Doppler Echocardiographic Assessment of Fetal Descending Aortic and Umbilical Blood Flows
Validation Studies in Fetal Lambs
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Background. Doppler ultrasound has been used to assess abdominal aortic and umbilical blood flows in the human fetus, but the accuracy of this approach has not been demonstrated in an animal model.

Methods and Results. We compared abdominal aortic and umbilical blood flows determined by Doppler echocardiography in 12 fetal lambs with invasive flow measurements using radionuclide-labeled microspheres and electromagnetic flow transducers. We varied fetal blood flow from 100 to 950 ml/min in the abdominal aorta and from 130 to 610 ml/min in the umbilical vein. Invasive and Doppler echocardiographic flow measurements correlated well at both sites \( y = 68 + 1.02x, r = 0.94\), and \( y = 33 + 1.1x, r = 0.91\), respectively. A slight overestimation of flow using the Doppler method may require a correction factor to assess actual flow. The degree of overestimation was not systematically related to measurements taken in smaller vessels or at larger angles of incidence.

Conclusions. The present study demonstrates that determination of abdominal aortic and umbilical venous flows by echocardiography and pulsed Doppler ultrasound is feasible. Doppler measurements appear to be suited to serial comparison of actual flow. (Circulation 1991;83:1731–1737)

Doppler echocardiography has been used in human subjects to noninvasively determine stroke volume and cardiac output.\(^1\)\(^–\)\(^3\) This method is based on the principle of volume flow and requires measurements of the cross-sectional area and the mean temporal velocity at the site at which Doppler interrogation is performed;\(^4\) these measurements may be taken from various sites within the heart and great arteries. Doppler echocardiographic flow determinations have been validated against several invasive methods of flow measurement in vitro experiments,\(^1\) animal models,\(^5\)\(^–\)\(^7\) and both children\(^2\)\(^,\)\(^7\) and adults.\(^13\)\(^,\)\(^8\)\(^,\)\(^9\) In children with congenital heart disease, the Doppler echocardiographic assessment of intracardiac shunt flow compared favorably with invasive measurements at cardiac catheterization.\(^10\)\(^–\)\(^12\) More recently, Doppler echocardiographic determinations of stroke volume and cardiac output have been reported in human fetuses\(^13\)\(^–\)\(^17\); this method has also been used to assess regional blood flow in the fetal descending aorta and umbilical vein.\(^18\)\(^–\)\(^21\)

Measurements of fetal descending aortic or umbilical blood flow may be important in assessing placental function and fetal well-being. The accuracy of Doppler echocardiographic measurements, however, may be limited because of small vessel size or distance to the transducer and has not been demonstrated in intact fetal animals. To determine how accurate the Doppler echocardiographic technique might be in the human fetus in the clinical situation, we compared blood flow measurements in the descending aorta and umbilical vein of fetal lambs by Doppler echocardiography with simultaneous invasive flow measurements using radionuclide-labeled microspheres and electromagnetic flow transducers.

Methods
Preparation of Study Animals
We studied 12 ewes of mixed Western breed carrying fetuses with mean gestational age of 126
days (approximately 85% gestation) and mean±SD weight of 3±0.8 kg. The ewes were fasted for 24 hours before surgery and underwent epidural anesthesia with 4 ml of 1% tetracaine HCl. Polyvinyl catheters (1.27 mm i.d.) were inserted into a maternal pedal artery and vein, and 10% dextrose in 0.9% NaCl was infused intravenously at a rate of 3 ml/min. Intravenous ketamine HCl (100 mg/10–15 min) was used for maternal sedation. With the ewe placed in the supine position, the uterus was exposed through a midline incision. A hysterotomy was performed on the proximal portion of the pregnant horn; subsequent fetal surgery was performed with 0.5% lidocaine HCl for local anesthesia. A fetal hind limb was exposed, and polyvinyl catheters (0.76 mm i.d.) were inserted into the anterior tibial vessels and advanced to the lower descending aorta and inferior vena cava, respectively.

