The fetus can be considered to be a highly sensitive model of long-term physiologic pulsatile perfusion, and fetal lamb preparations have been used extensively to test different patterns of bypass flows or pharmacologic manipulations. In previous experiments we have observed that pulsatile flow could overcome the progressive rise in peripheral and placental vascular resistances generated by steady-flow fetal bypass, and we have demonstrated that the use of 30 minutes of pulsatile-flow bypass may help in preventing the onset of fetal hypoxia in this setting.

In a second study, we hypothesized that nitric oxide (NO) production was increased under pulsatile flow conditions by comparison with steady-flow bypass.

**Objective:** Pulsatile flow was shown to overcome the progressive rise in peripheral and placental vascular resistances observed during steady-flow bypass, this rise being counteracted by inhibition of nitric oxide synthase. This study quantifies the release of endothelial vasoactive substances during a 60-minute in utero model of fetal bypass.

**Methods:** Fetuses were randomly allocated into 1 of 2 groups (steady flow, n = 8, or pulsatile flow, n = 13) and subjected to bypass through central cannulation and perfusion with either a centrifugal or pulsatile (125 beats · min⁻¹) blood pump.

**Results:** Lactate concentration was high, starting at fetal exteriorization and increasing during fetal preparation in the 2 groups. Once bypass was established, the rise was significant only in the steady-flow group. Plasma nitric oxide metabolites, similar before bypass, reached higher levels during pulsatile flow at the end of bypass (99 ± 9 vs 82 ± 23 µmol · L⁻¹; P = .037). Levels of urinary nitric oxide metabolites were significantly higher in the pulsatile-flow than in the steady-flow group (764 ± 4 vs 508 ± 240 µmol · L⁻¹; P = .005). Plasma cyclic guanosine monophosphate levels increased after 30 minutes of bypass in the pulsatile-flow group (25 ± 18 vs 12 ± 8 pmol · mL⁻¹; P = .004), and urinary cyclic guanosine monophosphate excretion was higher in the pulsatile-flow group (517 ± 450 vs 118 ± 78 pmol · mL⁻¹; P = .024). Plasma endothelin-1 levels increased in the 2 groups and were higher in the steady-flow group at 30 minutes (27 ± 5 vs 23 ± 2 pg · mL⁻¹; P = .04) and 60 minutes of bypass (39 ± 7 vs 32 ± 6 pg · mL⁻¹; P = .04). Plasma renin concentration increased significantly during bypass only in the steady-flow group (26 ± 10 vs 57 ± 42 in ng A1 · mL⁻¹ · h⁻¹; P = .04).

**Conclusions:** Improved placental and peripheral perfusion during fetal pulsatile-flow bypass may be mediated by preservation of fetal/maternal endothelial nitric oxide biosynthetic mechanisms and/or decreased activation of the fetal renin-angiotensin pathway. (J Thorac Cardiovasc Surg 2000;120:770-7)
Infusion of an inhibitor of NO synthase (Nω-nitro-1-arginine) during the second half of a 60-minute period of pulsatile bypass generated fetal systemic and placental vascular resistances at the level observed during steady-flow bypass. Thus, we had indirect proof that pulsatile flow enhances NO production during fetal bypass. Still, we had not completed direct measurements of either NO or NO metabolites.

In the current study, we have used a traditional model of fetal bypass in utero and have measured fetal hemodynamic parameters during a 60-minute bypass conducted under pulsatile or steady flow. Under these circumstances, we measured directly the level of endothelium-released vasoactive substances to confirm our previous hypothesis.

Materials and methods

Protocol. The fetuses (n = 24) were randomly allocated into 1 of the 2 groups according to the type of fetal bypass, either steady flow (SF group, n = 8) or pulsatile flow (PF group, n = 16). Three animals were excluded in the PF group (1 because of major maternal hypoxemia and hypercapnia during preparation, 1 because of severe fetal pulmonary hemorrhage, and 1 that was found dead from umbilical cord twisting). Thus, the results concern only the remaining animals (SF group, n = 8; PF group, n = 13).

