Chronic Hypoxia Alters Vasoconstrictive Responses of Femoral Artery in the Fetal Sheep

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The purpose of this study was to determine if mild hypoxia alters the responsiveness to vasoactive agents in the renal and the femoral arteries in the fetal sheep. Ten pregnant sheep were operated under halothane anesthesia at 116 to 124 days' gestation. A maternal tracheal catheter was placed for infusing compressed air (control group, n=5) or nitrogen (hypoxia group, n=5) starting on post operative day 6 and maintained for 5 days. Femoral and renal arteries were harvested from the fetus to study the constriction response to phenylephrine (PE 10^{-9} to 10^{-5} mol/L). To determine the involvement of nitric oxide as a modulator of vessel constriction, N-nitro-L-arginine methyl ester (L-NAME) was used at a concentration of 10^{-4} mol/L in parallel chambers. In the hypoxia group, maternal Pao_2 significantly decreased from a baseline of 110.4 ± 1.4 to 80.5 ± 1.6 (mmHg, $p<0.01$), fetal Pao_2 significantly decreased from a baseline of 20.9 ± 0.3 to 15.5 ± 0.1 (mmHg, $p<0.01$). Hypoxia was associated with a significant increase in PE maximal response in the absence (184.5 ± 6.6 vs. 146.2 ± 4.3) and presence (166.9 ± 6.3 vs. 145.0 ± 4.5) of L-NAME, and a decrease in EC_{50} in the absence (6.0 ± 1.1 vs. 27.0 ± 4.1) of L-NAME of femoral arteries. However, there were no significant differences in PE maximal response and EC_{50} in the absence and presence of L-NAME of renal arteries. We concluded that mild chronic hypoxia seems to increase the fetal femoral artery response to PE, but not in the fetal renal artery. This observation is consistent with a redistribution of cardiac output away from the carcass.

Key Words : *Anoxia; Femoral artery; Blood Vessels; Phenylephrine*

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INTRODUCTION

The fetus differs from the adult in that it increases blood flow to different organs during growth by adjusting the distribution of its cardiac output (1). During generalized fetal asphyxia, there is a significant redistribution of blood flow away from non-essential organs to maintain flow to the brain, the heart and the adrenals. This is thought to occur as a result of a altered response to sympathetic alpha receptors (2). Animal experimental data have shown that in the presence of hypoxia, there is a reduction in blood flow to visceral organs, muscle and skin while preserving flow to the heart, the brain, and the adrenals and away from non-essential organs such as the carcass, the skin and limbs (2, 3). The exact mechanism is not fully understood. Blood flow could be redistributed as a result of a selectively increased sensitivity of different vascular beds to vasoconstrictive agents, or a decrease response to vasodilatory agents. The aims of this study were: 1) to determine if maternal oxygen modification using a maternal tracheal infusion of nitrogen could be carried out safely for a five day period, 2) to determine the effect of mild hypoxemia on fetal and maternal pH, blood gases, and hematocrit, as well as fetal glucose, and lactate, 3) to determine if mild hypoxia alters the responsiveness to vasoconstrictive agents in the renal and the femoral arteries of the fetal sheep, and 4) to determine if this response is affected by the nitric oxide (NO) synthase inhibitor (N-nitro-L-arginine methyl ester, L-NAME).

MATERIALS AND METHODS

Animal preparation

All the procedures for housing, handling, surgical implantation of catheters, and postoperative management were approved by Wake Forest University's Institutional Care and Use Committee. Ten pregnant sheep (average weight 60 kg) were operated at 116 to 124 days' gestation (term, 145 days) under 0.75% halothane general anesthesia. Polyvinyl catheters were placed in the maternal carotid artery, jugular vein, and trachea. The tracheal catheter (3.5 mm outer diameter and 2.5 mm inner diameter) was introduced in the direction of the lungs to a distance of 15 cm into the trachea from a point approximately 2 to 3 cm below the cricoid (4). Polyvinyl catheters

were introduced into the fetal carotid artery, jugular vein, and amniotic cavity as previously described (5). All catheters were exteriorized through the flank of the ewe. All the animals received ampicillin (1 g intravenously) and gentamicin (80 mg intravenously) once postoperatively and were allowed to recover for 5 days.