Through the same hysterotomy, the other hind limb and the rump were extracted, and the left flank was exposed. An incision was made anterior to the spinal muscles along the lower lumbar vertebral column, and the abdominal muscles were carefully dissected. The lower descending aorta and the common umbilical artery were exposed as described previously.22 A cuff-type electromagnetic flow transducer (C&C Instruments, Culver City, Calif.) of appropriate size that had been precalibrated in vitro was placed on the descending aorta just proximal to the iliac bifurcation. Another flow transducer was placed on the common umbilical artery, which extends in the sheep fetus from the iliac bifurcation of the descending aorta to the bifurcation of the branch umbilical arteries, thus allowing measurement of total umbilical blood flow at one arterial site. In six animals, a small branch of the iliac artery was then used to introduce a polyvinyl catheter (0.4 mm i.d.). The tip of this catheter was positioned at the origin of the common umbilical artery, allowing embolization of the placental vascular bed with unlabeled 25-μm microspheres. In the other six animals, a balloon occluder that had been made in our laboratory was placed around the umbilical cord. The fetal incisions were closed in separate layers, and the fetus was returned to the uterine cavity. A catheter with multiple side-holes was placed into the amniotic cavity; lost amniotic fluid was replaced with warmed 0.9% NaCl solution, and the uterine incision was closed.

**Doppler Ultrasound**

Two-dimensional echocardiography and pulsed Doppler ultrasound were performed using an ATL Mark 600 instrument (Advanced Technology Laboratories, Bothell, Wash.) with a 5- or 3-MHz mechanical transducer placed directly on the uterine horn that contained the instrumented fetus. We demonstrated recently that this approach provides quantitative echocardiographic data comparable to that obtained in studies through the maternal abdominal wall23 while avoiding interference from maternal movements or intestinal gas. The lower descending aorta and the intra-abdominal portion of the umbilical vein were imaged in their long axes to measure the angle of incidence between the ultrasound beam and the direction of blood flow as accurately as possible (Figure 1). The Doppler sample volume was placed both in the descending aorta approximately 1 cm proximal to the flow transducer and in the intra-abdominal umbilical vein proximal to the ductus venosus. Because umbilical arterial flow equaled umbilical venous flow, we used the umbilical flow signal to represent umbilical arterial flow. The signal is both continuous and easier to integrate. Doppler velocity tracings selected for analysis had narrow spectral dispersions. Ultrasonographic observations were recorded on 1/2-in. videotape for later playback and quantitative analysis.

**Experimental Procedure**

The fetuses were studied acutely after surgery. After flow assessment at baseline, descending aortic and umbilical blood flows were varied. We first increased blood flow by injection of atropine (0.1 mg/kg estimated fetal wt) and infusion of norepinephrine (1 μg/kg/min). Subsequently, descending aortic and umbilical flows were decreased by stepwise occlusion of the cord or placental embolization using several injections of 2×10⁶ carbonized microspheres (25-μm diameter; 3M Co., St. Paul, Minn.). Descending aortic and umbilical blood flows were measured at baseline level and after each variation of flow using the radionuclide-labeled microsphere technique, in which the ratio of radioactive counts to the flow in the reference sample withdrawn from the artery is proportional to the ratio of organ count to organ flow as described previously.24,25 Labeled 15-μm microspheres (selected from 153Gd, 97Co, 114In, 51Cr, 113Sn, 85Sr, 95Nb, 54Mn, and 68Zn; New England Nuclear, Boston) were rapidly injected into the inferior vena cava. A reference sample was withdrawn from the descending aorta at a rate of 4 ml/min beginning immediately before the injection of microspheres and continuing for at least 40 seconds after completion of the injection. Removed fetal blood was weighed and replaced with an equal volume of fetal donor or maternal blood.