All procedures and protocols performed in this study were approved by the local animal care committee and in compliance with the Guide of the National Veterinary School for Laboratory Animal Studies.

Anesthesia and hemodynamic monitoring. Ile de France ewes between 140 and 146 days’ gestation (term is 147 days) were fasted for 36 to 48 hours before the operation. The animals were placed in the supine position on an operating table after induction of anesthesia with diazepam (0.25 mg · kg−1) and ketamine (5 mg · kg−1) intravenously administered in the jugular vein. Ewes were intubated and connected to a volume-cycled respirator (MMS 107 ventilator; MMS, Chelles, France) with a respiratory rate of 10 cycles · min−1 and a tidal volume of 15 mL.

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Fetal surgical procedure. After exposure of the uterus through a low midline laparotomy and a small hysterotomy, fetal surgery was carried out according to a previously described technique.2,3,6 Ketamine (50 mg · kg−1) was administered intramuscularly to the fetus. Through a fetal neck incision, catheters were inserted into the common carotid artery (20 gauge) for blood pressure monitoring and blood sampling and into the jugular vein (18 gauge) for fetal infusion. The umbilical cord was mobilized and a 4S to 6S ultrasonic flow probe was carefully placed around the umbilical artery for umbilical blood flow monitoring (T206 flowmeter; Transonic Systems, Inc, Ithaca, NY). After exteriorization, avoiding amniotic fluid losses, the fetus was placed on an isolating pad and the temperature was constantly monitored by a rectal probe. After a fetal midline sternotomy, the pericardium was opened, suspended, and the heart was exposed. The main pulmonary artery and the ascending aorta were dissected out, and tapes were placed around the right and left pulmonary arteries. An ultrasonic flowmeter (8S-10S) was placed around the descending aorta, downstream to the ductus. A 300 U · kg−1 dose of heparin was administered intravenously to the fetus. Normothermic bypass was instituted between the pulmonary artery and right atrium. The bypass circuit consisted of a bubble oxygenator (Hi-Flex D 7008; Dideco, Mirandola, Italy) incorporating a heat exchanger and a venous reservoir primed with 700 mL of freshly drawn heparinized adult sheep donor blood diluted with 300 mL of Ringer’s lactate solution. The gas flow through the oxygenator was a mixture of 21% oxygen and 79% nitrogen and was adjusted to maintain fetal oxygen and carbon dioxide tensions (P O₂ and P CO₂) within normal limits. After connection to the circuit, both right and left pulmonary arteries were occluded by the previously placed tapes. Bypass was conducted for a 60-minute period with either a centrifugal pump (Medtronic Bio-Medicus centrifugal system, BP 50 mL; Medtronic, Inc, Eden Prairie, Minn) or a Harvard pump (Harvard pulsatile blood pump, 1421A; Harvard Biosciences, Les Ulis, France) delivering pulsatile flow at a rate of 125 beats · min−1. After the onset of bypass, the fetal heart was electrically fibrillated to rule out any contribution of the heart to the bypass flow and pulsatility. In each experiment the pump flow was adjusted to deliver a fetal mean blood pressure within the physiologic range of 50 to 60 mm Hg. A third flowmeter was placed around the bypass outlet tubing to measure the bypass flow. Because the study was not aimed at fetal survival, we were not interested in the late outcome of the fetus after bypass. Therefore, just after cessation of bypass, the fetus was completely exteriorized, killed, and weighed. The ewe was then allowed to recover under sedation and antibiotic prophylaxis, receiving penicillin and colistin intramuscularly for 48 hours.

