Myocardial blood flow and coronary reserve in chronically anemic fetal lambs

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Davies, Lowell E., A. Roger Hohimer, and Mark J. Morton. Myocardial blood flow and coronary reserve in chronically anemic fetal lambs. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R306–R313, 1999.—Chronic fetal anemia produces large compensatory increases in coronary blood flow in the near-term fetal lamb. To determine if increased coronary flow in anemic fetuses is associated with decreased coronary flow reserve or, alternatively, an increase in coronary conductance, we measured maximal coronary arterial conductance during adenosine infusion before and during anemia. Systolic hemorrhage over 7 days reduced hematocrit from 30.6 ± 2.7 to 15.8 ± 2.4% (P < 0.02) and the oxygen content from 7.3 ± 1.4 to 2.6 ± 0.4 ml/dl (P < 0.001). Coronary blood flow increased from control (202 ± 60) to 664 ± 208 ml·min⁻¹·10⁰ g⁻¹ with adenosine to 726 ± 169 ml·min⁻¹·10⁰ g⁻¹ during anemia and to 1,162 ± 250 ml·min⁻¹·10⁰ g⁻¹ (left ventricle) during anemia with adenosine infusion (all P < 0.001). Coronary conductance, determined during maximal vasodilation, was 18.2 ± 7.7 before and 32.8 ± 11.9 ml·min⁻¹·10⁰ g⁻¹·mmHg⁻¹ during anemia (P < 0.001). Coronary reserve, the difference between resting and maximal myocardial blood flow interpolated at 40 mmHg, was unchanged in control and anemic fetuses (368 ± 142 and 372 ± 201 ml/min). Because hematocrit affects viscosity, anemic fetuses were transfused with blood to acutely increase the hematocrit back to control, and conductance was remeasured. Coronary blood flow decreased 57.3 ± 18.9% but was still 42.6 ± 18.9% greater than control. We conclude that in chronically anemic fetal sheep coronary conductance is increased and coronary reserve is maintained, and this is attributed in part to angiogenesis as well as changes in viscosity.

fetal anemia; coronary conductance

IN FETAL SHEEP, adaptation to chronic anemia includes a 30% increase in heart-to-body weight ratio, a 50% increase in stroke volume and cardiac output, and a nearly sixfold increase in coronary blood flow (5, 6). These changes, which exceed those that follow acute normovolemic anemia (13), are also accompanied by morphometric changes that include an increase in ventricular capillary diameter and maintenance of capillary density in the presence of cardiac hypertrophy. Molecular changes include the induction of hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF) (16). Taken together, these data suggest an increase in coronary vascular growth during chronic fetal anemia.

The slope of the relationship between myocardial blood flow during maximal vasodilatation and adenosine infusion and coronary pressure (coronary conductance) is a physiological measure of the total cross-sectional area of the coronary resistance vessels (14). In the adult, because there is no increase in cross-sectional area of the myocardial resistance vessels, coronary conductance is constant and is thought not to increase in response to stimuli such as left ventricular hypertrophy (14, 15). In the developing fetus, however, it is not known if coronary conductance can be accelerated by growth of resistance vessels during anemia. Coronary conductance can also be used to quantify coronary flow reserve, the difference between resting and maximal myocardial blood flow at a given perfusion pressure. Coronary conductance and coronary reserve of lambs are similar to those of adult sheep (11). However, in response to aortic banding, in adult sheep left ventricular hypertrophy results in a decrease in subendocardial capillary density, a decrease in maximal coronary conductance to adeno-

sine by two thirds, and a similar decrease in coronary reserve (11). In contrast, aortic banding in lambs does not change maximal coronary conductance, coronary reserve, or capillary density, suggesting proportionate angiogenesis in response to hypertrophy that is age dependent. Similarly, capillary density in response to pressure-induced left ventricular hypertrophy in humans is dependent on the age of onset with an increase in capillary-to-myocyte density in congenital aortic stenosis (23). Furthermore, in another animal model, young rats chronically exposed to carbon monoxide showed an increase in total cross-sectional area of large arteries when killed as adults (22). These data indicate that the developing fetus may be able to accelerate coronary conductance in certain circumstances.