At the end of the experiment, the ewe and fetus were killed by an overdose of pentobarbital followed by bilateral thoracotomies in accordance with the guidelines of the Committee for Animal Research at the University of California, San Francisco. The fetus was weighed and dissected; the lower carcass (below L4–5), uterus, and cotyledons were weighed and placed in formalin. The tissues were carbonized in an oven at 350°C, pulverized, and placed into plastic vials; radioactivity of each tissue and reference blood sample was counted in a 1,000-channel multichannel pulse-height analyzer (Norland Corp., Fort Atkinson, Wis.). The specific activity of each isotope within a sample was calculated by the least-squares method.26 Descending aortic and umbilical blood flows were then calculated according to the following formula:
FIGURE 1. Doppler echocardiographic assessment of blood flow in descending aorta (top) and umbilical vein (bottom) of a fetal lamb. Top left panel: Distal descending aorta (DAo) and two electromagnetic flow transducers (FTD). Sample volume of pulsed Doppler system is placed centrally within vessel. Top right panel: Spectral display of Doppler shift indicates blood velocity; vertical scale markers relate to velocity, and horizontal scale markers relate to time. Bottom left panel: Umbilical vein (UV) at its intra-abdominal course surrounded by liver tissue. Bottom right panel: Spectral display shows typical uniform velocity pattern with little changes throughout cardiac cycle within UV.

\[ Q = \frac{\text{reference flow} \times \text{organ counts}}{\text{reference counts}} \]

where \( Q \) is blood flow. Descending aortic and umbilical flows were also monitored with a Statham SP2202 electromagnetic flowmeter (Statham Instruments, Oxnard, Calif.) and continuously recorded with a Beckman eight-channel direct-writing recorder (Beckman Instruments, San Jose, Calif.). The electromagnetic flow measurements were adjusted to microsphere measurements and served as readings at any time between microsphere determinations for the comparison with simultaneous Doppler echocardiographic flow determinations.

Data Analysis

Doppler echocardiographic recordings were analyzed using a computer-assisted analysis system and commercially available software (Microsonics CAD 886, Indianapolis, Ind.). We measured from two-dimensional echocardiograms the largest diameter of the vessel at the site of Doppler interrogation by averaging 10 consecutive measurements. With axial resolution, measurements were taken from trailing edge to leading edge; with lateral resolution, measurements were taken from center to center of the echoes of the vessel wall. In addition, the angle of incidence (\( \Theta \)) between the ultrasound beam and the direction of flow was measured from two-dimensional echocardiographic images by averaging three to five measurements immediately before or after Doppler interrogation had been performed. From the spectral display of the Doppler shift, we measured the mean temporal velocity by tracing the modal velocities (i.e., the darkest portion of the spectral Doppler display). For descending aortic velocity, we averaged measurements of five consecutive beats; for umbilical venous velocity, we averaged 10 measurements taken randomly during a 2-second period.

Blood flow was calculated using the following formula:

\[ Q = \frac{\pi \times D^2}{4} \times Vm \times 60 / \cos \Theta \]

where \( Q \) is blood flow (ml/min), \( D \) is vessel diameter (cm), \( Vm \) is mean temporal velocity over the entire
cardiac cycle (cm/sec), and Θ is angle of incidence. The internal clock of the ultrasound machine and time marks on the recording paper served as coordinates for the comparison of ultrasonographic and invasive measurements simultaneously.

To assess intraobserver and interobserver variabilities, 15 measurements of diameter measurement and velocity for the calculation of flow were repeated by the same and a second observer, each unaware of previous results. Variability was expressed as difference from the mean of the two results in percent of the mean.

Statistics

Values are given as mean±SD. We used linear regression analysis to compare the descending aortic and umbilical venous flows calculated by echocardiography and Doppler ultrasound with simultaneous measurements by the flow transducer on the descending aorta and umbilical artery, respectively. Linear regression analysis was also used to assess the influence of angle and vessel diameter size on the accuracy of Doppler echocardiographic measurements. Student’s unpaired t test was used to compare the angle and diameter measurements and the degree of overestimation of flow by Doppler echocardiography at the descending aorta and the umbilical vein; a probability value of less than 0.05 was considered to indicate a significant between-group difference.

Results

Descending aortic flow ranged from 100 to 950 ml/min, and umbilical blood flow ranged from 130 to 610 ml/min; because of the ultrasonographic technique, descending aortic and umbilical flow measurements could not be taken simultaneously. Over these ranges of flow, we compared Doppler echocardiographic determinations (120 descending aortic and 58 umbilical venous) with simultaneous invasive flow measurements by radionuclide-labeled microspheres and electromagnetic flow transducers at the respective sites (Figures 2 and 3). Invasive measurements (x axis) and ultrasonographic determinations (y axis) correlated well for both descending aortic flow (Figure 2: y=68+1.02x; r=0.94; SEE, ±73 ml/min; p<0.001) and umbilical flow (Figure 3: y=33+1.1x; r=0.91; SEE, ±70 ml/min; p<0.001).

