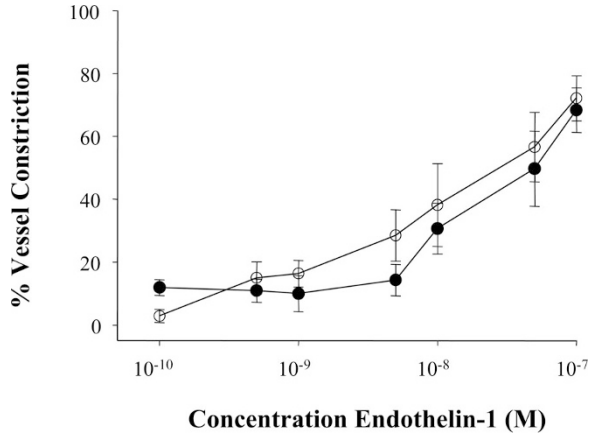

Figure 2. Carotid artery constrictor responses to endothelin-1. There was no significant difference in the magnitude of the carotid artery constrictor response to endothelin-1 between nutrient restricted (black circles, $n = 4$) and control (open circles, $n = 4$) fetuses.

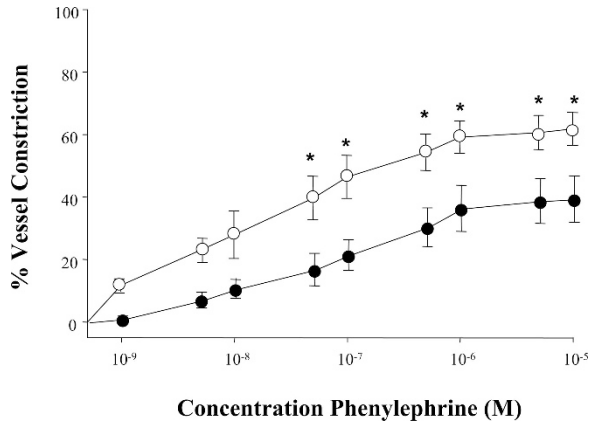

Figure 3. Carotid artery constrictor responses to phenylephrine. The carotid artery response to phenylephrine was significantly blunted in arteries from nutrient restricted fetuses (black circles, $n = 6$) compared with control (open circles, $n = 7$). $p < 0.001$, *$p < 0.05$ nutrient restricted *vs* controls, Holm-Sidak posthoc testing.

DISCUSSION

In this study we have demonstrated that global maternal nutrient restriction to 50% of control intake during pregnancy reduced fetal body weight by 21%. The changes in organ proportions in the nutrient restricted fetuses, a significant increase in relative brain weight in association with a significant decrease in relative liver weight, are consistent with a pattern of circulatory redistribution aimed at preservation of brain growth. These findings are consistent with published data demonstrating that alterations in feto-maternal nutrition either by isocaloric protein restriction or by global caloric restriction during pregnancy induces growth restriction with relative brain sparing in the offspring (20,24,25).

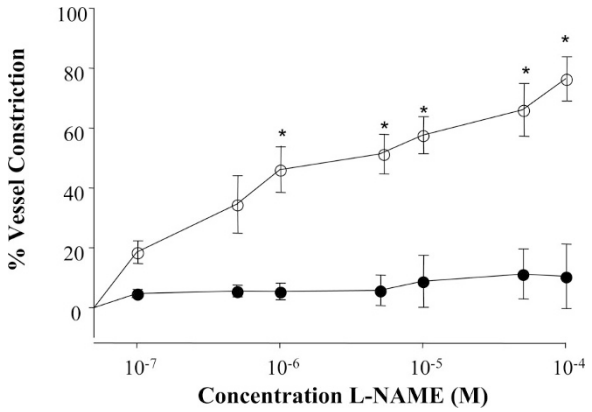

Figure 4. Carotid artery constrictor response to L-NAME. Increasing concentrations of L-NAME induced marked vasoconstriction in the control group (open circles, $n = 4$), but had little effect in the nutrient restricted group (black circles $n = 4$), $p < 0.001$, $*p < 0.05$ control *vs* nutrient restricted, Holm-Sidak posthoc testing.