Thus we studied coronary pressure flow relationships in utero to determine if chronic anemia can cause increased coronary conductance in the growing fetus. Because changes in viscosity with chronic anemia may also influence blood flow and coronary conductance, we also sought to determine the role of reduced viscosity by repeating coronary conductance measurements after acute transfusion of anemic fetuses.

MATERIALS AND METHODS

Surgical preparation. All care and procedures were reviewed and approved by the Oregon Health Sciences University Animal Care Committee. General anesthesia was induced in 10 fetal lambs at 118–121 days gestation with intravenous ketamine-diazepam and maintained with 1.5% halothane and a 50% nitrous oxide-50% oxygen mixture as previously described (6). A uterine incision was made, the
right side of the fetal neck was exposed, and polyvinyl catheters, V5 (0.86 mm ID; Bollab, Lake Havasu, AZ) and V8 (1.19 mm ID), were placed in the carotid artery and advanced into the ascending aorta. Two V8 catheters were placed in the jugular vein and advanced to the right atrium. A thoracotomy incision was made in the left fourth intercostal space, and a V5 Silastic-tipped catheter was placed in the hemiazygos vein in accordance with the technique described by Fisher et al. (9). The catheter tip was advanced ~1 cm to the coronary sinus, and care was taken to ligate any collateral branches. The ascending aorta was mobilized by blunt dissection, and an inflatable vascular occluder, generally 10 mm (In Vivo Metric Systems), was placed around the aorta distal to the ductus arteriosus. A second inflatable vascular occluder was placed around the inferior vena cava just outside the pericardium. A pericardial sac was opened and two V5 catheters with 4-mm-long V8 tips were placed in the left atrial appendage. A Doppler crystal (Crystal Biotech, Hopkinton, MA) was placed on the proximal left circumflex coronary artery. A pericardial catheter was left in place, the ribs were reapproximated with suture, and the thoracotomy incision was closed in layers. An amniotic fluid catheter was attached to the thorax, the uterine incision was closed, and the catheters were tunneled to a pouch on the ewe's flank. One million units of penicillin were given in the amniotic space.

Experimental protocol. After a 4-day recovery period, the fetus was studied under control nonanemic conditions. Hydrostatic pressures, including mean and peak carotid arterial, right and left atrial, pericardial, and amniotic fluid pressures were measured with transpac transducers (Abbott Critical Care Systems, Chicago, IL), and the Doppler signal (S45C-4 Doppler flowmeter; University of Iowa Bioengineering) and heart rate were recorded on a Beckman R611 polygraph. Data were digitized (Data Translation, Marlborough, MA) and stored online. All pressures were referenced by the computer to pericardial pressure, and all output data from the hard disk was checked against the original strip chart records.

Blood gases and oxygen content were drawn from the carotid artery and coronary sinus in 3-ml heparinized syringes at the same time and were measured by an IL 482 co-oximeter and 1L 1312 analyzer calibrated at 39°C. An aliquot of whole blood from the carotid artery and coronary sinus was stored at ~80°C for glucose and lactate analysis. Propranolol (1 mg/kg) and atropine (0.5 mg/kg estimated fetal weight) were given intravenously to the fetus, and the carotid arterial blood gases and oxygen content measurements were repeated. Blockade with propranolol and atropine was necessary to minimize baroreceptor-induced heart rate changes during the subsequent pressure-flow studies.

Coronary pressure flow and conductance measurements. Coronary arterial pressure-flow relationships were determined as follows. Left circumflex arterial blood flow was measured over a range of pressures from ~40 mmHg (resting mean arterial pressure) down to 20 mmHg or from ~40 to 65 mmHg by inflating either the inferior vena cava or aortic occluder over ~10 s in a randomized order. Mean hemodynamic pressures and the Doppler electronic signal were sampled every 10 ms, averaged every second, and recorded. After the occluder was released, the fetus was allowed to recover for ~20 min until mean arterial pressure, heart rate, and coronary blood flow had returned to baseline levels. Coronary blood flow was again measured while the other occluder was rapidly inflated in the same manner.

