Nitric oxide (NO) is a labile compound produced via the oxidation of a guanidino-nitrogen moiety on the amino acid L-arginine, a reaction catalyzed by the ubiquitous enzyme NO synthase (26). Within the peripheral vasculature, NO is constitutively produced within endothelial cells and plays an important role in regulating vascular resistance by stimulating soluble guanylate cyclase in subjacent smooth muscle cells, with consequent elevation of cGMP to produce vasorelaxation (26). Moreover, inhibition of NO synthase with L-arginine analogs in adult experimental animals is accompanied by a dose-dependent rise in blood pressure and fall in cardiac output, consistent with the notion that NO maintains a tonic vasodilatory influence within the circulation (26, 34). However, despite the fall in cardiac output and consequent reduction in systemic O2 delivery, inhibition of NO synthase is accompanied by a proportionally greater increase in tissue O2 extraction, so that whole body O2 consumption rises (34). In conjunction with in vitro observations, which indicate that NO inhibits mitochondrial respiratory processes (39), the latter provides evidence that NO normally exerts a suppressive effect on tissue oxidative metabolism in the adult. As in the adult, inhibition of NO synthesis increases fetal arterial blood pressure (5, 11, 27) and reduces fetal cardiac output (11), but it is unknown if NO additionally modulates fetal whole body O2 extraction or O2 consumption. The latter question is of particular relevance because of the major differences that exist between the adult and fetal circulations. Thus the low fetal blood O2 content not only requires a high level of cardiac output to maintain an adequate level of whole body O2 delivery (31) but may also limit any increases in systemic O2 extraction and therefore rises in fetal O2 consumption. Furthermore, in the “in series” adult circulation, the left ventricular (LV) output is distributed to systemic tissues and is equal to the right ventricular (RV) output, which is distributed to the lungs (32). By contrast, in the “in parallel” fetal circulation 1) RV output is 50–100% greater than the LV output (32, 36) and 2) the left and right ventricles both have a systemic distribution, with the combined ventricular output passing not only to the fetal body but also to the placenta, the site of fetal-maternal gas exchange (32). Thus an alteration in the balance between the LV and RV outputs, or the relative distribution of blood flow to the fetal body and placenta, may alter O2 delivery patterns to the fetus and thereby affect O2 usage by fetal tissues. However, it is unknown if NO influences the relative level of the LV or RV outputs or blood flow distribution between the fetal body and placenta.

Accordingly, the aims of the present study were to evaluate the role of NO in modulating 1) the relative levels of LV and RV outputs, 2) the distribution of the systemic output between the fetal body and placenta, and 3) fetal body O2 extraction and O2 consumption. Experiments were performed in chronically instrumented late-gestation fetal lambs, in which hemodynamic, blood flow, and blood gas measurements were performed after incremental inhibition of NO synthesis with the stereospecific and potent NO synthase inhibitor N\textsuperscript{-}nitro-L-arginine (L-NNA).

MATERIALS AND METHODS

Experiments were approved by the Monash University Animal Experimentation Committee and conducted in accord with guidelines established by the National Health and Medical Research Council of Australia.

Animal preparation. Eight fetuses, with known breeding dates were chronically instrumented under aseptic conditions at 128–134 days gestation (term 147 days). Fasted Border-Leicester cross ewes were anesthetized with propofol (5 mg/kg iv), intubated, and then mechanically ventilated with 1–3% halothane and a 2:1 nitrous oxide-oxygen mixture. The pregnant horn of the uterus was exposed through a midline laparotomy, and the fetal head, left forelimb, and upper thorax were delivered through a hysterotomy. A fetal thora-
cotomy was performed in the 3rd left interspace, and the 4th rib was removed to increase exposure of the heart and great vessels. After incision of the pericardium, an 8- or 10-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the pulmonary trunk. A Teflon cannula was inserted into the pulmonary trunk through an adventitial purse-string suture distal to the flow probe and connected to a polyvinyl catheter. After insertion of a polyvinyl catheter into the left atrial cavity through a purse-string suture in the appendix, the pericardium was loosely closed and the overlying muscle layers were repaired. The left auxiliary artery and vein were then exposed, and catheters were passed via these vessels into the brachiocephalic trunk and superior vena cava, respectively. All catheters were exteriorized, and after a wide-bore catheter was sutured to the skin of the anterior chest wall for measurement of amniotic fluid pressure, the fetal skin and maternal uterine incisions were closed.

