Doppler ultrasound evaluation of ductus venosus blood flow during acute hypoxemia in fetal lambs

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ABSTRACT

It has been demonstrated with invasive techniques in fetal lambs that the ratio of ductus venosus to umbilical vein blood flow rate (DV/UV ratio) increases during hypoxemia and infusion of catecholamines. Recently it was found in human fetuses using pulsed wave Doppler ultrasound equipment that the DV/UV ratio in fetuses with intrauterine growth restriction was significantly increased. The aim of the present study was to show in fetal lambs whether routine Doppler ultrasound devices were capable of determining the DV/UV ratio with sufficient reliability.

The experiments were performed on seven near-term instrumented fetal lambs using pulsed wave Doppler ultrasound to measure flow rates (derived, in milliliters per min, from the intensity-weighted mean velocity ($V_{\text{mean}}$) and the vessel’s cross-sectional area) in the ductus venosus and intra-abdominal umbilical vein. Fetal hypoxemia was induced by administering a low-oxygen gas to the ewe (5–7% oxygen, 2% carbon dioxide). Fetal arterial $P_{\text{O}_2}$ and heart rate decreased significantly during maternal hypoxia. The proportion of umbilical venous return passing through the ductus venosus in controls was $36 \pm 5\%$ (mean $\pm$ SD). This increased to $53 \pm 6\%$ ($p < 0.001$) because the umbilical venous blood flow fell during late hypoxemia when the heart rate had decreased by 20%. Severe hypoxemia tended to reduce the mean velocity ($V_{\text{mean}}$) and the minimum velocity ($V_{\text{min}}$) (based on the maximum velocity envelope curve) in the ductus venosus, descending aorta and inferior vena cava. The pulsatility index of the umbilical artery significantly increased at the end of hypoxemia. We conclude that determination of the proportion of umbilical vein blood flow entering the ductus venosus by Doppler ultrasound in a clinical setting may contribute to the detection and evaluation of fetal distress.

INTRODUCTION

The effects of acute hypoxemia on the fetal circulation, especially in the arterial system, have been studied extensively. On the venous side, it was demonstrated in instrumented fetal lambs that shortage of oxygen affects blood flow through the ductus venosus. Normally, 38–55% of blood flow in the umbilical vein is directed to the ductus venosus, allowing for preferential oxygen delivery to the fetal brain and heart. During acute hypoxemia, this proportion, or the ductus venosus/umbilical vein (DV/UV) ratio, increases to 60–65%, and the redistribution of the venous return may be a possible adaptation to maintain the supply to these vital organs. Infusion of norepinephrine (noradrenaline) or epinephrine (adrenaline) into fetal lambs increases the resistance of umbilical and hepatic veins. Because the latter are arranged in parallel to the ductus venosus, these resistance changes may cause the redistribution of venous flow during hypoxemia which is well known to raise fetal plasma catecholamine levels. It thus appears that an increase of the DV/UV ratio is related to fetal stress.

In fetal lambs with intrauterine growth restriction (IUGR), plasma catecholamine concentrations are typically above normal levels; similarly, in human IUGR fetuses, amniotic fluid catecholamine concentrations tend to be elevated. By Doppler ultrasound technology, it has recently been shown that the DV/UV ratio in human IUGR fetuses is increased. Measuring blood flow rates in fetal vessels with pulsed wave Doppler ultrasound systems is difficult and inherently variable, which makes these results open for debate. Because it had been proven in instrumented fetal lambs by invasive techniques (microsphere injection) that redistribution of venous return does occur during hypoxia, it was our main purpose to investigate in...
Ductus venosus blood flow in fetal lambs

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METHODS

Animal preparation

The experiments were approved by the local animal protection authorities and performed in seven mixed-breed pregnant sheep between 120 and 132 gestational days (term 145 days). Anesthesia was induced by intravenous injection of barbiturate (0.5 g Trapanal®; external jugular vein catheter) and maintained after intubation by ventilation with 1–2% halothane in oxygen. A fetal hindlimb was delivered through a midline laparotomy and uterotomy. Polyethylene catheters (1.0 mm OD) were placed into the femoral artery and vein and advanced to the descending aorta and to the lower inferior vena cava, respectively. The fetuses were instrumented in addition with inflatable bilateral carotid occluders for other experimental purposes. Both catheters and an additional amnion catheter were fixed to the skin, and ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously. The fetus was returned to the uterus, and fetal and maternal wounds were closed. All catheters and the ECG electrodes were placed subcutaneously.