Experimental protocol

The experimental protocol began on the sixth day after surgery and continued for a period of 5 days. On the day before the experiment (day 0), no gas was infused into the maternal trachea. On the first experimental day (day 1), either compressed air (control, n=5) infused at a rate of 4.5 L/min or nitrogen (hypoxia, n=5) were infused through the tracheal catheter. Nitrogen flow was titrated (3-6 L/min) in order to achieve a 25% drop in the fetal Pao_2 without causing fetal acidosis. Maternal hypocarbia due to hyperventilation was avoided by the simultaneous infusion of CO_2 at a rate of 0.25-0.5 L/min to the tracheal catheter. Blood samples were obtained at 30-min intervals for 3 hr and at 6 hr after initiation of nitrogen gas infusion. Blood gases were measured in the morning and evening on each subsequent day. Throughout the experiment, fetal and maternal heart rate, fetal and maternal blood pressure, and amniotic cavity pressure were continuously monitored. Heparinized arterial blood samples (1 mL) were obtained for measurement of pH, blood gases, and hematocrit. Additional venous samples (1.5 mL) were obtained for measurement of plasma glucose and lactate. The blood gases were measured by means of a Radiometer (Copenhagen ABL5™, Skanderborg, Denmark) maintained at a temperature of $39°C$. Hematocrit was measured by an Adams Readacrit micro-hematocrit centrifuge (Clay Adams, Inc., Chatsworth, California, U.S.A.). Plasma glucose and lactate were measured by specific enzymatic spectrophotometric method using glucose and lactate reagent (Sigma Diagnostics, St. Louis, Missouri, U.S.A.).

Tissue collection

On experimental day 6, the fetuses were removed by cesarean section under halothane anesthesia and the ewe euthanized. The fetal sheep were sacrificed by injecting a lethal dose of pentobarbital sodium (50 mg) into the umbilical vein. Fetal renal and femoral arteries were collected, placed in ice-cold saline solution $(4°C)$ and carefully dissected free of visible adventitia.

Arterial ring preparation

The femoral artery and the first branch of the renal artery proximal to the first bifurcation were cut into rings of 3 mm long and suspended by use of two stainless steel wires that were carefully inserted in the lumen of the ring. Each ring

was mounted in 20 mL organ chambers (Radnoti, Monrovia, CA, U.S.A.) filled with modified Krebs-Henseleit solution composed of (mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, and 11.1 glucose, containing 10^{-5} mol/L indomethacin warmed at $37°C$ and gassed with 95% oxygen and 5% carbon dioxide. In our experiments, we used indomethacin in the arterial ring to eliminate any contribution of locally produced prostaglandins. Isometric tension was measured continuously with a force transducer and recorded on a Grass Instrument Co. (Quincy, Massachusetts, U.S.A.) No.7 polygraph. Before the start of the experiment each chamber was washed three times and the rings were allowed to equilibrate for at least 60 min. The rings were stretched to their optimum length-tension relationship by repeated stimulation with potassium chloride (40 mM/L) over a 1-hr period. To study the constriction response, the vessel was progressively constricted using phenylephrine (PE 10^{-9} to 10^{-5} mol/L). To determine the involvement of nitric oxide as a modulator of vessel constriction, L-NAME was used at a concentration of 10^{-4} mol/L in parallel chambers. The femoral and renal arteries from the same animals were studied concurrently. Each vessels had a recorded response to KCl (KCl_{max}) which was used to index the maximum response to PE. The percent maximal response to each dose of PE and the EC_{50} (the PE dose yielding 50% of the maximum contraction) were calculated from computer generated log curves. EC_{50} values were derived from nonlinear least square regression analysis. Vessels were analyzed and grouped by normoxic or hypoxic conditions, and the presence or absence of L-NAME.

Statistical analysis

Data are presented as mean±SEM throughout the article. Comparisons over the entire experimental time were performed by using repeated-measures analysis of variance and the Student-Newman-Keuls methods. The response to PE is expressed as the percent of the KCl-induced maximum constriction. EC_{50} was derived from the "linear portion of the sigmoidal response curve" using Sigma Plot Scientific Graph System (Jandel Scientific, San Rafael, California, U.S.A.). Comparisons between hypoxia and control groups for all variables in vascular response were performed by using a paired t test. A p value of <.05 was considered statistically significant.