After delivery of the fetal hindlimbs through a second hysterotomy, polyvinyl catheters (ID 1 mm, OD 1.5 mm) were inserted into a posterior tibial artery bilaterally and into a lateral saphenous vein and advanced into the abdominal aorta and inferior vena cava, respectively. A polyvinyl catheter was inserted into a cœlydovenous vein, and the tip was advanced into a major umbilical vein. The fetus was returned to the uterus, and all incisions were closed. Vascular catheters were filled with sodium heparin solution (1,000 IU/ml) and sealed. The catheters were tunneled subcutaneously to the right flank of the ewe and secured with elastic netting. Postoperatively, vascular catheters were flushed daily and refilled with concentrated sodium heparin. Antibiotics (500 mg streptomycin and 5 × 10⁶ units penicillin) were instilled into the amniotic cavity at the time of surgery and on each subsequent postoperative day.

Experimental protocol. Ewes were placed in a mobile laboratory cart 3–4 days after surgery and allowed free access to feed and water. Fetal brachiocephalic trunk blood pressure, heart rate, and pulmonary trunk flow were recorded, and 0.4-ml blood samples were collected anaerobically from the brachiocephalic trunk, pulmonary trunk, abdominal aorta, and the umbilical vein for hemoglobin and blood gas analysis. Fetal ventricular outputs and blood flows to the fetal body and placenta were then measured with radioactive microspheres using the reference sample method (18). After baseline measurements, NO synthesis was inhibited with the stereospecific NO synthase inhibitor L-NNA (Sigma Chemical), which was dissolved in normal saline to a concentration of 5 mg/ml and infused continuously through the hindlimb venous catheter at a rate of 0.68 mg·kg⁻¹·min⁻¹ to nominal cumulative doses of 10 and 25 mg/kg, assuming a fetal body weight of 4 kg. At each L-NNA dose, hemodynamics were allowed to stabilize over a 5-min period, and blood pressure, heart rate, blood flow, and blood gas measurements were then repeated.

Physiological measurements. Brachiocephalic trunk blood pressure was referenced to amniotic fluid pressure. Both pressures were monitored with silicon-chip pressure transducers (model CDX 111; COBE Laboratories, Lakewood, CO), which were calibrated against a water manometer before each experiment. Pulmonary trunk flow was measured continuously with an ultrasonic flowmeter (model T208, Transonic Systems). The outputs from the pressure transducers and flowmeter were amplified using a programmable signal conditioner (Cyberamp model 380; Axon Instruments, Foster City, CA), and signals were continuously displayed on a paper recorder (model 8002Z; Neomedix Systems, Sydney, Australia).

At baseline and the two L-NNA doses, a 30-s segment of hemodynamic data was also digitized with an analog-to-digital converter at a sampling rate of 200 Hz and stored on computer hard disk for subsequent off-line analysis using customized interactive software.

Blood pH, P O₂, P CO₂, and base excess were measured at 40°C with a blood analyzer (model ABL 500; Radiometer, Copenhagen, Denmark). Blood hemoglobin concentration and hemoglobin O₂ saturation were measured in duplicate with a hemoximeter (model OSM2, Radiometer).

Radioactive microsphere technique. Radioactive microspheres, 15 µm in diameter and labeled with one of five gamma-emitting isotopes (¹⁴¹Ce, ¹¹³Sn, ⁸⁵Sr, ⁹⁵Nb, or ⁴⁶Sc; NEN, Boston, MA) were ultrasonicated for 10–15 min before injection and injected over 30 to 45 s with 5 ml isononic saline. At baseline and at an L-NNA dose of 25 mg/kg, two different microsphere labels were injected simultaneously, one into the left atrium to measure LV output and the other into the superior vena cava to measure RV output (37), whereas reference samples were drawn simultaneously from the pulmonary trunk, brachiocephalic trunk, and descending aorta for determination of fetal body and umbilical-placental flows. At an L-NNA dose of 10 mg/kg, LV output was obtained with a single microsphere label injected into the left atrium, whereas reference samples were withdrawn from the brachiocephalic trunk and descending aorta to obtain fetal body and placental flows. Approximately 0.5–1 × 10⁶ microspheres were injected per radiolabel, and reference samples were withdrawn at a rate of 4.1 ml/min with a mechanical pump (model 901A; Harvard Apparatus, South Natick, MA). Reference sample collection was commenced 5–10 s before injection of microspheres and continued for an additional 75 s after the end of injection. Blood withdrawn in the reference samples was simultaneously replaced with maternal blood.