Data acquisition. Maternal and fetal hemodynamic parameters were continuously monitored with blood pressure transducers (Abbott Transpac pressure set; Abbott Healthcare, Rungis, France) and a data acquisition system (Biopac Data Acquisition System for Windows, ACK100 Acqknowledge Software for Windows; Harvard Biosciences).
Fetal hemodynamic parameters (pressure and flows) were collected after exteriorization and insertion of a catheter into the common carotid artery (baseline time, Tb), before onset of bypass (cannulation time, Tc), and every 10 minutes after initiation of bypass (T10-T60). Fetal systemic vascular resistances were therefore calculated by dividing mean fetal arterial blood pressure by corresponding pump flow, and placental vascular resistances were calculated by dividing the same numerator by the corresponding placental blood flow. Values were indexed on the fetal weight in kilograms.

Blood gases sampled from the fetal carotid artery were collected at Tb, Tc, T10, T30, and T60 and immediately analyzed (PaO2, PaCO2, CO3H−1, oxyhemoglobin saturation, and pH) on a Radiometer 2400 gas analyzer (ABL 330; Radiometer A/S, Copenhagen, Denmark). Fetal hemoglobin concentration was measured with a hemoglobin photometer (HemoCue, Ängelholm, Sweden) at Tb, T10, and at the end of bypass. Plasma sampling for osmolality, endothelin-1, plasma renin concentration (PRC), and cyclic guanosine monophosphate (cGMP) measurements were made immediately after the beginning of bypass and at 30 and 60 minutes of bypass. For technical reasons NO metabolites were sampled at the beginning and at the end of bypass. Arterial blood samples (4.5 mL) were withdrawn into ethylenediaminetetraacetic acid glass tubes and immediately stored in ice. Blood samples were then centrifuged at 1250g and 4°C for 10 minutes, and plasma was stored at −20°C until assayed. Endothelin-1 was determined by radioimmunoassay. Nitrate assays were performed after dilution with distilled water and incubation with nitrate reductase and flavine adenosine. PRC was measured by radioimmunoassay, after incubation of plasma with an excess of angiotensinogen. PRC was expressed as nanograms of angiotensin I per milliliter of plasma and hour of incubation (ng A1 · mL−1 · h−1); cGMP was measured by radioimmunoassay and expressed as picomoles per milliliter (pmol · mL−1) (Kit Nex 133; NEN Life Sciences Products, Paris, France).

Blood was collected on fluoride ethylenediaminetetraacetic acid at Tb, T10, and T60, was centrifuged, and enzymatic determination of lactate was performed immediately on plasma samples (Lactate PAP; Biomerieux, Marcy-L’Etoile, France).

Statistical analysis. Data were stored on a spreadsheet database with a Power Macintosh 7600/132 computer (Apple Computer, Cupertino, Calif) and then analyzed with a statistical package (StatView; Abacus Concepts, Inc, Berkeley, Calif). Values were expressed as mean ± SD. Between-group differences were compared with 1-way analysis of variance and repeated-measures 2-way analysis of variances. Within-group differences were analyzed with t tests for dependent samples. Statistical significance was established at the 5% level.

Results

The 2 groups were comparable with regard to the average weight of ewes (SF group, 65 ± 17 kg; PF group, 74 ± 18 kg) and fetuses (SF group, 4.2 ± 1 kg; PF group, 4.1 ± 0.8 kg).

Arterial blood gases. The fetal values of arterial pH, Pco2, Po2, CO3H−1, and oxyhemoglobin saturation were not significantly different between groups (Table I). The pH and CO3H−1 values decreased significantly (respectively, P = .004 and P = .0012) only in the SF group, as a consequence of metabolic acidemia devel-