RESULTS

Maternal blood gases, pH, and hematocrit

As shown in Table 1 there was no significant difference in maternal baseline Pao_2 between the "control" group and the "hypoxic" group (111.4±1.8 vs. 110±1.4 mmHg respectively). Maternal Pao_2 in the group receiving the continuous nitrogen infusion significantly decreased from a baseline value

of 110 ± 1.4 to a value of 81.4 ± 4.4 mmHg at day 3 ($p<0.01$) and 76.0 ± 3.9 mmHg at day 5 ($p<0.01$).

Maternal $Paco_2$ in the two groups was not different at baseline (36.5 ± 0.8 vs. 35.7 ± 0.6 mmHg) or during the study (Table 1). Similarly, maternal pH was not significantly different at baseline between the two groups (7.47 ± 0.01 vs. 7.47 ± 0.01), at day 3 (7.46 ± 0.02 vs. 7.47 ± 0.02) or at day 5 (7.44 ± 0.01 vs. 7.47 ± 0.01) control versus hypoxia respectively.

No differences in maternal hematocrit between the two groups were observed at baseline (27.0 ± 3.1 vs. $27.4\pm2.1\%$) or during the study period (Table 1).

Maternal plasma glucose and plasma lactate

Maternal plasma glucose levels were not different during the study period between groups at baseline (59.0 ± 7.1 vs. 47.2 ± 3.8 mg/dL). During the experimental period mater-

nal plasma glucose levels were not different at day 3 (59.8 ± 3.4 vs. 54.9 ± 3.5 mg/dL) or at day 5 (60.7 ± 4.6 vs. 56.4 ± 3.5 mg/dL). In either the controls or the hypoxic group maternal plasma glucose levels did not change significantly. Maternal plasma lactate levels were not different between the two groups at baseline (6.12 ± 0.10 vs. 4.63 ± 0.85 mg/dL) or during the study (Table 1).

Fetal blood gases, pH and hematocrit

Fetal Pao_2, $Paco_2$, pH, and hematocrit were not significantly affected during the experimental period in control group. At baseline there was no difference in either the Pao_2, $Paco_2$, pH, or hematocrit between the controls and the hypoxic group. In the hypoxic group, values for fetal Pao_2 at day 3 (15.4 ± 0.5 mmHg) and day 5 (15.5 ± 0.4 mmHg) were significantly lower than fetal Pao_2 obtained at baseline for that group (20.9 ± 0.3 mmHg; $p<0.01$). Fetal $Paco_2$, pH, and hematocrit were not

Table 1. Maternal arterial blood gases, hematocrit, and metabolites in control and hypoxic fetal sheep

	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day of necropsy	
	C	H	C	H	C	H	C	H	C	H	C	H	C	H
Pao_2, mmHg	111.4± 1.8	110.4± 1.4	112.1± 3.5	80.1± 3.1*	111.5± 3.2	83.4± 3.3*	116.3± 3.0	81.4± 4.4*	115.9± 3.8	83.0± 3.7*	116.2± 4.4	76.0± 3.9*	114.6± 1.5	80.5± 1.6*
$Paco_2$, mmHg	36.5± 0.8	35.7± 0.6	37.9± 1.5	35.7± 0.9	37.6± 1.4	35.5± 0.8	36.5± 1.4	35.6± 0.9	36.1± 0.7	34.8± 0.7	37.3± 1.0	35.2± 0.6	37.0± 0.5	35.4± 0.2
pH	7.47± 0.01	7.47± 0.01	7.46± 0.02	7.47± 0.01	7.46± 0.02	7.48± 0.01	7.46± 0.02	7.47± 0.02	7.45± 0.01	7.47± 0.02	7.44± 0.01	7.47± 0.01	7.45± 0.01	7.47± 0.01
Hct, %	27.0± 3.1	27.4± 2.1	27.0± 2.5	27.8± 2.0	26.5± 2.6	28.8± 1.8	26.3± 2.7	27.6± 1.8	25.7± 3.2	27.9± 2.2	25.5± 3.0	28.1± 1.9	26.3± 1.0	28.2± 0.8
Lactate, mg/dL	6.12± 0.10	4.63± 0.85					4.66± 0.59	6.85± 0.89			5.50± 0.85	5.75± 0.41	5.09± 1.09	6.30± 0.50
Glucose, mg/dL	59.0± 7.1	47.2± 3.8					59.8± 3.4	54.9± 3.5			60.7± 4.6	56.4± 3.5	60.3± 2.8	55.6± 2.4

Values are mean±SE. C, Control; H, Hypoxic group; Hct, Hematocrit.
*$p<0.05$ repeated-measures analysis of variance and Student-Neuman-Keuls test.