After hemodynamic and Doppler flow returned to baseline levels, an adenosine infusion into the left atria was started. A steady-state coronary blood flow response as gauged from the Doppler signal was generally reached within 4 min. A dose response curve was obtained by increasing the infusion rate to assure maximal vasodilation to adenosine, and an infusion rate was then selected for the remainder of the study. This dose averaged 147 ± 80 µg·kg⁻¹·min⁻¹. When a steady-state coronary blood flow response to adenosine was reached at baseline mean arterial blood pressure, carotid arterial blood and coronary sinus samples were drawn for arterial blood gases and oxygen content. The adenosine infusion was stopped, and the fetus was allowed to recover for 20 min. A maximal conductance curve was then determined as follows. The adenosine infusion was restarted, and once a steady state was reached either the inferior vena cava or aortic occluder (in randomized order) was inflated rapidly over 10 s to reach a mean arterial pressure of 20 or 65 mmHg. Microspheres were injected into the left atrium when the animal recovered at steady-state conditions without an adenosine infusion (1.5 million, 15 µm diameter; NB, Cr, or S) while a carotid arterial reference sample was withdrawn for 2.5 min at a rate of 4.26 ml/min (5).

Anemia and daily sampling. The next day an isovolemic hemorrhage was performed by removing 100 ml of blood and replacing the blood with isotonic saline (5, 6). The blood was collected in a sterile fashion in citrate phosphate dextrose adenine solution and stored refrigerated with penicillin. On each subsequent day the coronary blood flow was recorded, and, carotid arterial and coronary sinus blood gases, oxygen content, hematocrit, and arterial samples for lactate and glucose were taken. An isovolemic hemorrhage was then repeated. Over 6.9 ± 0.9 days the fetal hematocrit was reduced to ~16%, and carotid oxygen content was reduced to ~2.5 ml/dl. At this point, as on the first day of the study, hemodynamic data before and after blockade and during adenosine infusion were recorded, and coronary blood flow, coronary pressure studies before and after adenosine infusion were repeated. As Doppler flow was recorded, a second set of microspheres was injected while adenosine was infused to obtain the highest coronary flow possible. This allowed left ventricular blood flow measurements to be correlated with the Doppler flow signal and to interpolate and not extrapolate blood flow. Seven of the ten fetuses completed the study with functioning catheters and had conductance relationships studied. Three fetuses had a conductance curve measured during adenosine infusion as previously described. To differentiate the effects of the change in viscosity from vessel growth on coronary conductance, we also studied the maximal coronary conductance after red blood cell transfusion in five fetuses. Red blood cells that were stored as described were warmed and then filtered using a Pall rcx1L high-efficiency leukocyte filter (Pall Biomed, East Hills, NY) to remove aggregate material. Over 6.9 ± 0.9 days the fetal hematocrit was reduced to 32%. One hour later, when a steady-state coronary blood flow response to adenosine was reached at baseline mean arterial blood pressure, carotid arterial blood and coronary sinus samples were drawn for arterial blood gases and oxygen content. The adenosine infusion was stopped, and the fetus was allowed to recover for 20 min. A maximal conductance curve was then determined as follows. The adenosine infusion was restarted, and once a steady state was reached either the inferior vena cava or aortic occluder (in randomized order) was inflated rapidly over 10 s to reach a mean arterial pressure of 20 or 65 mmHg. Microspheres were injected into the left atrium when the animal recovered at steady-state conditions without an adenosine infusion (1.5 million, 15 µm diameter; NB, Cr, or S) while a carotid arterial reference sample was withdrawn for 2.5 min at a rate of 4.26 ml/min (5).