### Table I. Values of fetal blood gases before and during bypass

<table>
<thead>
<tr>
<th>Group</th>
<th>After extraction</th>
<th>Before bypass</th>
<th>10-min bypass</th>
<th>30-min bypass</th>
<th>60-min bypass</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SF</td>
<td>7.25 ± 0.08</td>
<td>7.23 ± 0.07</td>
<td>7.23 ± 0.8</td>
<td>7.21 ± 0.07</td>
<td>7.21 ± 0.09*</td>
</tr>
<tr>
<td>PF</td>
<td>7.24 ± 0.07</td>
<td>7.25 ± 0.06</td>
<td>7.25 ± 0.08</td>
<td>7.25 ± 0.08</td>
<td>7.23 ± 0.1</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>19.1 ± 3.5</td>
<td>22.4 ± 7.2</td>
<td>68.7 ± 27.0</td>
<td>45.4 ± 13.4</td>
<td>59.0 ± 25.4</td>
</tr>
<tr>
<td>PF</td>
<td>22.0 ± 4.7</td>
<td>21.1 ± 4.4</td>
<td>50.2 ± 16.7</td>
<td>52.6 ± 17.9</td>
<td>44.7 ± 10.6</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>34.7 ± 8.2</td>
<td>41.0 ± 8.5</td>
<td>31.7 ± 9.1</td>
<td>32.7 ± 8.2</td>
<td>32.5 ± 10.9</td>
</tr>
<tr>
<td>PF</td>
<td>40.8 ± 10.9</td>
<td>40.8 ± 12.7</td>
<td>33.9 ± 13.0</td>
<td>33.9 ± 12.7</td>
<td>32.3 ± 10.8</td>
</tr>
<tr>
<td>CO3H−1 (mmol · L−1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>17.2 ± 4.1</td>
<td>16.8 ± 3.2</td>
<td>13.3 ± 4.2</td>
<td>13.9 ± 3.9</td>
<td>12.7 ± 3.2*</td>
</tr>
<tr>
<td>PF</td>
<td>15.5 ± 4.9</td>
<td>15.6 ± 3.8</td>
<td>14.7 ± 2.8</td>
<td>14.6 ± 2.5</td>
<td>14.0 ± 3.6</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>15.8 ± 5.4</td>
<td>22.3 ± 11.1</td>
<td>62.1 ± 28.4</td>
<td>54.7 ± 20.2</td>
<td>70.7 ± 20.6</td>
</tr>
<tr>
<td>PF</td>
<td>21.3 ± 7.5</td>
<td>19.6 ± 6.4</td>
<td>60.2 ± 20.7</td>
<td>66.1 ± 18.1</td>
<td>61.4 ± 15.5</td>
</tr>
</tbody>
</table>

*Within group differences, between “after extraction” and “60-min bypass.”

PF, Pulsatile flow; SF, steady flow; PaO2, arterial oxygen tension; PaCO2, arterial carbon dioxide tension; CO3H−1, bicarbonate; SaO2, arterial oxygen tension; values are given as mean ± SD.
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oping between the exteriorization of the fetus and the end of bypass. Lactate concentration was high right after fetal exteriorization in both groups (Fig 1) and continued to increase significantly during fetal preparation and cannulation in the 2 groups (SF group, \( P = .0002 \); PF group, \( P < .0001 \)). Once under bypass, the rise was significant only in the SF group (\( P = .049 \)).

After induction of anesthesia, hemoglobin values were similar in the 2 groups (SF group, 12.8 ± 1.5 g · dL⁻¹; PF group, 12.6 ± 1.2 g · dL⁻¹) and decreased at the beginning of bypass due to the priming dilution. It remained stable afterward throughout the experiment (SF group, 9.6 ± 0.8 g · dL⁻¹; PF, 9.7 ± 0.9 g · dL⁻¹).