Table 2. Fetal arterial blood gases, hematocrit, and metabolites in the control and hypoxic groups

	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day of necropsy	
	C	H	C	H	C	H	C	H	C	H	C	H	C	H
Pao_2, mmHg	19.8± 0.7	20.9± 0.3	20.0± 0.5	15.5± 0.2*	20.5± 0.5	15.6± 0.3*	19.1± 0.4	15.4± 0.5*	19.0± 1.0	15.7± 0.3*	19.1± 0.3	15.5± 0.4*	19.6± 0.2	15.5± 0.1*
$Paco_2$, mmHg	50.1± 1.0	48.5± 0.5	50.8± 1.4	48.0± 0.7	50.8± 1.2	48.2± 0.6	50.9± 1.1	47.1± 0.8	51.2± 1.1	48.2± 0.8	51.1± 1.0	49.5± 0.6	50.9± 0.5	48.2± 0.3
pH	7.34± 0.01	7.36± 0.01	7.34± 0.01	7.36± 0.01	7.35± 0.02	7.36± 0.01	7.34± 0.01	7.39± 0.01	7.35± 0.01	7.36± 0.00	7.34± 0.01	7.35± 0.01	7.34± 0.00	7.36± 0.00
Hct, %	31.5± 2.8	34.6± 0.6	30.0± 2.5	34.0± 0.5	30.0± 2.1	34.2± 0.8	30.5± 1.8	35.2± 1.0	30.0± 0.6	34.4± 1.5	30.8± 2.1	35.1± 2.1	30.3± 0.6	34.7± 0.5
Lactate, mg/dL	14.5± 0.7	13.0± 0.8					14.2± 1.2	22.6± 2.6*			18.2± 0.9	28.8± 2.8*	16.2± 0.9	25.7± 2.0*
Glucose, mg/dL	14.3± 2.9	14.2± 0.8					16.9± 2.2	18.7± 1.7			16.1± 2.2	16.2± 1.3	16.9± 1.5	17.4± 1.1

Values are mean±SE. C, Control; H, Hypoxic group; Hct, Hematocrit.
*$p<0.05$ repeated-measures analysis of variance and Student-Neuman-Keuls test.

significantly different at day 3 and at day 5 from baseline during maternal infusion of nitrogen (Table 2).

Fetal plasma glucose and plasma lactate

There were no significant differences in the fetal plasma glucose and lactate levels between the control and the hypoxic groups at baseline (Day 0). In the control group, plasma glucose and lactate levels did not significantly change on day 3 or day 5. In the hypoxic group, fetal plasma lactate levels rose significantly from 13.0 ± 0.7 at baseline to 22.6 ± 2.6 (mg/dL; $p<0.01$) on day 3 and continued to increase at day 5 (28.8

Table 3. Responsiveness of isolated fetal sheep renal and femoral arteries to phenylephrine. Arteries were studied with and without L-NAME 10^{-4} M in the bath. Contractile response is expressed as percentage of KCl maximal response for each individual vessel

Artery	L-NAME	Maximal response		EC$_{50}$ ($\times 10^{-8}$ M)	
		Control (n=5)	Hypoxia (n=5)	Control (n=5)	Hypoxia (n=5)
Renal	Without	110.9 ± 2.5	119.7 ± 2.5	29.1 ± 3.1	35.5 ± 3.0
Femoral	Without	146.2 ± 4.3	$184.5 \pm 6.6*$	29.8 ± 3.9	34.4 ± 4.7

*$p<0.01$.

Table 4. esponsiveness of isolated fetal sheep renal and femoral arteries to phenylephrine. Arteries were studied with and without L-NAME 10^{-4} M in the bath. Contractile response is expressed as percentage of KCl maximal response for each individual vessel