At the end of the experiment, the ewe was killed with an intravenous overdose of sodium pentobarbitone, and the position of the catheters was carefully checked at autopsy. The placenta was removed from the uterus by gentle traction, placed in 10% Formalin fixative for 7–10 days, and then carbonized at a temperature of 280°C in a vented box furnace. The carbonized tissue was subsequently ground into a coarse powder, which was packed into plastic counting vials to a height of <2 cm. The radioactive microsphere blood flows were counted in a gamma counter (model 1282 CompuGamma; LKB-Wallac, Turku, Finland) at the appropriate window settings, and the photopeaks of individual isotopes were separated by an on-line computer program.

Calculation of fetal ventricular outputs, fetal body, and placental flows. Radioactive microsphere blood flow measurements were calculated using the general relation

\[ Q_{\text{Tissue}} = \frac{Q_{\text{Reference}} \times R_{\text{Tissue}}}{R_{\text{Reference}}} \]

where \( Q \) is flow (ml/min) and \( R \) is radioactivity (counts/min). LV and RV outputs and systemic flows were obtained using an adaptation of this general relation for the fetal circulation (37). Thus, fetal LV output \( Q_{\text{LV}} \) was equal to \( Q_{\text{Reference}} \times R_{\text{LV}} \times R_{\text{LA}} \times R_{\text{BCT}} \), where \( R_{\text{LA}} \) is the radioactivity of the label injected into the left atrial cavity and \( R_{\text{BCT}} \) is the radioactivity of the same label collected in the brachiocephalic trunk reference sample. Fetal RV output \( Q_{\text{RV}} \) was equivalent to \( Q_{\text{Reference}} \times R_{\text{RV}} \times R_{\text{PT}} \), where \( R_{\text{PT}} \) is the radioactivity of venous label passing into the right ventricle, calculated as the injected radioactivity of this label minus that portion crossing the foramen ovale to appear in the LV output and \( R_{\text{RV}} \) is the radioactivity of the venous label in the pulmonary reference sample (37). RV output at an L-NNA dose of 10 mg/kg was obtained by interpolation from the measured pulmonary trunk flow, using baseline and 25 mg/kg L-NNA ultrasonic flow probe measurements of
pulmonary trunk flow and radioactive microsphere determinations of RV output.

Placental blood flow (Q_p) was calculated as (Q_{Reference} \times R_p)/R_{AA}, where R_p is the radioactivity of the placenta and R_{AA} is the radioactivity of the microsphere label in the abdominal aortic reference sample. Note that placental blood flow at baseline and 25 mg/kg L-NNA comprised the average of the flow values obtained from the different microsphere labels injected into the left atrium and superior vena cava, whereas only the single label injected into the left atrium was used at an L-NNA dose of 10 mg/kg. Blood flow to the fetal body at baseline and after L-NNA was calculated as the combined ventricular output (i.e., Q_{LV} + Q_{RV}) minus the placental flow.

Blood gas calculations. The O_2 content of arterial or venous blood (ml O_2 per dl blood) was calculated as (1.36 \times HbS \times Hb/100) + (0.003 \times P_{O2}), where HbS is hemoglobin O_2 saturation (%), Hb is hemoglobin level (g/dl), and P_{O2} is O_2 tension (mmHg).

Systemic O_2 delivery to the fetal body was calculated as [(Q_{LV} \times C_BTOT) + (Q_{RV} \times C_PTO)] - (Q_{O2} \times C_DAO), where C_BTOT, C_PTO, and C_DAO are the O_2 contents in brachiocephalic trunk (which is representative of blood in the ascending aorta), descending aorta, and pulmonary trunk, respectively. Whole body O_2 consumption (FB_{MV,O2}) at baseline and after L-NNA was calculated according to the Fick principle as Q_p - (C_BTOT - C_PTO), where C_BTOT is umbilical venous O_2 content (31, 36). Average arterial O_2 content of the fetal body (FAO_2) was computed as [(Q_{LV} \times C_BTOT) + (Q_{RV} \times C_PTO)] - (Q_{O2} \times C_DAO)]/(Q_{LV} + Q_{RV} - Q_p), the fetal arteriovenous O_2 content difference (FA_{A,V,O2}) as FB_{MV,O2}/Q_{FB}, fetal mixed venous O_2 content as FAO_2 = FA_{A,V,O2}, and the fetal O_2 extraction coefficient as FA_{A,V,O2}/FB_{AO_2} (36).