Experimental protocol

The animals were allowed to recover for at least 3 days after surgery. Pressures (fetal descending aorta, inferior vena cava and amniotic fluid) were measured with Gould transducer amplifiers and P23XL pressure transducers (Ohmeda, Oxnard, CA, USA). Amniotic pressure was used as the zero pressure reference. Fetal heart rate was derived from arterial pressure pulses (Biotach®, Gould, Dietzenbach, Germany) or the ECG signal. Fetal signals were recorded on a paper chart (Servogor 462, Metrawatt, Nürnberg, Germany) and with a data acquisition system (Biopac MP100WS, Santa Barbara, CA, USA, sampling rate 250 Hz).

In the inferior vena cava, Doppler measurements were performed close to the heart, and aortic blood flow was determined in the diaphragmatic region of the descending aorta.

For measurement of the blood flow rate in the umbilical vein, a straight middle segment of the intrahepatic part of the umbilical vein was selected. The resulting signal was visualized in an oblique or a mid-sagittal section of the fetal abdomen. The vessel was identified by audio recognition of its Doppler signal, and by its characteristic velocity waveform (Figure 1). Measurements from the isthmic portion were accepted when at least four identical consecutive typical waveforms could be obtained.

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Doppler ultrasound measurements

Doppler measurements were made with a Combison 420 color-coded pulsed wave Doppler ultrasound system, with a 5-MHz convex transducer, or with a Combison 320-5 Doppler ultrasound system (external Doppler probe, 2.25 MHz; Kretztechnik, Austria), and results were obtained from the video screen printouts. Measurements were accepted when the fetus did not breathe or move. The Doppler ultrasound systems calculated the average blood flow rate (ml/min) as the intensity weighted mean of the frequency spectrum (averaged over the cardiac cycle) multiplied by the vessel’s cross-sectional area ($\pi (\text{diameter}/2)^2$). The Doppler sample volume was positioned to cover the vessel, and Doppler angles were less than 45° (average value in the ductus venosus 27°, range 15–45). The diameter was measured in frozen B-mode to the nearest tenth of a millimeter by carefully placing the calipers at right angles to the vessel axis. In the isthmic portion of the ductus venosus, the color mode had to be turned off, because the pixels usually covered the lumen and vessel wall. Care was taken to obtain the largest diameter of the longitudinal vessel sections. Blood flow rates were normalized for fetal body weight. Flow values for each fetus were taken as the mean of two to six (average three) repeated measurements, which included the repeated measurements of vessel diameters; only measurements with instantaneous variation less than 10% were accepted for the analysis.

Peak velocity ($V_{\text{peak}}$), minimum velocity ($V_{\text{min}}$), mean velocity ($V_{\text{mean}}$) and pulsatility index (PI) derived from the maximum velocity envelope curve were recorded whenever feasible.

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Data were collected during three experimental phases: control (about 45 min), 5 min after onset of reduced maternal fractional inspired oxygen concentration (FiO₂; see below), and when the fetal heart rate had decreased by 20% from control levels, typically 15–20 min into the hypoxic phase. Fetal blood gas values were determined immediately before and after the hypoxic period.

Fetal hypoxemia was induced by administering a gas mixture containing 5–7% oxygen and 2% carbon dioxide in nitrogen to the ewe via a loosely fitting plastic hood placed over its head.

At the end of the experiment, the ewe and fetus were sacrificed by intravenous injection of T61® (Hoechst), and the fetuses were weighed towel-dry.