Vessel diameters at the site of Doppler interrogation measured by two-dimensional echocardiography ranged from 0.52 to 0.74 cm in the descending aorta and from 0.44 to 0.76 cm in the umbilical vein (mean, 0.62±0.05 versus 0.63±0.07 cm; p=0.26). The angle of incidence at the site of Doppler interrogation ranged from 27° to 61° in the descending aorta and from 0° to 66° in the umbilical vein (mean, 47.5±6.7° versus 24.8±18.5°; p<0.0001). The overestimation of flow by the Doppler echocardiographic method was expressed as the quotient of the simultaneous flow measurements by Doppler echocardiography and flowmeter; this quotient averaged 1.18±0.16 for descending aortic flow and 1.19±0.17 for umbilical flow (p=0.70). There was no significant influence on the magnitude of the overestimation by vessel diameter or angle of incidence in either the descending aorta or the umbilical vein (Table 1). Both interobserver and intraobserver variabilities were 5% or less.

Discussion

Results from the present study demonstrate that the determination of descending aortic and umbilical blood flows in the fetus with the use of Doppler echocardiography is feasible. Over a wide range of flows, we found a linear relation between the invasive flow measurements and simultaneous ultrasonographic determinations, suggesting that such measurements can be used to assess placental flow. These results are in accordance with the excellent agreements between Doppler echocardiographic and invasive measurements of blood flow reported in several previous studies in animals5-7 and humans.1-3,8-12 In
these studies, however, Doppler interrogation was performed within the heart or proximal great arteries of larger individuals. We have demonstrated that the method can also be used for flow determinations in relatively small fetal vessels. Doppler echocardiographic determination of flow in the descending aorta or umbilical vein of a fetus requires that vessel size and angle of incidence be meticulously assessed because small errors in these measurements will result in larger errors of flow calculation.\(^{29}\) We studied fetal lambs at approximately 85% of gestation, which corresponds to approximately 34 weeks in the human fetus; because of the induced changes in blood flow, vessel diameter varied between 4.4 and 7.6 mm. In younger fetuses, a smaller vessel diameter could limit the ability of the ultrasonographic approach to determine descending aortic and umbilical flows.\(^{29}\)

The Doppler echocardiographic method overestimated actual flow at both measurement sites. Although the mean angle of incidence was significantly smaller for the Doppler measurements taken within the umbilical vein, the magnitude of flow overestimation by the ultrasonographic method was not different at the descending aortic and umbilical levels. There also was no tendency toward a larger overestimation by the Doppler approach when smaller vessel size or larger angle of incidence had to be considered (Table 1). The overestimation by the Doppler method may be a result of intrinsic difficulties with the sector format of the duplex scanning system that we used. Doppler interrogation in either vessel at small angles of incidence is optimal for velocity determination, but it makes echocardiographic diameter measurements less accurate because the vessel wall is imaged with more lateral than axial resolution. On the other hand, imaging a vessel with axial resolution is optimal for diameter measurements but less so for velocity determination because exact measurement of the angle of incidence exerts a large effect on the calculation of flow. Overestimation of the vessel diameter, especially when imaged in lateral resolution, may therefore have led to a small overestimation of flow calculated by the Doppler echocardiographic method.

Measuring the largest vessel diameter during systole may also contribute to overestimation of the Doppler echocardiographic flow calculation because the cross-sectional area may be smaller during diastole. Other researchers\(^{21,28}\) have therefore suggested using a time-distance recorder to assess the pulsatile vessel diameter changes. However, no pulsatile changes of vessel diameter occur within the umbilical vein, where we observed the same degree of overestimation by Doppler echocardiography as in the pulsating descending aorta (Figure 1). Previous studies\(^{30,31}\) have also shown little variation of the cross-sectional area during the cardiac cycle in pulsating

**Figure 3.** Scatterplots of regression analysis correlating umbilical flow measured invasively by radionuclide-labeled microspheres and an electromagnetic flow transducer at umbilical artery (UA, x axis) and by Doppler echocardiography at umbilical vein (UV, y axis). Solid line is regression line; broken line is line of identity.