Viscosity effects. To differentiate the effects of the change in viscosity from vessel growth on coronary conductance, we also studied the maximal coronary conductance after red blood cell transfusion in five fetuses. Red blood cells that were stored as described were warmed and then filtered using a Pall rcx1L high-efficiency leukocyte filter (Pall Biomed, East Hills, NY) to remove aggregate material. Over 6.9 ± 0.9 days the fetal hematocrit was reduced to ~16%, and carotid oxygen content was reduced to ~2.5 ml/dl. At this point, as on the first day of the study, hemodynamic data before and after blockade and during adenosine infusion were recorded, and coronary blood flow, coronary pressure studies before and after adenosine infusion were repeated. As Doppler flow was recorded, a second set of microspheres was injected while adenosine was infused to obtain the highest coronary flow possible. This allowed left ventricular blood flow measurements to be correlated with the Doppler flow signal and to interpolate and not extrapolate blood flow. Seven of the ten fetuses completed the study with functioning catheters and had conductance relationships studied. Three fetuses had a conductance curve measured during adenosine infusion as previously described. Analysis. The ewe and fetus were then killed with an intravenous injection of pentobarbital sodium. The left and right atria, septum, and ventricles were dissected free and separately weighed, and the radioactivity of the tissues and reference arterial samples were determined by a Micrad gamma scintillation spectrometer. Blood flow was calculated as (radioactive counts/min of 100 g tissue/arterial reference
counts/min) (withdrawal rate). To correct for heart growth over the 7 days of the study, the fetal weight was calculated backward by using the equation \( W = W_0 \cdot e^{(0.08 \text{ days})} \), where \( W \) was the weight at autopsy and \( W_0 \) was the weight on the first day of the study (5). On the assumption of a heart/body weight ratio of 7.3 g/kg fetus, the heart weight was corrected accordingly, and the left and right atria, septum, and ventricular weights were adjusted proportionally. We believe these corrections to be valid as heart weight growth parallels blood volume changes in fetal sheep (1). We have previously measured the rate of growth of blood volume (0.8 ± 0.3%/day) in anemic fetuses and have found that nonanemic fetuses have a heart/body weight ratio of 7.3 ± 1.1 g/kg (5).

Blood lactate and glucose concentrations were measured with membrane-bound enzymatic analysis (Yellow Springs Instruments 23A, Yellow Springs, OH). Lactate and glucose samples from the carotid artery and coronary sinus were measured in duplicate and averaged, and they were repeated if there was more than a 2% difference. Left ventricular consumption of oxygen and substrate fluxes of lactate and glucose were determined from the arterial-venous difference multiplied by the left ventricular free wall blood flow.

In each fetus, linear regression was used to correlate the Doppler Hertz signal to left ventricular blood flow as measured by microspheres. Pressure-flow relationships were described by best-fit polynomial or linear regression to interpolate resting blood flow at 40 mmHg. Coronary reserve at 40 mmHg was determined as the difference between left ventricular blood flow interpolated at 40 mmHg and the interpolated blood flow under resting conditions as (interpolated blood flow at 40 mmHg when anemic) – (interpolated blood flow after transfusion)/(interpolated blood flow when anemic – interpolated blood flow on the first day of study). The remainder was attributed to growth. Data such as coronary reserve, slope of the adenosine conductance, and lactate and glucose consumption in fetuses on the first day of study that were not anemic and in fetuses that were studied after 7 days of anemia were compared by means of paired t-tests. Hemodynamic data taken at baseline and after blockade and adenosine infusion were compared by means of ANOVA for repeated measures and post hoc Student-Newman-Keuls test when an \( F \) statistic was significant. Comparisons between nonanemic and anemic fetuses before blockade and after blockade during adenosine infusion were made by paired t-tests and corrected with the Bonferroni inequality. All data are presented as means ± SD, and means are considered different at \( P < 0.05 \).

