Coronary flow regulation in the fetal sheep

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Thornburg, Kent L., and Mark D. Reller. Coronary flow regulation in the fetal sheep. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1249–R1260, 1999.—The two ventricles of the fetal sheep heart have anatomic and biochemical differences that account for their differing functional capabilities and blood flows. Coronary flows to both ventricles have been measured using radiolabeled microspheres [or left ventricular (LV) flow, by Doppler sensor on the circumflex coronary artery] during experiments of pressure loading and chronic and acute hypoxemia. Blood flow to the left ventricle with its lower wall tension is about two-thirds the flow per gram compared with the right ventricle (RV). Acute systolic pressure loading of the RV to its maximal work capability stimulates flow to double (from ~250 to 500 ml·min⁻¹·100 g⁻¹), but to a level less than stimulated by adenosine (750 ml·min⁻¹·100 g⁻¹). At all RV work loads, LV flow remains at two-thirds RV flow. Resting myocardial flow levels in fetuses that have been chronically hypoxemic are similar to maximal adenosine-stimulated flows of normal fetal sheep. This flow augmentation is evidently due to vascular remodeling because a normal “flow reserve” of ~500 ml·min⁻¹·100 g⁻¹ during adenosine administration remains. Acute hypoxemia stimulates myocardial flow to extraordinary levels (~1.5 l·min⁻¹·100 g⁻¹), levels larger than can be obtained with chemical dilation alone. LV flows do not exceed adenosine-stimulated flows when nitric oxide synthase is antagonized. We conclude 1) fetal RV coronary flow increases with RV work but to levels less than during adenosine stimulation; 2) the fetal heart is designed to accommodate extremely high flows in response to acute hypoxemia, partially through large production of nitric oxide; and 3) the fetal coronary tree is dramatically remodeled in response to chronic hypoxemia.

Fetal heart; nitric oxide; adenosine; law of Laplace; fetal hypoxemia; wall stress; heart ventricle; coronary remodeling

B E F O R E D I S C U S S I N G the regulation of blood flow to immature myocardium, it may be helpful to recall the physiological features that underlie fetal cardiac physiology. The technology to study the chronically prepared fetal heart was not available until the 1970s. Therefore, many aspects of physiology of the normal fetal circulation were not determined until the 1970s and 1980s. Even now our understanding of fetal cardiovascular physiology is primitive compared with adult cardiovascular physiology. The fetal sheep heart has been studied, perhaps more than any immature mammal heart, and, unless so indicated, the data presented herein come from the sheep model during the last 15 days of gestation. There are undoubtedly differences between the immature sheep and human hearts, but many of the salient features that have been determined in sheep are known to be applicable to the human heart as well as to other species.

F E T A L C I R C U L A T I O N

The fetal circulation operates as two parallel circuits, differing from the adult arrangement where pulmonary and systemic circuits are in series (16). Therefore, by convention, the output of the fetal heart is reported as the combined output of the two ventricles. As is the case in the adult mammal, all of the upper body flow in the fetus is returned to the right ventricle via the superior vena cava. However, a substantial portion of the well-oxygenated inferior vena cava flow is shunted away from the right ventricle and into the left ventricle through the foramen ovale where it is distributed to the upper body (21). Therefore, the heart and brain are assured of receiving oxygen-rich blood. Blood flowing from the right ventricle has a low oxygen saturation and output joins aortic flow via the ductus arteriosus and perfuses lower body and placenta for reoxygenation (66).
DIFFERENCES BETWEEN RIGHT AND LEFT VENTRICLES

Throughout medical history the two fetal ventricles were viewed to be anatomically identical. Dawes summarized the current thinking of 1968 (16)

The two sides of the foetal heart are of much the same shape and size, like the twin kernels of a nut as Harvey put it, and thus very different from the adult. So the right and left sides of the heart have about the same capacity (as Pohlman showed in the foetal piglet, 1909; 58), are filled at approximately the same pressure, eject blood against the same arterial pressure and so might reasonably be expected to have about the same output.

It should not be surprising that investigators initially felt that the ventricles were similar. Compared with the adult heart, where the thin-walled right ventricle looks like an architectural afterthought next to the dominant thick-walled left ventricle, the two ventricular chambers and free walls of the fetal mammalian heart look relatively similar. On closer look, however, substantial anatomic and cytological differences between the two ventricles are evident and these carry important physiological consequences.