Hemodynamics. There were no significant differences in either mean or differential blood pressure between the 2 groups during fetal extraction (SF group, 63 ± 10 mm Hg and 28 ± 11 mm Hg; PF group, 65 ± 7 mm Hg and 26 ± 5 mm Hg) or just before bypass (PF group, 59 ± 9 mm Hg and 23 ± 7 mm Hg; SF group, 61 ± 6 mm Hg and 24 ± 5 mm Hg). During bypass the mean blood pressure was comparable in the 2 groups, set around 60 mm Hg, according to the protocol (Table II). In the PF group, mean differential pressure during bypass was 13.8 ± 2.6 mm Hg (13-15 mm Hg). Heart rate was similar in the 2 groups during fetal exteriorization (SF group, 142 ± 20 beats · min⁻¹; PF group, 128 ± 21 beats · min⁻¹) and before bypass (SF group, 148 ± 18 beats · min⁻¹; PF group, 135 ± 25 beats · min⁻¹). Since the heart was fibrillating in each group during bypass, the pulse pressure disappeared in the SF

Table II. Values of fetal blood pressure before and during bypass

<table>
<thead>
<tr>
<th>Group</th>
<th>After extraction</th>
<th>Before bypass</th>
<th>10-min bypass</th>
<th>20-min bypass</th>
<th>30-min bypass</th>
<th>40-min bypass</th>
<th>50-min bypass</th>
<th>60-min bypass</th>
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</thead>
<tbody>
<tr>
<td>SAP (mm Hg)</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>SF</td>
<td>80 ± 16</td>
<td>72 ± 10</td>
<td>58 ± 9</td>
<td>57 ± 6</td>
<td>59 ± 10</td>
<td>60 ± 10</td>
<td>62 ± 10</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>PF</td>
<td>82 ± 9</td>
<td>76 ± 8</td>
<td>68 ± 8*</td>
<td>70 ± 9*</td>
<td>68 ± 7*</td>
<td>70 ± 8*</td>
<td>71 ± 6*</td>
<td>72 ± 6*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>63 ± 10</td>
<td>59 ± 9</td>
<td>57 ± 9</td>
<td>57 ± 6</td>
<td>58 ± 9</td>
<td>60 ± 10</td>
<td>61 ± 10</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>PF</td>
<td>65 ± 7</td>
<td>61 ± 6</td>
<td>60 ± 7</td>
<td>61 ± 8</td>
<td>61 ± 8</td>
<td>62 ± 8</td>
<td>62 ± 6</td>
<td>61 ± 6</td>
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<tr>
<td>DAP (mm Hg)</td>
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</tr>
<tr>
<td>SF</td>
<td>53 ± 7</td>
<td>49 ± 3</td>
<td>56 ± 9</td>
<td>56 ± 6</td>
<td>58 ± 9</td>
<td>58 ± 10</td>
<td>60 ± 10</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>PF</td>
<td>56 ± 7</td>
<td>53 ± 6</td>
<td>53 ± 6</td>
<td>55 ± 8</td>
<td>54 ± 7</td>
<td>56 ± 8</td>
<td>59 ± 6</td>
<td>58 ± 5</td>
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<tr>
<td>PP (mm Hg)</td>
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<td></td>
</tr>
<tr>
<td>SF</td>
<td>28 ± 11</td>
<td>23 ± 7</td>
<td>0.5 ± 0.9</td>
<td>0.2 ± 0.3</td>
<td>0.4 ± 0.7</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>PF</td>
<td>26 ± 5</td>
<td>24 ± 5</td>
<td>15 ± 3*</td>
<td>15 ± 3*</td>
<td>14 ± 3*</td>
<td>14 ± 3*</td>
<td>13 ± 2*</td>
<td>13 ± 3*</td>
</tr>
</tbody>
</table>

SAP, Systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; PP, pulse pressure or differential pressure; PF, pulsatile flow; SF, steady flow. Values are given as mean ± SD.

* \( P < .05 \).

Fig 1. Fetal plasma level of lactate (mmol · L⁻¹): Measurements were made after fetal exteriorization, on bypass, and at the end of bypass. Black circle, Within-group differences, \( P < .05 \).