Statistics. Changes in hemodynamics, blood flows, and blood gas variables were analyzed with repeated-measures one-way ANOVA (36). The sums of squares were partitioned into individual degrees of freedom, and the significance of changes was evaluated using the Bonferroni procedure, as appropriate, for multiple tests (41). Results are reported as means ± SE, and P < 0.05 was considered significant.

RESULTS

Fetoplacental weight was 3.9 ± 0.2 kg, so that the nominal doses of 10 and 25 mg/kg L-NNA corresponded to infusion rates of 10.5 ± 0.5 and 26.3 ± 1.3 mg/kg, respectively.

Hemodynamics and blood gases. Mean brachiocephalic trunk blood pressure increased by 13.5 ± 1.5 mmHg (P < 0.005), and heart rate fell by 20 ± 3 beats/min (P < 0.005) after administration of 10 mg/kg L-NNA, but neither changed further at 25 mg/kg L-NNA dose. With the exception of an increase in hemoglobin concentration (P < 0.005) and a reduction in pH (P < 0.05), blood gas variables in the brachiocephalic trunk were unchanged between baseline and 10 mg/kg L-NNA. The higher dose of L-NNA was accompanied by a further rise in hemoglobin concentration (P < 0.025) and a fall in pH (P < 0.025), whereas P_{CO2} increased (P < 0.005) and hemoglobin O_2 saturation decreased (P < 0.005) compared with the baseline level. Neither P_{O2} nor O_2 content changed significantly with L-NNA (Table 1).

Ventricular outputs. The baseline combined ventricular output was 505 ± 16 ml·min⁻¹·kg⁻¹ with the contribution of LV output (41.3 ± 1.4%) being less than RV output (58.7 ± 1.4%, P < 0.005). The combined ventricular output fell to 405 ± 11 ml·min⁻¹·kg⁻¹ at 10 mg/kg L-NNA (P < 0.005), and then declined further to 350 ± 19 ml·min⁻¹·kg⁻¹ at 25 mg/kg L-NNA (P < 0.05, Fig. 1A). However, the proportional contribution of the left and right ventricles to the combined ventricular output (Fig. 1B) was unchanged between baseline and 10 mg/kg L-NNA.

Table 1. Ascending aortic hemodynamics and blood gases before and after N^•-nitro-L-arginine

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>L-NNA (10 mg/kg)</th>
<th>L-NNA (25 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>47.3 ± 0.6*</td>
<td>60.8 ± 1.3</td>
<td>62.2 ± 1.9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>167 ± 3*</td>
<td>147 ± 2</td>
<td>145 ± 4</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>9.6 ± 0.3*</td>
<td>10.3 ± 0.3†</td>
<td>11.0 ± 0.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.394 ± 0.01t†</td>
<td>7.372 ± 0.012†</td>
<td>7.354 ± 0.014‡</td>
</tr>
<tr>
<td>Hb O_2 saturation, %</td>
<td>53.2 ± 2.4</td>
<td>49.8 ± 2.5</td>
<td>47.6 ± 3.0</td>
</tr>
<tr>
<td>P_{O2}, mmHg</td>
<td>23.4 ± 0.8</td>
<td>23.0 ± 0.8</td>
<td>23.3 ± 1.0</td>
</tr>
<tr>
<td>P_{CO2}, mmHg</td>
<td>50.2 ± 0.9</td>
<td>51.1 ± 0.7</td>
<td>52.7 ± 0.8§</td>
</tr>
<tr>
<td>O_2 content, ml/dl</td>
<td>7.0 ± 0.2</td>
<td>7.0 ± 0.2</td>
<td>7.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 fetuses. L-NNA, N^•-nitro-L-arginine; MABP, mean arterial blood pressure. *P < 0.005, †P < 0.05 baseline vs. 10 mg/kg L-NNA. §P < 0.025 baseline vs. 25 mg/kg L-NNA.