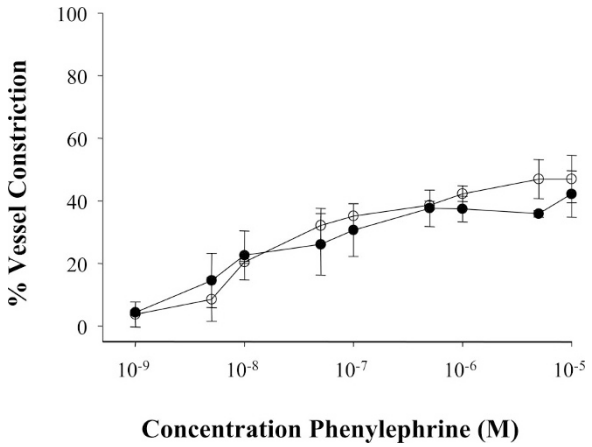

Figure 5. Effect of L-NAME incubation on phenylephrine induced carotid constriction in restricted fetuses. This figure shows the response to phenylephrine, as measured by the change in carotid artery diameter from baseline diameter in the presence or absence of L-NAME. There was no change ($p > 0.05$) in the nutrient restricted carotid artery constrictor response to phenylephrine before (open circles, $n = 3$) and after (black circles, $n = 3$) incubation with L-NAME (10^{-4}mol/L).

In association with these changes in fetal growth, our results demonstrate that maternal nutrient restriction during pregnancy alters fetal carotid artery function. Carotid artery constriction to the α1-adrenergic agonist PE was attenuated in nutrient restricted fetuses. This effect is consistent with vascular adaptation aimed at the conservation of brain blood flow. The reduced vasoconstrictor response to PE was not a reflection of an overall decreased constrictor capacity in the nutrient restricted group as increasing doses of the vasoconstrictor ET-1 induced marked vasoconstriction in carotid arteries from both groups with no difference in the magnitude of this constrictor response. These data suggest that nutrient restriction specifically attenuates α1-adrenergic constrictor responses in the carotid artery. Interestingly, an agonist for this receptor, norepinephrine, has been shown to be increased in fetal sheep plasma when pregnant ewes were fasted (26). Circulating norepinephrine concentrations during an acute stress *in utero* were also greater in chronically hypoglycemic fetal sheep than control (27). The reduced carotid artery responsiveness to α1-adrenergic receptor activation observed in this study may therefore be an important fetal adaptation to maternal undernutrition to maintain blood flow to the brain despite increases in circulating norepinephrine.

The mechanisms involved in reducing the constrictor response to PE in nutrient restricted fetuses may be independent of alterations in the nitric oxide pathway. Nitric oxide production was previously demonstrated to be increased in the fetal guinea pig carotid artery after a chronic reduction in maternal oxygen supply (22). In our study, increasing L-NAME (NOS inhibitor) concentrations produced a marked contractile response in carotid arteries from control fetuses, demonstrating an important role for nitric oxide modulation of basal tone in the fetal rat carotid artery. In contrast to our hypothesis, however, fetal carotid arteries from nutrient restricted dams demonstrated significantly reduced nitric oxide modulation of basal tone. Furthermore, L-NAME did not alter the PE response of carotid arteries from nutrient restricted fetuses, indicating that the attenuated α1-adrenergic constrictor response in this group was not a consequence of increased modulation by the vasodilator nitric oxide. The changes in vascular responses to PE observed may be related to enhanced production of other vasodilators such as prostaglandins or endothelial-derived hyperpolarizing factor, or may relate to decreased α1-adrenergic receptor expression. Further studies are warranted to understand the mechanisms producing these effects.