Fig 2. Mean pump flow evolution (mL · min⁻¹ · kg⁻¹) and between-group comparison. T10, 10 minutes of bypass; T60, 60 minutes of bypass. Intergroup differences, pulsatile-flow versus steady-flow group; \( P < .05 \).
Mean pump flow was significantly higher in the PF group than in the SF group after 20 minutes of bypass and until the end of bypass ($P = .0004$) (Fig 2). Distal fetal aortic flow rate was significantly higher in the PF group than in the SF group after 40 minutes of bypass and until the end of bypass ($P = .0376$) (Fig 3). In both groups the umbilical flow decreased significantly at the beginning of bypass (SF group, $P = .001$; PF group, $P < .0001$) but was significantly higher after 40 minutes of bypass in the PF group than in the SF group ($P = .030$) (Fig 4).

Fetal systemic vascular resistances were significantly lower after 40 minutes of bypass in the PF group ($P = .402$) (Fig 5). Placental vascular resistances increased in the 2 groups immediately after the beginning of bypass (SF group, $P = .0086$; PF group, $P < .0001$). After 40 minutes of bypass and until the end of bypass, the placental vascular resistances were significantly higher in the SF group than in the PF group ($P = .0271$) (Fig 6).

**Release of vasoactive substances.** Fetal plasma (Table III) and urinary osmolality (SF group, $221 \pm 77$ mOsm · kg$^{-1}$ H$_2$O; PF group, $231 \pm 72$ mOsm · kg$^{-1}$ H$_2$O) were not significantly different between the groups before or after bypass. Total fetal urinary creatinine excretion was higher in the PF group (SF group, $207 \pm 70$ mg · L$^{-1}$; PF group, $340 \pm 92$ mg · L$^{-1}$; $P = .0427$). Plasma NO metabolites were also similar before bypass, increased significantly during bypass in the 2?
Vasactive substance release and plasma osmolality level during continuous (SF) or pulsatile bypass flow (PF)

<table>
<thead>
<tr>
<th></th>
<th>Start bypass</th>
<th>30-min bypass</th>
<th>60-min bypass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SF</td>
<td>PF</td>
<td>SF</td>
</tr>
<tr>
<td>PRC (ng A1 · mL⁻¹ · h⁻¹)</td>
<td>26 ± 10</td>
<td>26 ± 13</td>
<td>44 ± 36</td>
</tr>
<tr>
<td>NO₂⁻NO₃ (µmol · L⁻¹)</td>
<td>68 ± 24</td>
<td>74 ± 11</td>
<td>82 ± 23</td>
</tr>
<tr>
<td>cGMP (µmol · mL⁻¹)</td>
<td>12 ± 5</td>
<td>12 ± 8</td>
<td>20 ± 12</td>
</tr>
<tr>
<td>Endothelin-1 (pg · mL⁻¹)</td>
<td>18 ± 5</td>
<td>15 ± 3</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm · kg⁻¹ H₂O)</td>
<td>308 ± 10</td>
<td>298 ± 15</td>
<td>311 ± 7</td>
</tr>
</tbody>
</table>

PRC, Plasma renin concentration; NO₂⁻NO₃, nitric oxide metabolites; cGMP, cyclic guanosine monophosphate.
*Within group differences between "start bypass" and "30-min bypass" and "start bypass" and "60-min bypass."
†Intergroup differences.
‡Within group differences between 30-min and 60-min bypass.

The current study generally confirms the hemodynamic and oximetric data obtained by our previous experiments: after 1 hour of in utero bypass, overall fetal endothelial function is better preserved under pulsatile than steady flow. Pulsatile flow prevents the drop in placental flow and limits the rise in placental vascular resistance observed with steady flow. We have also hypothesized that the addition of pulsatile flow during fetal bypass, through the shear stress of blood on the arterial wall, acted by stimulation of the NO release. However, at the time of the last study, evidence of enhanced NO release from the endothelium with pulsatile-flow bypass was only indirectly available. This was accomplished through Nω-nitro-L-arginine blockade. The currently available direct measurements of endothelium-released vasoactive substances and their metabolites add to the evidence of the beneficial effects of pulsatility during fetal bypass.