Fig. 1. Changes in combined ventricular output (A) and proportion of combined ventricular output constituted by left (open bars) and right (filled bars) ventricular outputs (B) at baseline and after 10 and 25 mg/kg N^•-nitro-L-arginine (L-NNA). ***P < 0.005 left vs. right ventricular output.
output was unchanged between baseline and 10 and 25 mg/kg L-NNA (Fig. 1B).

Fetal body and placental blood flows. Baseline fetal body blood flow (330 ± 19 ml·min⁻¹·kg⁻¹) fell to 228 ± 16 ml·min⁻¹·kg⁻¹ at 10 mg/kg L-NNA (P < 0.005) and was not statistically different at 25 mg/kg L-NNA (Fig. 2A). By contrast, placental blood flow was unchanged between baseline (175 ± 9 ml·min⁻¹·kg⁻¹) and 10 mg/kg L-NNA (177 ± 12 ml·min⁻¹·kg⁻¹), but fell to 140 ± 9 ml·min⁻¹·kg⁻¹ at 25 mg/kg L-NNA (P < 0.005, Fig. 2B). As a result of these divergent changes, the ratio of fetal body to placental blood flow fell from 1.93 ± 0.17 at baseline to 1.40 ± 0.23 at 10 mg/kg L-NNA (P < 0.05) and was not significantly different at 25 mg/kg L-NNA (1.53 ± 0.11; Fig. 2C).

Fetal body arterial and venous O₂ contents. Average arterial fetal body O₂ content was 6.3 ± 0.2 ml/dl at baseline and was unaltered at both 10 and 25 mg/kg L-NNA (Fig. 3A). By contrast, the calculated average venous O₂ content fell from 4.3 ± 0.2 ml/dl at baseline to 2.7 ± 0.5 ml/dl at 10 mg/kg L-NNA (P < 0.005), and was not statistically different at 25 mg/kg L-NNA (Fig. 3B). As a result, fetal body O₂ extraction increased from 2.1 ± 0.2 ml/dl to 3.6 ± 0.4 ml/dl at 10 mg/kg L-NNA (P < 0.005), and did not change further at 25 mg/kg L-NNA (Fig. 3C).

Fig. 2. Changes in fetal body (A) and placental (B) blood flow and fetal body-to-placental blood flow ratio (C) at baseline and after 10 and 25 mg/kg L-NNA.

Fig. 3. Changes in average fetal arterial (A) and venous (B) O₂ contents and fetal O₂ extraction (C) at baseline and after 10 and 25 mg/kg L-NNA.

was not statistically different at 25 mg/kg (Fig. 3B). As a result, fetal body O₂ extraction increased from 2.1 ± 0.2 ml/dl to 3.6 ± 0.4 ml/dl at 10 mg/kg L-NNA (P < 0.005), and did not change further at 25 mg/kg L-NNA (Fig. 3C).

Fetal body O₂ delivery, O₂ consumption, and O₂ extraction coefficient. Average fetal body O₂ delivery fell from 20.6 ± 0.9 ml·min⁻¹·kg⁻¹ at baseline to 14.4 ± 1.3 ml·min⁻¹·kg⁻¹ at 10 mg/kg L-NNA (P < 0.002) and did not fall further at 25 mg/kg L-NNA (Fig. 4A). By contrast, fetal body whole body O₂ consumption increased from 6.7 ± 0.4 to 7.7 ± 0.5 ml·min⁻¹·kg⁻¹ between baseline and 10 mg/kg L-NNA (P < 0.05) and then fell to a near baseline value of 6.4 ± 0.5 ml·min⁻¹·kg⁻¹ at 25 mg/kg L-NNA (P < 0.01, Fig. 4B). These changes in whole body O₂ consumption were accompanied by alterations in the fetal body O₂ extraction coefficient, which increased from 0.33 ± 0.03 at baseline to 0.58 ± 0.08 at 10 mg/kg L-NNA (P < 0.005) and was not altered further at 25 mg/kg L-NNA (Fig. 4C).
DISCUSSION

Three main findings have emerged from this study, which has examined the effect of NO synthase inhibition on ventricular outputs, fetoplacental blood flow distribution, as well as whole body O2 extraction and O2 consumption in chronically instrumented late-gestation fetal sheep. First, whereas NO synthase inhibition reduced the fetal combined ventricular output, it did not alter the relative balance between the LV and RV outputs. Second, because a reduction in fetal body blood flow preceded and was of greater magnitude than a decrease in placental blood flow, NO synthase inhibition was associated with a systemic flow redistribution away from the fetal body and toward the placenta. Last, despite a fall in fetal body perfusion, NO synthase inhibition was accompanied by a proportionally greater increase in fetal body O2 extraction, which initially supported a rise in fetal whole body O2 consumption and subsequently maintained fetal whole body O2 consumption at near baseline levels after a reduction in placental perfusion.