Figure 1 shows examples of measurements of right ventricle output as a fraction of the biventricular output in early studies of the sheep heart. It was nearly 30 years ago that Assali and colleagues (3) first showed that the right ventricle had a larger stroke volume than the left ventricle, whereas Dawes et al. (17) had shown the opposite. Most experts now agree that the right ventricle ejects between 60 and 70% of the biventricular output in sheep with a lesser but clear-cut dominance by measuring simultaneous outputs of the two ventricles using calibrated electromagnetic flow sensors. It should be noted that the output measured from the left ventricle could not include coronary flow because the sensors were necessarily placed distal to the coronary ostia. It was possible to change the filling pressures of the two ventricles by adding and withdrawing blood and/or saline so that simultaneous function curves could be constructed (30). The ventricular function curve is one way of investigating the relationship between the filling of the ventricle (preload) and the output of the ventricle (10). Our function curves (Fig. 2) were not “pure” because there were concomitant changes in arterial pressure during the generation of the curves and because it was difficult to get perfect transmural pressure measurements in chronically prepared animals (75, 76). Nevertheless, these reproducible function curves were highly instructive. These experiments indicated that right ventricular stroke volume is greater than is the left because the right ventricle operates on a completely separate and elevated function curve where the right stroke volume is higher at all filling pressures (Fig. 2).

To further investigate the elevation of the right ventricular function curve, we made anatomic measurements of hearts that had been fixed at their resting filling pressures. Table 1 shows that the right ventricular chamber is larger than the left chamber as suggested by pressure-volume curves (55, 67). That is, the right ventricular curve is always right-shifted compared with the left (56) so that the right ventricular chamber contains more blood than the left chamber at any given common filling pressure. With similar ejection fractions, the larger right ventricle ejects up to 50% more blood each beat.

The anatomic differences between the ventricles affect their function in other ways. The law of Laplace predicts that the right ventricle will have a higher resting wall stress due to its larger radius of curvature-to-free wall thickness ratio (r/h, Table 1). Figure 3 shows the rapid decrease in fetal right ventricular...
Stroke volume with increasing pulmonary arterial pressure compared with the left ventricle, which is hardly affected by the same increases in aortic pressure. This finding is important because it demonstrates that the right ventricle is much less able to eject against increasing arterial pressure than is the left. These data also suggest that the fetal right ventricle performs more work than the left and must meet more severe metabolic demands whenever fetal arterial pressures are increased.

The histological features of the ventricles also differ before birth to serve their separate pump functions (46). The working myocytes of the right ventricle are larger than those of the left, and the capillary luminal area is greater (73). These differences between the ventricles reverse after birth so that left ventricular myocytes become larger than those on the right side (73) as left ventricular work load is increased dramatically during postnatal life (72).

In summary, the right ventricle is different from the left, with a larger chamber volume, a larger radius-to-wall thickness ratio, a higher free wall stress, and greater sensitivity to increases in arterial pressure. The right ventricle performs more work and has higher metabolic requirements in the face of stresses when arterial pressure is increased.

### REGULATION OF CORONARY FLOW

Hemodynamics. The regulation of coronary flow in adult myocardium has been studied extensively and reviewed frequently (8, 22, 34, 38, 49). Intensive investigation of coronary control has been driven by scientific curiosity and the reality that coronary disease is the major cause of death in western societies. However, our present state of knowledge is based almost exclusively on study of mature myocardium. The fact that coronary development may predispose adults for coronary disease (4) suggests that further investigation of prenatal coronary physiology may be important. The extent to which adult coronary findings apply to embryonic and fetal myocardium must yet be determined.

The principal determinants of mean flow through any organ are driving pressure and resistance to flow. In the heart, resistance to flow is carefully regulated by integrating a number of interdependent factors (22, 38), including tissue pressure, myocardial metabolism, myogenic responses, neural and humoral stimulation, and flow-dependent shear stress (53). Chilian and colleagues (38) have provided good evidence that there are "microdomains" (vessels in different size categories) that are regulated uniquely from neighboring domains. Thus microvessels of one size may constrict under a stimulus that dilates larger vessels and vice versa. The integration of all microdomains under a given set of circumstances determines the actual resistance to flow that becomes physiological reality.