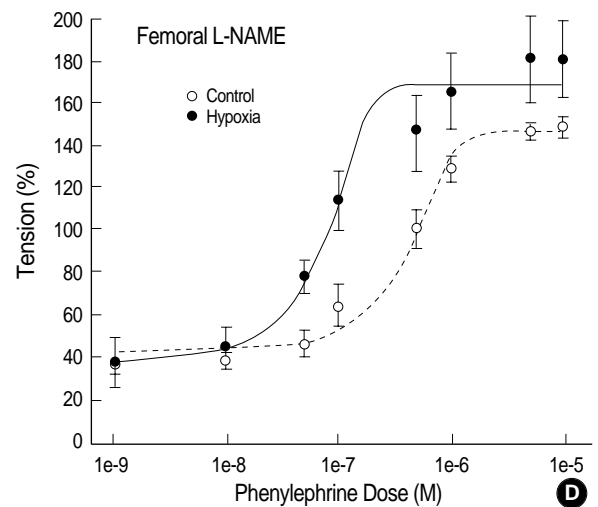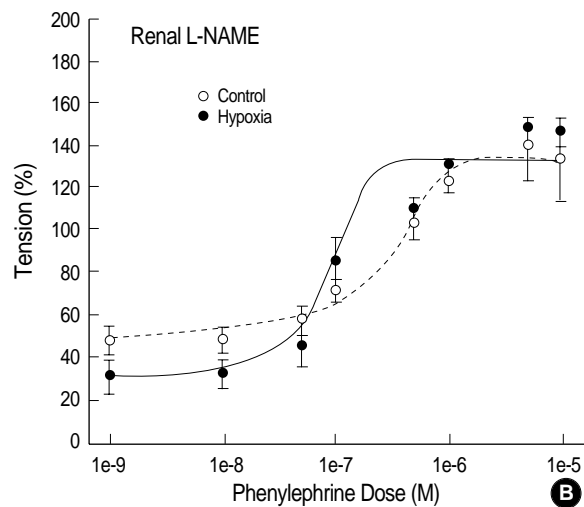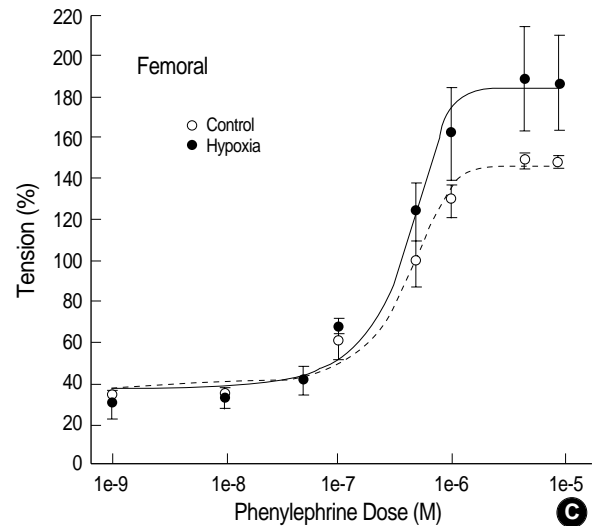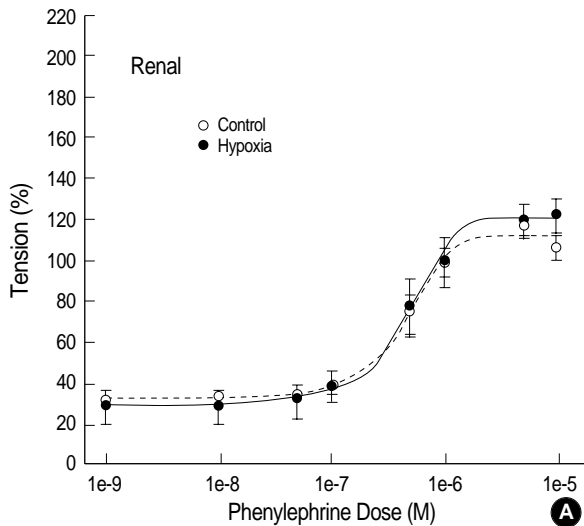| Artery	L-NAME	Maximal response		EC$_{50}$ ($\times 10^{-8}$ M)	
		Control (n=5)	Hypoxia (n=5)	Control (n=5)	Hypoxia (n=5)
Renal	With	134.5 ± 3.7	133.4 ± 7.3	14.9 ± 3.3	7.5 ± 1.8
Femoral	With	145.0 ± 4.5	$166.9 \pm 6.3*$	27.0 ± 4.1	$6.0 \pm 1.1^{\dagger}$

*$p<0.05$; $^{\dagger}p<0.01$.

Fig. 1. Concentration-effect curves for phenylephrine (PE 10^{-9} to 10^{-5} mol/L) in isolated fetal sheep renal and femoral arteries obtained from normoxic (control group n=5) and hypoxic fetuses (hypoxia group n=5). Arteries were studied with and without L-NAME in the bath. Results are expressed as a percentage of the maximal response to KCl in each vessel and presented as mean±SEM.