The reduced constriction in response to PE, with no change in the response to ET-1 in NR fetal arteries in this study may further suggest that nutrient restriction specifically alters adrenergic, but not ET-1 dependent carotid artery function. ET-1 may act *via* ET_A receptors on vascular smooth muscle cells to induce constriction (28), while simultaneously acting *via* ET_B receptors on the endothelium to induce vasodilation (29). A difference in the ET-1 response may therefore reflect a shift in the balance of constrictor to dilator effects. In this study there was no difference in the response to ET-1 in carotid arteries from control or NR groups, although the L-NAME data clearly demonstrate that nitric oxide modulation of vascular tone is greater in the control than NR fetus. The similar responses to ET-1 in the two groups may therefore imply that endothelial ET-1 receptors do not play a significant role in the fetal carotid artery. Alternatively, the endothelial component of the response to ET-1 may be mediated by non-NOS–dependent mechanisms that are unaffected in NR fetuses. The latter is unlikely, because the substantial NOS contribution to basal artery tone in the control offspring suggests that NOS also contributes substantially to endothelial function in this group. It has recently been reported, however, that big endothelin-1 (ET-1 precursor) increased plasma prostaglandin F1α concentrations, but not nitrite/nitrate levels in the fetal sheep (30). Experimentally, one method to address this issue is the removal of the endothelium; however the delicacy of the fetal rat carotid arteries prevented this approach in the current study.

Overall, few studies have investigated the effects of maternal undernutrition on fetal vascular function. Two studies have determined the effects of maternal undernutrition on the functioning of a peripheral vascular bed in fetal sheep during mid

and late gestation (10,11). The authors demonstrated that undernutrition impaired endothelial-dependent relaxation without affecting constriction to norepinephrine in branches of the femoral artery (10,11). The response to exogenous nitric oxide was also reduced in this study after nutrient restriction. Our results demonstrate that in fetal carotid arteries both constriction to PE, and nitric oxide modulation of vascular tone was reduced by dietary restriction. We speculate that reduced constriction to α1-adrenergic receptor activation in the carotid artery, but not in peripheral vascular beds may represent one mechanism involved in mediating the redistribution of cardiac output in the compromised fetus. Interestingly, it appears that maternal undernutrition may impair nitric oxide modulation of vascular responses both within vessels supplying brain blood flow, and in peripheral vessels.

The reduced nitric oxide contribution to carotid artery tone in the NR fetus may reflect reduced production, release, or vascular smooth muscle sensitivity to nitric oxide. Several possible factors may be involved in this reduced nitric oxide contribution, including a decreased L-arginine availability, resulting from decreased dietary intake with global nutrient reduction. This may contribute *in vivo*; however, all the *in vitro* vascular function experiments utilized a buffered culture medium containing L-arginine, making this a less likely explanation. It has previously been reported that undernutrition in pregnant sheep reduced sensitivity to the nitric oxide donor sodium nitroprusside in femoral artery branches from both mid (11) and late gestation fetal lambs (10). Although a similar decrease in vascular smooth muscle sensitivity to nitric oxide may be involved in the current study, differences in femoral *versus* carotid artery function may also be significant. The effects of maternal undernutrition on fetal carotid artery function have not previously been examined. Interestingly, chronic maternal hypoxia increased the nitric oxide contribution in carotid arteries from fetal guinea pigs (22). The differential effects of nutrient restriction in the current study suggest that the vascular mechanisms maintaining fetal brain blood flow in compromised pregnancies may differ depending on the *in utero* environment. One such mechanism that may be involved is a change in the expression of NOS. It has been demonstrated that the expression of mRNA for endothelial NOS is lower in aorta from adult male offspring of undernourished dams (31). The effects of maternal undernutrition on NOS expression in fetal arteries are currently unknown, however investigation of these effects through future studies may be warranted based on the current data.

In summary, we demonstrate that global maternal nutrient restriction during pregnancy alters fetal growth with a decrease in body weight and an alteration in the proportion of fetal organ weight consistent with a pattern of preservation of brain growth. Carotid artery vascular function demonstrates blunted constrictor responses to the α1 adrenergic agonist PE, concurrent with a decrease in nitric oxide modulation of basal tone. Improved understanding of the factors contributing to the alterations in regional vascular tone in fetal IUGR will assist in design of appropriately targeted therapeutic interventions.

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