**TABLE 1.** Correlation Between Angle of Incidence and Diameter Measurements and Degree of Overestimation of Flow by Doppler Echocardiography

<table>
<thead>
<tr>
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<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Angle (\theta) at DAo versus (Q_{\text{Dopp}}/Q_{\text{MSFM}})</td>
<td>-0.002</td>
<td>1.26</td>
<td>-0.067</td>
<td>0.47</td>
</tr>
<tr>
<td>Angle (\theta) at UV versus (Q_{\text{Dopp}}/Q_{\text{MSFM}})</td>
<td>-0.001</td>
<td>1.21</td>
<td>-0.077</td>
<td>0.56</td>
</tr>
<tr>
<td>DAo diameter versus (Q_{\text{Dopp}}/Q_{\text{MSFM}})</td>
<td>-0.016</td>
<td>1.19</td>
<td>-0.004</td>
<td>0.96</td>
</tr>
<tr>
<td>UV diameter versus (Q_{\text{Dopp}}/Q_{\text{MSFM}})</td>
<td>-0.223</td>
<td>1.33</td>
<td>-0.095</td>
<td>0.48</td>
</tr>
</tbody>
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DAo, descending aorta; \(Q_{\text{Dopp}}/Q_{\text{MSFM}}\), ratio of simultaneous flow determination by Doppler echocardiography to invasive measurements by radionuclide-labeled microspheres and electromagnetic flowmeter; UV, umbilical vein.
arteries. In addition, time-distance recorders may indicate changes in vessel size that are results of movements of the pulsating vessel relative to the fixed direction of the ultrasound beam. Using the largest diameter at least ensures that the measurements are probably taken from the central chord of the vessel cross section.

An additional possible source of overestimation by the Doppler echocardiographic approach may relate to measuring the modal velocities in the center of the vessels, which may not represent the spatial average velocity because the flow velocity profile may be parabolic. Previous reports, however, suggest that the velocity profile in the descending aorta is flat in systole; this has been demonstrated both in an in vitro model of the human aorta and by film anemographic measurements in dogs. Although the velocity profile in the descending aorta may become parabolic in diastole, this should not lead to considerable overestimation of flow per cardiac cycle or per minute because diastolic flow is less important than systolic descending aortic flow (Figure 1).

In a recent report, flow measured by an electromagnetic flowmeter in the proximal (thoracic) fetal descending aorta compared favorably with the pulsed Doppler determination of flow. In this study, the Doppler method did not overestimate actual flow but instead tended to underestimate it. The discordant finding may be because the authors did not integrate the entire spectral Doppler tracing in systole and diastole, thus disregarding the portion of diastolic forward flow in the upper descending aorta. In the lower abdominal descending aorta, this segment of diastolic flow is larger and cannot be disregarded.

The overestimation of blood flow by the Doppler approach appears less likely to have resulted from inaccurate measurements of the actual flow by the invasive reference method that we chose. Although all methods for blood flow measurement have potential sources of error, the radiolabeled-microsphere technique has been shown to give accurate results as validated by the controlled roller-pump method, which is believed to be the most accurate measurement of blood flow. Excellent agreement was also found when measurements of cardiac output in newborn lambs by the microsphere technique were compared with those by other methods used widely in clinical practice, such as the Fick or thermodilution method.

In conclusion, assessment of descending aortic and umbilical flows in the fetus by echocardiography and pulsed Doppler ultrasound is feasible. The ultrasonographic approach may slightly overestimate actual flow, but it is still clinically useful. When assessment of actual flow is sought, a correction factor may be applied to Doppler echocardiographic determinations of fetal descending aortic and umbilical blood flows. Because serial studies of individual human fetuses thought to be at risk of developing placental dysfunction will demonstrate relative changes in flow, these calculations would not be subject to such considerations.

References


KEY WORDS • echocardiography, fetal • prenatal care • fetal diseases
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