After discussing the role of the main substances involved during the typical 60-minute bypass period (NO-cGMP, endothelin-1, renin-angiotensin), we will also envision the limitations still attached to this type of perfusion, along with the possible various modalities of fetal bypass.

**Discussion**

As demonstrated by many studies, fetal bypass with standard steady-flow pumps rapidly triggers a severe placental dysfunction dominated by a strong vasoconstrictive reaction. Thus, some authors have used pharmacologic manipulations (generally based on vasodilators) to overcome the rise in placental vascular resistances, with subsequent decreased placental blood flow and acidosis. Our approach has been more on the hemodynamic side in trying to use the potentially beneficial effects of pulsatile flow on global endothelial function.

The current study generally confirms the hemodynamic and oximetric data obtained by our previous experiments: after 1 hour of in utero bypass, overall fetal endothelial function is better preserved under pulsatile than steady flow. Pulsatile flow prevents the drop in placental flow and limits the rise in placental vascular resistance observed with steady flow. We have also hypothesized that the addition of pulsatile flow during fetal bypass, through the shear stress of blood on the arterial wall, acted by stimulation of the NO release. However, at the time of the last study, evidence of enhanced NO release from the endothelium with pulsatile-flow bypass was only indirectly available. This was accomplished through Nω-nitro-L-arginine blockade. The currently available direct measurements of endothelium-released vasoactive substances and their metabolites add to the evidence of the beneficial effects of pulsatility during fetal bypass.

After discussing the role of the main substances involved during the typical 60-minute bypass period (NO-cGMP, endothelin-1, renin-angiotensin), we will also envision the limitations still attached to this type of perfusion, along with the possible various modalities of fetal bypass.

NO is the physiologic activator of the soluble guanylate-cyclases responsible for cGMP production, and the levels of plasma and urinary NO and of urinary cGMP concentration were significantly higher in the PF group. These facts confirm that the use of pulsatile flow appears to be better than nonpulsatile flow in preserving the NO biosynthetic pathway. This is also concordant with the work of Reddy and associates, who compared the vasoactive effects of 2 vasodilators, acetylcholine (endothelium-dependent) and nitroprusside (endothelium-independent) before and after bypass to evaluate the ability of the endothelium to produce NO: steady-flow bypass gen-
erates a selective impairment of endothelium-depend-
ent vasodilatation.

Similarly, pharmacologic activators of the soluble
guanylate-cyclases, like nitro-compounds nitroprusside
or nitroglycerin, were commonly used during fetal
bypass by other authors \(^{1,5}\) to avoid hypertension due to
high steady blood flows (400 mL · kg \(^{-1}\)). The addition
of pulsatile-flow bypass has a similar effect and seems
to be well tolerated (60 minutes in our last 2 series vs
30 minutes in other reports).

Endothelin-1, the most potent vasoconstrictor pep-
tide, \(^{13}\) is also known to be an important regulator of
placental vascular tone. \(^{14,15}\) In the current study, the
plasma level of endothelin-1 at the beginning of
bypass, as well as its rise after 30 minutes of bypass,
were similar to those observed in the Reddy study. \(^{12}\)
These authors also observed that the rise in placental
resistance during steady-flow fetal bypass was limited
after injection of a nonspecific endothelin-1 receptor
blocker (PD 145065). In the current study, the plasma
measurement of endothelin-1 at 60 minutes was signif-
ically higher than the initial level at the beginning of
bypass in the 2 groups but was also significantly higher
in the SF group.