Previous studies addressing the role of NO in the fetal circulation have generally examined hemodynamic and blood gas responses at single dose of NO synthase inhibitor (5, 27). The use of an incremental infusion regimen in this study has pointed to a number of dose-related differences between the responses of hemodynamic blood flow and blood gas variables to fetal NO synthase inhibition. Thus a rise in blood pressure associated with NO synthase inhibition attained a plateau by 10 mg/kg L-NNA. Given the wide acceptance of the concept that blood vessel tone represents the overall balance between circulating and locally released vasodilator and vasoconstrictor influences acting on the vessel wall (26), this increase in blood pressure was most likely related to the loss of a significant endogenous vasodilator mechanism counteracting the vasoconstrictor effects of the sympathetic nervous system (24) and circulating agents such as endothelin-1 (1, 6), ANG II (21), and norepinephrine (7). Inhibition of NO synthase was also accompanied by a fall in heart rate which, because baroreceptor responses are present in the late-gestation fetus (24), most likely represented a reflex response to the rise in blood pressure.

Inhibition of NO synthase in the present study also resulted in a progressive rise in hemoglobin concentration. Because no significant release of erythrocytes occurs from storage sites such as the spleen in chronically instrumented fetal sheep (2), the most plausible explanation for this finding was a reduction in plasma volume associated with a fluid shift from intravascular to extravascular compartments. One factor that presumably contributed to such a fluid shift was the high permeability of fetal capillaries, which predisposes to transudation of fluid across the capillary membrane with increases in hydrostatic pressure (3). Consistent with this proposal, increases in hemoglobin concentration accompanied by falls in fetal blood volume (6, 7, 21) have been reported after elevation of fetal blood pressure via infusion of vasoconstrictor compounds such as endothelin-1 (1, 6), norepinephrine (7, 29), epinephrine (29), or ANG II (29). However, the presence of a progressive rise in hemoglobin concentration in the face of similar blood pressure levels at 10 and 25 mg/kg L-NNA (Table 1) suggests that NO synthase inhibition may have also directly augmented vascular permeability (22).

The observed changes in fetal blood pressure, heart rate, and hemoglobin concentration that accompanied inhibition of NO synthase are particularly relevant because increases in arterial blood pressure (17, 40), reductions in heart rate (32), hemoconcentration (12), and falls in circulating blood volume (14) all reduce fetal cardiac output in the fetus. It is therefore likely that these alterations contributed to the fall in combined ventricular output evident with inhibition of NO synthesis in the present study. On the other hand, the contribution of the left and right ventricles to the combined ventricular output were unaffected after
L-NNA. Taken together, these results suggest that, via its hemodynamic effects, NO supports the maintenance of a high level of cardiac output characteristic of the fetal circulation (32) but is not involved in the regulation of the relative magnitudes of the fetal LV and RV outputs.

In this study, a decline in combined ventricular output evident at 10 mg/kg L-NNA was related to a fall in fetal body blood flow, whereas a further decline in combined ventricular output apparent at 25 mg/kg L-NNA was predominantly due to a reduction in placental perfusion. It is likely that this differing pattern of blood flow changes, which effectively resulted in a redistribution of blood flow away from the fetal body and toward the placental compartment, was related to at least three factors. The first was that the sensitivity of NO synthase inhibition with L-NNA was greater in the fetal body than in the placenta, a scenario that is compatible with the recent report that gene expression of the inducible form of NO synthase is normally present in the fetal circulation and is particularly prominent in the placenta (4), and the pharmacological evidence pointing to a greater effect of L-NNA on constitutive compared with inducible NO synthase (13, 28). The second factor is that basal release of NO accounted for a lesser portion of the vasodilator influence in the placenta compared with the fetal body, a proposal in accord with observations suggesting that the vasodilatory role of NO diminishes in late gestation in the umbilical-placental circulation (35). Finally, it is possible that vasoconstriction after inhibition of NO synthesis was less pronounced in the placenta than in the fetal body because of the absence of innervation of the umbilical and placental vessels (30) and the consequent lack of a tonic sympathetic neural influence.