**Data analysis and statistics**

Average data are presented as mean ± SD. Means were compared by paired Student’s t test when appropriate. Differences were considered significant at levels of \( p < 0.05 \). Standard linear regression analysis was used to calculate linear correlations. All 45 measurements are included in Figure 2, but only the first measurement within each experimental period for each animal was used for statistical description and analysis.

**RESULTS**

The average fetal weight was 3.3 ± 0.6 kg. Blood gas values during the control phase (Table 1) were in the normal range for chronically instrumented sheep fetuses, and fetal arterial \( pO_2 \) and oxygen content decreased significantly during maternal hypoxia (Table 1). Fetal heart rate decreased significantly but there was no change in mean arterial blood pressure. It is well known¹ that fetal plasma catecholamine levels are raised in this situation, and this was confirmed in two animals (from 880 pg/ml to 12,130 pg/ml of norepinephrine).

Figure 2 illustrates the correlations of umbilical venous blood flow rate (ml/min per kg) with flow rates in the fetal ductus venosus, descending aorta and inferior vena cava during the control phase. This was an anticipated finding which indicates that Doppler ultrasound flow measurements were consistent.

The proportion of umbilical venous return passing through the ductus venosus (percentage DV/UV ratio) in the control group was 36.2 ± 5.2%. This increased to 39.8 ± 13.4% (not significant) within 5 min after onset of reduced maternal FiO₂, and to 52.6 ± 5.8% (\( p < 0.001 \)) at the end of hypoxemia (Figure 3).

Table 2 summarizes the results of Doppler blood flow measurements in controls and during late hypoxemia.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Hypoxemia (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descending aorta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.33 ± 0.24</td>
<td>7.21 ± 0.14</td>
</tr>
<tr>
<td>( pO_2 ) (mmHg)</td>
<td>18.1 ± 1.9</td>
<td>12.1 ± 2.3*</td>
</tr>
<tr>
<td>( pCO_2 ) (mmHg)</td>
<td>50.4 ± 3.6</td>
<td>39.8 ± 17.6</td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>41.9 ± 16.8</td>
<td>26.3 ± 16.9</td>
</tr>
<tr>
<td>Mean aortic pressure (mmHg)</td>
<td>46.8 ± 3.4</td>
<td>48.8 ± 3.4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>198 ± 10</td>
<td>142 ± 33*</td>
</tr>
</tbody>
</table>

**Table 1** Blood gas values, blood pressure and heart rate before and immediately after hypoxemia. Values presented as mean ± SD. Significant differences from controls are indicated by asterisks (*, \( p < 0.05 \)); \( n \), number of experimental animals. Owing to catheter malfunction in one animal, \( n \) is one less than 7

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¹. Tchirikov et al., Ultrasound in Obstetrics and Gynecology.
Compared to controls, a significant change was the decrease of the intensity-weighted mean blood velocity and blood flow rate in the umbilical vein ($p < 0.05$). Flow rates tended to decrease in the aorta ($p = 0.053$) and inferior vena cava also. At 5 min into the hypoxic phase, flow measurements showed considerable variability, with average values of flow rates in the ductus venosus (106.4 ± 38.6 ml/min per kg) and umbilical vein (291.1 ± 149.1 ml/min per kg) being higher than the control values (not significant).

Severe hypoxemia tended to reduce blood flow velocity during heart diastole ($V_{\text{min}}$) in the ductus venosus, aorta and inferior vena cava, and to increase the PI in the ductus venosus. The PI was significantly increased in the umbilical artery (Table 3).

**DISCUSSION**

The proportion of umbilical venous blood perfusing the ductus venosus varies considerably in instrumented fetal lambs (36–64%) as well as in instrumented rhesus monkeys (30–71%) and in acute experiments in human fetuses (8–92%). Our DV/UV ratios were in the lower range of values reported, which may be due to methodological reasons. We found, in accordance with previous studies, that this proportion was clearly increased (from 36.2 ± 5.2% to 52.6 ± 5.8%) at the end of hypoxemia (Figure 3). The DV/UV ratio increase appeared to be due mostly to the reduction of the umbilical vein flow rate at the end of hypoxemia, whereas the ductus venosus blood flow rate did not change significantly.