± 2.8 mg/dL; $p<0.01$). Fetal plasma glucose levels did not significantly change on day 3 or day 5 in the hypoxic group (Table 2).

Effects of hypoxia on fetal renal and femoral artery constriction by phenylephrine

There was a significant increase in the femoral artery maximal response to PE in the absence (184.5 ± 6.6 vs. 146.2 ± 4.3) and presence (166.9 ± 6.3 vs. 145.0 ± 4.5) of L-NAME in the hypoxic group (Table 3, 4). As shown in Fig. 1, this difference was evident whether or not the vessel had been pretreated with the NOS inhibitor, L-NAME. However, there were no significant differences in PE maximal responses of renal arteries between the control and hypoxia groups in the presence or absence of L-NAME. A significant left-shift in the femoral artery dose response relationship to PE was also observed in the hypoxic group when pretreated with L-NAME in Fig. 1. As shown in Table 4, the PE EC_{50} in the hypoxic group was significantly lower than that of the control group in the presence of L-NAME (6.0 ± 1.1 vs. $27.0\pm4.1\times10^{-8}$ M; $p<0.01$). There were no significant differences of PE EC_{50} in renal and femoral arteries in the absence of L-NAME between control and hypoxia group (Table 3).

DISCUSSION

We found that five days of mild fetal hypoxemia using maternal tracheal administration of nitrogen is well tolerated by the mother and the fetus. At the onset of the nitrogen infusion, we observed a mild decrease in maternal $Paco_2$ as the mother hyperventilated in response to the fall in Pao_2. This decrease was easily adjusted by adding CO_2 to the nitrogen infusion, and values were not different than those seen at baseline. In this protocol, we aimed to decrease fetal Pao_2 by 25% which achieved and was well tolerated by the mother. Towell et al. (6) investigated the response to a decrease in fetal arterial Pao_2 of 5 mmHg or less sustained for a period of 24 hr in the chronically instrumented fetal lamb. They showed that the fetal lamb at 122 to 139 days' gestation was highly sensitive to small decrements in Pao_2 of 5 mmHg or less. Fetal hypoxemia was not associated with any change in fetal pH. During exposures to hypoxemia, fetal plasma lactate and cortisol were increased above preliminary values. The normal range of blood gases and acid-base balance in fetal sheep of 120-126 days' gestation were Pao_2 21.4 ± 0.9 mmHg, Pco_2 46.0 ± 0.9 mmHg, pH 7.36 ± 0.01 (7). We found that mild hypoxia did not significantly affect maternal and fetal pH, nor did it affect fetal $Paco_2$. There was no significant change in fetal glucose plasma concentration during the entire experiment. Plasma lactate levels progressively increased in the hypoxic fetuses from baseline to a maximum on day 5. This finding is consistent with that of Towell et al. (6). Thus, despite

the absence of significant changes in fetal pH and $Paco_2$, the fetus had a reduction in oxygen availability sufficient to cause anaerobic metabolism and lactate production. Maternal lactate plasma levels were not significantly increased in the controls or the hypoxic animals suggesting that the elevation in fetal plasma lactate levels was not due to transport of maternal lactate across the placenta. We cannot eliminate the possibility that placental lactate production was increased and thus contributed to the observed increase in fetal lactate levels. We found that this simple method has the major advantage of causing mild fetal hypoxia while the mother is eating and drinking freely without obvious discomfort during the entire course of nitrogen infusion. Selected specimens of maternal trachea were examined at the time of necropsy. There were only minimal areas of inflammation at the site of the catheter into the trachea and no signs of granulation tissue or obvious infection were noted. We consider that the tracheal nitrogen administration is a simple experimental tool to cause mild fetal hypoxia that can potentially be carried out for much longer study periods. We suggest that this model could be helpful to study the effect of long term mild hypoxemia on the cardiovascular system in studies related to fetal programming. Fetal programming is an area of great interest and this model of prolonged mild hypoxemia may well serve as a model to study the growth impaired fetus during infancy and during adult life.