Several reports have also brought some evidence of
an active renin-angiotensin system in the human fetal
circulation that may modulate placental perfusion and
function under physiologic conditions. \(^{16}\) When com-
pared with the value given in reference before and after
hemorrhage in fetal sheep, \(^{17}\) the PRC on bypass was
high, even in the PF group. Even though levels of renin-
angiotensin activity measured between groups were not
significantly different, there was a significant increase
of PRC in the SF group. Is this difference due to a pri-
mary endothelial dysfunction or a widespread response
to increased fetal renin-angiotensin activity?

Some arguments will support the hypothesis of a
primary bypass-induced endothelial dysfunction that in
turn triggers a stimulation of the renin-angiotensin
mechanism. In glomerular arterioles, endothelial and
juxtaglomerular cells are closely related. NO has been
shown to inhibit renin secretion. \(^{18}\) Therefore,
endothelial cells, when stimulated through shear
stress to produce NO, could also represent the
intrarenal baroreceptors. Meanwhile, recent studies \(^{19}\)
have shown that on the top of a baseline, endotheli-
dependent vasoconstrictive tonus, there is a positive
interaction between angiotensin II and endothelin-1.
The latter was significantly increased in our study
during nonpulsatile bypass.

In support of the hypothesis that endothelial dysfunc-
tion is actually due to renin-angiotensin system activi-
ty, previous studies \(^{20}\) have shown a strong positive cor-
relation between the level of angiotensin II and that of
peripheral vascular resistance. The TREND study \(^{21}\)
and Vanhoutte, Boulanger, and Monbouli \(^{22}\) also demon-
strated that substances inhibiting angiotensin II synthesis
can restore endothelium-dependent vasodilatation.
Finally, placenta has been considered as a major site for
the conversion of angiotensin, which in turn could con-
trol the local placental flow. \(^{23}\) Whereas angiotensin
constricted the umbilical-placental and the renal circu-
lations (to redistribute blood flow to the myocardium), \(^{24}\)
the renin-angiotensin system may also be an
important factor in the fetal response to stress.

Even though our data confirm the benefits of pul-
satile flow for up to 1 hour of bypass, the perfusion
remains lower than optimal, as indicated by the level
of lactate. Significant lactic acidosis developed as
soon as hysterotomy, fetal mobilization, and inci-
sions for arterial and venous line placement were
performed, and it kept increasing after sternotomy and
cannulation. This was most certainly the conse-
quence of metabolic debt due to fetal stress, \(^{25}\)
including anesthetic procedures. \(^{26}\) Hypoxemia and
anemia due to fetal blood replacement by adult
hemoglobin with a lower affinity for oxygen than
fetal hemoglobin are other contributing factors. \(^{27}\)
Under pulsatile-flow bypass, the lactate level
remained stable as opposed to the continuous
increase observed in steady-flow bypass. As lactate
release into the maternal circulation was not differ-
ent between the initiation of bypass and the end of
bypass, the rise in fetal lactate comes from the fetus
itself. The placenta may play an important role in
regulating circulating lactate concentrations under
hypoxic conditions, \(^{28}\) allowing the fetus to maintain
stable lactate concentration. However, a reduction in
placental blood flow impedes lactate clearance from
the fetal circulation.

Given the cannula size limitation, high physiologic
blood flows (400 mL · kg \(^{-1}\)) that would possibly con-
trol the lactate level as described by Hawkins and
associates \(^{29,30}\) could not be achieved and total flows
ranged to a maximum of two thirds of normal expect-
ed flow. Finally, the choice of the pulse frequency
was dictated by the characteristics of the Harvard
pump, with an important residual volume and a back-
flow that imposed on us a relatively low frequency, the
physiologic heart rate being 140 to 180 beats ·
in these animals.

A different pump concept offering high beat rate, low
prime pulsatile flow, and limited backflow is currently
being developed for use in such specific settings.
REFERENCES


22. Vanhoutte PM, Boulanger CM, Monbouiu JV. Endothelium-derived relaxing factors and converting enzyme inhibition. Am J Cardiol 1995;76:3E-12E.