Although it was not measured directly, the liver blood flow rate may be estimated as the difference between the umbilical vein and the ductus venosus blood flow rates (neglecting the contribution from the hepatic artery and the portal vein). The results imply that, during hypoxemia, the
blood flow in the liver may be reduced. Under normal conditions, the liver blood flow rate is in the range of 151 ml/min per kg body weight. This appeared to be reduced in hypoxia to about 93 ml/min per kg, a 39% reduction (cf. Table 2). Paulick and colleagues\textsuperscript{15} demonstrated in fetal lambs that the flow in the intra-abdominal part of the umbilical vein had a mean velocity of 30 ± 3 cm/s (\(V_{\text{mean}}\) based on the maximum velocity envelope curve). We measured an umbilical vein intensity-weighted \(V_{\text{mean}}\) (27.7 ± 5.6 cm/s, Table 2) which was, as expected, slightly smaller.

As shown in Table 3, there was only a slight increase of the PI in the umbilical artery, and a tendency of \(V_{\text{min}}\) in the duc tus venosus and umbilical vein to decrease. The decrease of the PI may reflect a rise of the umbilical–placental resistance as judged from the reduction of the umbilical vein (Table 2) and aortic blood flow rate (not significant).

Morrow and associates\textsuperscript{16} found no alterations of the umbilical artery flow velocity waveform in fetal lambs during hypoxemia. In human fetuses seriously afflicted by IUGR, and in small-for-gestational-age fetuses with abnormal umbilical artery flow, \(V_{\text{min}}\) in the duc tus venosus was reduced\textsuperscript{15,21}. In less severe cases, a significant decrease of \(V_{\text{min}}\) was not detected\textsuperscript{10}.

We conclude that the determination of the DV/UV ratio using pulsed wave Doppler ultrasound equipment reflects the redistribution of blood flow. This ratio increases with fetal hypoxemia and therefore may be more reliable than blood velocity measurements for the detection and evaluation of fetal distress.

**REFERENCES**


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### Table 3 Blood velocity parameters of the ductus venosus, umbilical artery, descending aorta and inferior vena cava in controls and during late hypoxemia (mean ± SD).

<table>
<thead>
<tr>
<th>Blood velocity</th>
<th>Control ((n = 7))</th>
<th>Hypoxemia ((n = 7))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ductus venosus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{\text{peak}}) (cm/s)</td>
<td>79.3 ± 15.8</td>
<td>81.0 ± 25.0</td>
</tr>
<tr>
<td>(V_{\text{min}}) (cm/s)</td>
<td>45.0 ± 14.4</td>
<td>30.3 ± 24.8</td>
</tr>
<tr>
<td>(V_{\text{mean}}) (cm/s)</td>
<td>62.1 ± 13.5</td>
<td>55.7 ± 19.6</td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.8</td>
</tr>
<tr>
<td><strong>Umbilical artery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>1.0 ± 0.4</td>
<td>1.2 ± 0.4*</td>
</tr>
<tr>
<td><strong>Descending aorta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{\text{peak}}) (cm/s)</td>
<td>100.7 ± 25.4</td>
<td>101.7 ± 25.6</td>
</tr>
<tr>
<td>(V_{\text{min}}) (cm/s)</td>
<td>37.7 ± 15.7</td>
<td>32.3 ± 12.6</td>
</tr>
<tr>
<td>(V_{\text{mean}}) (cm/s)</td>
<td>69.2 ± 18.8</td>
<td>66.6 ± 16.6</td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td><strong>Inferior vena cava</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{\text{peak}}) (cm/s)</td>
<td>61.3 ± 20.0</td>
<td>50.8 ± 12.4</td>
</tr>
<tr>
<td>(V_{\text{min}}) (cm/s)</td>
<td>17.2 ± 12.6</td>
<td>8.1 ± 9.9</td>
</tr>
<tr>
<td>(V_{\text{mean}}) (cm/s)</td>
<td>39.5 ± 15.8</td>
<td>29.5 ± 9.7</td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.6</td>
</tr>
</tbody>
</table>