Pregnancies that are complicated with chronic hypoxia are associated with several perinatal problems including fetal growth restriction and increased mortality and morbidity (8-10). The growth restriction is asymmetric with brain and heart weight being reduced less than other organs. This asymmetry has been ascribed to redistribution of fetal cardiac output during hypoxia favoring the brain and heart. Redistribution of blood flow may occur as a result of altered response to sympathetic alpha receptors (2). We found that mild hypoxia increased the sensitivity to PE in the femoral artery but not in the renal artery. This increase in contractile response seemed to be part due to alteration in NO production as the presence of the nitric oxide synthase inhibitor L-NAME did significantly affect the response to PE under basal and hypoxic condition. The contractile response to PE when L-NAME was added to the bath would suggest that NO production and or release may be involved in the fetal femoral arteries. The effect of hypoxia on the expression of the endothelial cell isoform of NOS (eNOS) has been evaluated in cultured endothelial cells. The results of these studies are however conflictive. In endothelial cells from human umbilical vein, hypoxic incubation inhibits eNOS expression (11) whereas it is associated with increase in both eNOS protein and mRNA (12) in endothelial cells from bovine aorta. Vascular reactivity to the sodium nitroprusside was increased by lowering Pao_2 in the tissue bath. Sensitivity also increased after endothelium denudation and L-NAME treatment, suggesting that endothelium-derived nitric oxide inhibits nitrovasodilator relaxation in

isolated rat aortas (13). In fetal guinea pigs, acute hypoxia inhibited endothelium-dependent relaxation in response to acetylcholine and A23187, and increased sensitivity to sodium nitroprusside. Chronic hypoxia inhibited maximal relaxation of arteries in response to acetylcholine but not relaxation of arteries in response to sodium nitroprusside with respect to relaxation seen in arteries from normoxic fetuses. They concluded that hypoxia may alter relaxation of fetal arteries by decreasing the availability of oxygen for nitric oxide production and causing vascular adaptations related to altered nitric oxide release (14).

The present results are consistent with the result found by Millard et al. (15) in the fetal lamb during acute hypoxia. In that experiment, acute fetal hypoxia was induced by administering a mixture of 10% oxygen in nitrogen to the mother for 10 min. They showed that during hypoxia, there was an immediate vasoconstriction of the iliac vessels whereas the renal bed did not constrict initially, and in some experiments, the renal bed actually dilated in response to acute hypoxia. After blockade of prostaglandin synthesis, the renal vascular bed behaved like the iliac bed, showing an immediate and marked vasoconstriction. Even under this protocol, 5 days of mild fetal hypoxia without hypocarbia and alkalosis, did not alter the responsiveness of these renal vessels to PE. It is possible that greater degree of hypoxia could change the responsiveness of these vessels to vasoconstrictive agents such as PE. Other factors, such as endothelial-derived hyperpolarizing factors or endothelin-1 which we have not tested could potentially be involved and affect one particular vessel more than the others. Exposure to normobaric hypoxia has previously been shown to increase levels of the endogenous vasoconstrictor endothelin in plasma and aorta of Sprague-Dawley rats (16).

In the present study, the increased contractile response to PE by the femoral artery in the hypoxic fetuses is different from what has been reported in nonpregnant rats exposed to chronic hypoxia (17). Studies of the effects of chronic hypoxia on in vivo systemic vasoreactivity have yielded conflicting data. Aoki and Robinson (18) found augmented pressor responses to adrenoreceptor agonists in the hindlimb of chronically hypoxic rats. In contrast, Jin et al. (19) have demonstrated impaired responses to vasopressin infusion in hypoxia-adapted rats. Fukuda et al. (20) have reported that changes in systemic vascular resistance during infusion of phenylephrine and angiotensin II were not different in chronically hypoxic guinea pigs and normoxic controls. In these two studies chronic hypoxia decreased the contractile sensitivity to PE by downregulating α1-adrenergic-receptors number and by altering the excitation-contractile coupling in vascular smooth muscle (21). This difference may be due to the fact that these studies were done in adults and that these particular vascular beds were not studied.

In conclusions, five days of continuous maternal tracheal nitrogen infusion can induce fetal mild hypoxia without fetal

acidosis. Mild chronic hypoxia seems to increase vasoconstrictive responses of femoral arteries in the fetal sheep. In addition, L-NAME pretreatment significantlly decreased EC$_{50}$, along with the increased maximal contraction response. Therefore, the increase in contractile response may at least in part be due to alteration in NO production. Further studies are needed to confirm the effects of mild chronic hypoxia on renal and femoral vessel reactivity in the fetal sheep.

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