Despite the absence of any significant changes in arterial hemoglobin O2 saturation, P02 and O2 content, the initial dose of 10 mg/kg L-NNA had striking effects on fetal whole body oxygenation variables in the present study. Thus O2 delivery to fetal body tissues fell by 30%, a change that was entirely attributable to a reduction in fetal body blood flow. Moreover, the mixed venous O2 content fell markedly in association with a reduction in the O2 extraction coefficient to a level (0.58) that was even greater than the 0.53 attained with a 50% umbilical blood flow reduction produced via cord occlusion (19). Importantly, however, the magnitude of the increase in fetal systemic O2 extraction after 10 mg/kg L-NNA exceeded the fall in fetal body blood flow, so that fetal whole body O2 consumption increased by ~15%. The latter finding is consistent with the notion that, as in the adult (34), constitutive production of NO has an inhibitory effect on fetal oxidative metabolism. However, two important differences were evident between fetal and adult whole body O2 consumption data following NO synthase inhibition. First, the magnitude of the increase in whole body O2 consumption in the present study was about one-half the increase (27%) seen in adult dogs (34), a difference primarily related to a rise in O2 extraction (134%) that was nearly double the 71% increment observed in fetal lambs. Second, the stimulatory effect of NO inhibition on whole body O2 consumption in the fetus appeared to depend on the preservation of placental perfusion, because in the presence of a reduced placental blood flow evident at 25 mg/kg L-NNA, a still-present increase in O2 extraction then served to maintain whole body O2 consumption at near baseline levels.

The pattern of changes in fetal whole body O2 extraction and O2 consumption occurring in this study in the setting of a reduction in both the combined ventricular output and fetal body blood flow cannot be readily accounted for by hemodynamic or neurohumoral factors. Thus the increases in fetal whole body O2 extraction and consumption were unlikely to have been related to systemic vasoconstriction per se, because infusion of vasoconstrictors such as endothelin-1 is associated with an unchanged fetal O2 extraction and a decrease in whole body O2 consumption (1). Furthermore, although infusion of the sympathetic neurotransmitter norepinephrine can elevate fetal whole body O2 extraction and consumption (25), the increases in the latter variables observed in the present study were unlikely to have been related to sympathetic mechanisms because evidence from adult rabbits indicates that NO synthase inhibition is associated with a baroreceptor-mediated decrease in sympathetic nerve activity (15). Accordingly, the pattern of changes in fetal whole body O2 extraction and O2 consumption observed in this study appear to be specific to NO synthase inhibition.

The circulatory responses that occurred with NO synthase inhibition are of particular interest because they resemble the hemodynamic and metabolic changes observed during fetal hypoxemia in a number of respects. Thus acute hypoxemia results in bradycardia and hypertension (9, 31), an increase in systemic O2 extraction which serves to maintain fetal O2 consumption (10) and, if severe enough, a redistribution of cardiac output from fetal body to placenta (9). The hypertension appears in large part to be related to hypoxia-induced increases in the circulating levels of a range of vasoconstrictor compounds, including norepinephrine (8), endothelin-1 (16), vasopressin (33), as well as ACTH and cortisol (20). The increase in circulating levels of norepinephrine occurring in fetal hypoxemia may also augment systemic O2 extraction (25). However, as NO also requires molecular O2 for its formation (23), it is possible that in the low O2 environment of the fetus the level of tissue oxygenation may be a critical limiting step in NO production. With a diminution in tissue O2 delivery, a reduction in NO production may therefore constitute an additional mechanism contributing to a redistribution of blood flow from the fetal body to the placenta and, via disinhibition of fetal oxidative metabolism, an increase in O2 extraction.

In conclusion, in the fetus, inhibition of NO synthesis results in a redistribution of blood flow to favor the placenta, and an increase in fetal body O2 extraction, which initially increases whole body O2 consumption and then maintains whole body O2 consumption at near baseline levels following a fall in placental perfusion. 
REFERENCES