RESULTS

Hemodynamic data in seven fetuses at the start and end of the study are shown in Table 1. Baseline hemodynamic data on the first day of the study were similar to data in chronically instrumented fetal sheep at a similar gestational age (5, 6, 24, 25). Blood pressures and heart rate were unaffected in control and anemic fetuses by either blockade or adenosine infusion, with the exception of a slight increase in right atrial pressure after adenosine infusion on the first day of the study. Baseline carotid arterial pH was lower in anemic fetuses compared with controls (7.31 ± 0.06 vs. 7.36 ± 0.02, \( P < 0.05 \)), and as expected the PO\(_2\) was less (17.6 ± 1.4 vs. 21.6 ± 2.5 mmHg, \( P < 0.01 \)), as was the oxygen content (2.6 ± 0.4 vs. 7.3 ± 1.4 ml/dl, \( P < 0.001 \)). The anemic fetuses had a lower hematocrit (15.8 ± 2.4%) compared with controls (30.6 ± 2.7%, \( P < 0.02 \)). On the first day of the study, resting left ventricular coronary blood flow increased with adenosine infusion from 202 ± 60 to 664 ± 208 ml·min\(^{-1}\)·100 g\(^{-1}\) (\( P < 0.001 \)). By the last day of the study, resting left ventricular coronary blood flow in the anemic fetuses (726 ± 169 ml·min\(^{-1}\)·100 g\(^{-1}\)) was similar to the maximally induced flow in controls and increased further with adenosine infusion to 1,162 ± 250 ml·min\(^{-1}\)·100 g\(^{-1}\) (\( P < 0.001 \)). As expected, left ventricular oxygen extraction decreased with adenosine infusion. The product of heart rate and aortic systolic pressure was not affected, although left ventricular oxygen consumption increased with adenosine infusion in the control fetuses from 421 ± 113 to 551 ± 178 µM·min\(^{-1}\)·100 g\(^{-1}\) (\( P < 0.02 \)) and was higher in the anemic fetuses at baseline compared with control baseline values before blockade.

In Fig. 1 pressure flow measurements in a single fetus are shown, illustrating coronary blood flow changes in response to adenosine infusion before and after anemia.

Gestational age, fetal heart weight, and metabolic data obtained in control and anemic fetuses on the

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Table 1. Hemodynamic data in control and anemic fetuses

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Anemic</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Blockade</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>44.6 ± 3.1</td>
<td>45.3 ± 4.5</td>
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<tr>
<td>Right atrial pressure, mmHg</td>
<td>3.2 ± 1.3</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>3.9 ± 1.4</td>
<td>4.1 ± 1.1</td>
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<tr>
<td>Heart rate, bpm</td>
<td>185 ± 16.5</td>
<td>173 ± 18.9</td>
</tr>
<tr>
<td>Carotid arterial pH</td>
<td>7.36 ± 0.02</td>
<td>7.35 ± 0.06</td>
</tr>
<tr>
<td>Paco(_2), mmHg</td>
<td>50.5 ± 3.7</td>
<td>50.4 ± 2.8</td>
</tr>
<tr>
<td>PaO(_2), mmHg</td>
<td>50.6 ± 3.2</td>
<td>50.9 ± 3.0</td>
</tr>
<tr>
<td>Oxygen content, ml/dl</td>
<td>7.3 ± 1.4</td>
<td>7.1 ± 1.4</td>
</tr>
<tr>
<td>LV blood flow, ml·min(^{-1})·100 g(^{-1})</td>
<td>202 ± 60</td>
<td>621 ± 72</td>
</tr>
<tr>
<td>LV oxygen consumption, µM·min(^{-1})·100 g(^{-1})</td>
<td>421 ± 113</td>
<td>411 ± 137</td>
</tr>
<tr>
<td>LV oxygen extraction, %</td>
<td>65 ± 2.5</td>
<td>66 ± 6.5</td>
</tr>
<tr>
<td>HR-peak pressure product, bpm·mmHg</td>
<td>10,659 ± 583</td>
<td>10,619 ± 815</td>
</tr>
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</table>

Data are means ± SD; \( n = 7 \) fetuses. *\( P < 0.05 \) compared with baseline values within each group; †\( P < 0.05 \) compared with control baseline values. Blockade was with atropine-propranolol. LV, left ventricular; bpm, beats per minute.
Steady-state resting blood flow, hematocrit, myocardial oxygen consumption, and lactate and glucose consumption across the left ventricle measured on a daily basis are shown in Fig. 3. The daily isovolemic hemorrhage reduced the hematocrit in a gradual manner. Oxygen consumption remained relatively constant while left ventricular blood flow increased. Of note, in only 2 of 46 samples was there net lactate production. Both these instances were on the day before the final study; the carotid arterial pHs were 7.38 and 7.39, and the carotid lactate levels were 6.8 and 4.1 mM, respectively. In all other circumstances, despite blood lactate levels that reached 17 mM, on a net basis lactate was consumed. The mean arterial-venous difference for lactate throughout the study, 0.374 ± 0.029 mM (mean ± SE), was greater than zero (P < 0.001). This was calculated by averaging the arterial-venous differences for lactate drawn on different days for each fetus to obtain a mean arterial-venous difference for each animal.

Hemodynamic data in five anemic fetuses in which stored red blood cells were transfused to restore the hematocrit to control levels is shown in Table 3. Two hours after transfusing the fetus to a hematocrit of 29.8 ± 2.9%, resting left ventricular blood flow fell to 314 ± 143 ml·min⁻¹·100 g⁻¹, and mean arterial and atrial pressures as well as left ventricular oxygen consumption were similar to data obtained before the transfusion. In response to adenosine infusion, left ventricular blood flow at 40 mmHg was significantly less than in the anemic state (751 ± 210 ml·min⁻¹·100 g⁻¹). The change in viscosity attributed to 57.3 ± 18.9% of the total increase in maximal conductance induced by anemia. Conductance changes in a single fetus in response to adenosine infusion on the first day of the study at a hematocrit of 32%, on the last day of the study at a hematocrit of 16.5%, and after transfusion to a hematocrit of 28% are shown in Fig. 4. In these five fetuses, the slope of the conductance after transfusion.
(21.7 ± 6.5 ml·min⁻¹·100 g⁻¹·mmHg⁻¹) was less than the slope of the conductance in the anemic state (30.7 ± 13.8 ml·min⁻¹·100 g⁻¹·mmHg⁻¹, P < 0.025), but not significantly different from the slope on the first day of the study (17.7 ± 8.9 ml·min⁻¹·100 g⁻¹·mmHg⁻¹).

Finally, to test if adenosine infusion produced maximal vasodilation, in two other studies we placed a plastic bag over the maternal ewe’s head while 10% oxygen and 3% carbon dioxide was infused into the bag at a rate of 10 l/min for 10 min. This lowered the fetal carotid arterial oxygen content from 8.3 to 3.0 ml/dl in one study and from 6.7 to 2.4 ml/dl in the other. We then

Table 3. Hemodynamic data obtained 2 h after transfusing anemic fetuses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value ± SD</th>
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<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>49.0 ± 2.9</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>171 ± 10.2</td>
</tr>
<tr>
<td>Carotid arterial pH</td>
<td>7.30 ± 0.04</td>
</tr>
<tr>
<td>Pco₂, mmHg</td>
<td>49.8 ± 3.5</td>
</tr>
<tr>
<td>Pao₂, mmHg</td>
<td>21.2 ± 0.8</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>29.8 ± 2.9</td>
</tr>
<tr>
<td>Oxygen content, ml/dl</td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>LV resting blood flow, ml·min⁻¹·100 g⁻¹</td>
<td>314 ± 143</td>
</tr>
<tr>
<td>LV oxygen consumption, µM·min⁻¹·100 g⁻¹</td>
<td>505 ± 85</td>
</tr>
<tr>
<td>LV blood flow at 40 mmHg transmural with adenosine infusion, ml·min⁻¹·100 g⁻¹</td>
<td>751 ± 210</td>
</tr>
<tr>
<td>LV blood flow increase at 40 mmHg transmural, %</td>
<td>57.3 ± 18.9</td>
</tr>
<tr>
<td>Attributed to viscosity</td>
<td>42.6 ± 18.9</td>
</tr>
<tr>
<td>Attributed to growth</td>
<td></td>
</tr>
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</table>

Data are means ± SD; n = 5 fetuses.
repeated the conductance measurements during adenosine infusion and hypoxia to determine if hypoxia further increased coronary blood flow. A representative response is shown in Fig. 5. The conductance relationships were not significantly altered, suggesting that at the dose infused adenosine did induce maximal coronary vasodilation.

**DISCUSSION**

The primary observation of this study was that, with a large reduction in oxygen content and hematocrit brought about by inducing a state of chronic anemia, coronary conductance increased and coronary reserve was maintained. These data are physiologically relevant inasmuch as resting coronary blood flow, adjusted for growth and expressed per unit weight of left ventricle, was greater than maximal left ventricular blood flow on the first day of the study and increased further with adenosine infusion. Thus these data reflect a significant adaptation of the fetus to chronic anemia. Other cardiac adaptations that are necessary for the fetus to survive during graded reductions in hematocrit include 30–50% increases in right ventricular stroke volume, cardiac output, heart-to-body weight ratio, capillary dimensions, and a nearly sixfold rise in coronary blood flow (5, 6). In this study, we found that the heart to body weight ratio was not elevated, despite a 3.5-fold increase in resting blood flow with anemia. It is likely that this is a result of the fact that the degree of anemia was not as severe as in previous studies, in which the mean oxygen content was reduced to 1.8 and 2.1 ml/dl (5, 6).

Several prior observations suggest increased coronary angiogenesis in fetuses made hypoxic and/or anemic. First, VEGF protein expression increased 4.5-fold, messenger ribonucleic acid expression increased 3.2-fold, and HIF increased 3.8-fold in anemic fetal heart tissue (16). HIF-1, a heterodimeric basic helix-loop-helix transcription factor regulated by oxygen tension, has been shown to initiate VEGF transcription by binding to a hypoxia response element –1 kb 5′ to the start site of the human VEGF gene (12). Second, in studies of fetal sheep made chronically hypoxic by lowered maternal inspired oxygen fraction, the maximal coronary blood flow response to adenosine infusion was nearly 50% greater than in normoxic fetuses (25). Third, in both fetal sheep and young rats made chronically anemic, morphometric changes in the coronary vessels include an increase in capillary luminal diameter and either maintenance or increase in capillary density along with a decrease in capillary luminal diameter and either maintenance or increase in capillary density along with a decrease in capillary density (10). Finally, we observed that measurements of steady-state coronary blood flow, conductance, and flow interpolated at 40 mmHg all showed increases with anemia. Significantly, despite the increase in resting coronary blood flow with anemia, coronary reserve interpolated at 40 mmHg was maintained. Taken together, these data suggest increased coronary vascular growth of resistance vessels. This is supported by the recent observation that maximal coronary blood flow responses to adenosine infusion increased 20% in young pigs that underwent exercise training for 16 wk (28). Capillary density increased at 3 wk and then returned to baseline levels as small arterioles (20–30 µm) increased at 16 wk. These investigators concluded that capillaries developed into arterioles.

The large increase in resting myocardial blood flow we measured was necessary to maintain oxygen consumption because left ventricular oxygen extraction in normal fetal sheep is 65% (9). We observed that left ventricular oxygen extraction did not change during anemia as the oxygen content fell by two thirds. In the anemic fetuses, left ventricular oxygen consumption actually increased. Similar results have been described in chronically hypoxic fetal sheep (10) as well as in adult dogs made chronically anemic (2). In these studies, coronary blood flow increased, whereas oxygen extraction and consumption stayed unchanged. For comparison, the oxygen extraction rate in fetal sheep brain normally is 34% (3). At a similar degree of anemia, cerebral oxygen consumption is maintained, whereas cerebral blood flow only doubles (5). Thus vascular responses vary among different organs, implying local regulatory control of vessel growth.

We also found that there was no change in the product of peak systolic pressure and heart rate despite an increase in left ventricular myocardial oxygen consumption between control and anemic baseline states. The rate-pressure product is an index of ventricular power and should correlate with oxygen consumption (9). One likely explanation of this discrepancy is that the increase in oxygen consumption reflects changes in heat production and contractility that are not measured by indexes of external work (8). Measurements of internal work would necessitate pressure volume loop studies, which we did not perform. We also observed net
consumption of lactate despite marked reductions in arterial oxygen content and increases in lactate blood levels. Although lactate consumption on the final day of the study in the anemic fetuses could not be distinguished from zero, we believe the large standard deviation we found is in part caused by the high left ventricular blood flow rate. Net lactate consumption is supported by the finding that arterial-venous differences for lactate were significantly greater than zero. This suggests that aerobic metabolism was maintained. In agreement, levels of lactate dehydrogenase activity are increased in chronically hypoxic fetal sheep (20), as are levels of HIF-1 (16). The latter is known to increase transcription of lactate dehydrogenase in cultured cells (26, 27). Also, in fetal lambs in which arterial oxygen content has been reduced by 50%, myocardial consumption of oxygen, glucose, and lactate are not altered, with lactate accounting for 60% of the substrate (9, 10). Thus the chronically anemic fetal heart continues to consume peripherally generated lactate.

To investigate the role of reduced viscosity on blood flow in vivo, we studied conductance relationships in anemic fetuses transfused to a normal hematocrit and found that maximal coronary blood flow fell approximately by half. This is in agreement with a previous study in which half of the increase in cardiac output seen with chronic anemia was calculated to be attributed to decreases in viscosity (5). Baer et al. (2) studied conductance relationships in adult dogs made chronically anemic. In paced, open-chest animals, when the hematocrit was increased from 17% to 34%, these investigators noted that resting mean circumflex flow decreased from 113 to 70 ml · min⁻¹ · 100 g⁻¹ (heart), and coronary conductance fell from 10.7 to 8.6 ml · min⁻¹ · 100 g⁻¹ · mmHg⁻¹. Maximal coronary vascular conductance decreased linearly as hematocrit increased over a range from 12 to 80%. Drossner and Aversano (7) measured diastolic coronary-pressure flow relationships in adult dogs and found that reduction in hematocrit from 46 to 32% increased conductance 60% at 40 mmHg. Similarly, peak reactive hyperemic flow to coronary occlusion (a measure that is less than adenosine-induced coronary vasodilation) increased from 135 to 225 ml/min when the hematocrit was reduced from 41 to 19% acutely (4). Taken together, these data suggest that ~50% of the increase in coronary blood flow observed in this study can be attributed to decreases in red blood cell content.

In agreement with the findings of Flanagan et al. (11) that lambs with aortic stenosis maintained maximal coronary conductance and coronary reserve compared with adult sheep with aortic stenosis in which left ventricular maximal coronary conductance and coronary reserve decreased by two-thirds, we found that coronary reserve per gram of left ventricle was conserved. Coronary reserve in this study was similar to measurements made in lambs (11). Maximal coronary conductance however, in the nonanemic fetuses (18.2 ml · min⁻¹ · 100 g⁻¹ · mmHg⁻¹) was approximately twice that observed by Flanagan et al. (11) in lambs (8 ml · min⁻¹ · 100 g⁻¹ · mmHg⁻¹). We are aware of only one other study that measured coronary conductance in fetal sheep; the conductance measured in a single fetus was 25 ml · min⁻¹ · 100 g⁻¹ · mmHg⁻¹ (25). This might suggest that the still-growing fetus is capable of coronary angiogenic responses to hypoxia that are greater than those of the newborn lamb or adult. In support of this, fetal sheep compared with adults have the same myocardial oxygen consumption but twice the resting coronary blood flow at one-half the mean arterial pressure (9, 11, 25).

One factor that we did not investigate was the role of endothelial cell-dependent effects on the observed increase in resting blood flow and conductance. Both nitric oxide and adenosine are known to mediate coronary vasodilation. Coronary adenosine release is increased after nitro-L-arginine methyl ester (L-NAME) administration in adult dogs (17), and coronary conductance as measured from hyperemic responses in an adult rabbit Langendorff heart preparation decreased after L-NAME infusion (21). Reller et al. (24) studied fetal sheep and found that basal coronary blood flow (231 ml · min⁻¹ · 100 g⁻¹) increased to a greater extent after acute hypoxia than after adenosine infusion (1,020 vs. 802 ml · min⁻¹ · 100 g⁻¹). Myocardial blood flow fell after L-NAME infusion to levels achieved with adenosine infusion. Nitric oxide has also been reported to increase basic fibroblast growth factor in coronary venular endothelium, which would provide another mechanism linking flow and angiogenesis (29). These data suggest that nitric oxide may play a role in fetal coronary responses to hypoxia and raise the possibility of vasodilation leading to angiogenesis through mechanisms parallel to HIF-1 or VEGF.

We conclude that coronary conductance is increased in the fetus made chronically anemic and that, in addition to an increase in resting blood flow, coronary reserve is maintained. The fall in viscosity can account for half of the increase in conductance; the remainder is attributed to angiogenesis. The increase in coronary blood flow allows myocardial oxygen consumption and cardiac function to be conserved.

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