The standard textbook explanation of flow as the ratio of driving pressure (inflow minus outflow pressure) and resistance is useful for understanding mean flow through a stationary hypothetical organ. However, it is hard to imagine an organ being further from the hypothetical than is the beating heart. For example, the driving pressure for the myocardium is difficult to define for any given portion of the cardiac cycle, because, although the inflow pressure to the coronary tree is aortic pressure, the outflow pressure is not known with certainty for any part of the cycle. Tissue pressure acts as a powerful "surrounding pressure" during myocardial contraction, making the outflow pressure moot for that moment. Tissue pressure is also dependent on the degree of hydration of the extracellular compartment. The driving pressure may be better defined as the difference between aortic and diastolic pressure, where flow becomes zero. In the adult, this may be as high as 40–50 mmHg (6).
Autoregulation. Increasing inflow pressure to the coronary bed does not cause a proportional increase in flow to working heart muscle. Instead, flow tends to stay constant over a wide range of perfusion pressures through autoregulation (52). The autoregulation term was defined by Johnson (1964) (37) as "the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure." In adult dog, autoregulation may be effective over a pressure range of some 70 mmHg (33). The autoregulatory range for the fetal heart is unknown. The mechanisms that underlie autoregulation in adult hearts are not known with certainty. The autoregulatory response is thought to be mediated via locally produced metabolic factors that directly affect vascular smooth muscle, primarily vessels of $<150 \mu$m diameter (38). Although most investigators are convinced that tissue adenosine is very important as a regulator of vascular resistance in the heart, evidence also points to other unidentified local regulators that may be crucial participants in the autoregulatory mechanism (18). However, myogenic responses (constriction stimulated by increased intraluminal pressure and relaxation with decreased pressure) may also be simultaneously invoked at all levels throughout the myocardium (38).

Matching flow to metabolic need. In adult myocardium, coronary flow is closely linked to metabolic need. It is well known that the total oxygen demand includes a requirement for basal metabolic function and excitation-contraction coupling, as well as the potential energy in the myocardial wall after ejection. Changes in factors that increase metabolic need such as wall stress, heart rate, or contractility will stimulate increases in coronary flow through resistance changes in the coronary bed (12, 19). In accordance with the adenosine hypothesis, as proposed by Berne in 1963 (7), adenosine is the key regulator of metabolically activated flow alterations. In this model, adenosine is increasingly released as working myocardial cells increase metabolic activity or as oxygen becomes in short supply. Adenosine then diffuses among microcirculatory elements, causing coronary vasodilation. Recent evidence indicates a powerful relationship between interstitial adenosine concentration and coronary flow (74). Thus adenosine could be the primary tissue signaling molecule for matching flow to metabolic need. There are a number of factors generated within the myocardium that alter coronary resistance that are potential candidates for participating in the autoregulatory mechanism. These include nitric oxide (NO; 23, 29), endothelin (79), prostacyclin (23), atrial natriuretic factor (28), bradykinin (23), and angiotensin II (29). Recent experiments showed that cocaine administration to pregnant ewes causes dilatation of the fetal coronary bed without fetal hypoxemia (14, 56).

Coronary reserve. If the coronary bed can be dilated to its maximum by an exogenous chemical agent, then the difference between the resting coronary flow and the maximum can be defined as the coronary vascular reserve (2, 11, 33). Figure 4 shows two theoretical pressure flow curves in the adult dog, one at maximal chemical dilation (curve D) and one while the coronary bed is under autoregulatory control (curve A). At any pressure, the difference between the A curve and the D curve is the flow reserve (e.g., $R_1$ or $R_2$, Fig. 4). This figure illustrates several points. 1) The calculated flow reserve value is highly dependent on the perfusion pressure chosen for the measurement. Thus a reserve value ranging from 0 ml/min at 25 mmHg perfusion pressure to ~400 ml/min at 125 mmHg can be obtained in the same heart preparation, depending on which pressure is chosen. The take-home lesson is that the perfusion pressure must be defined for comparisons between experimental conditions. 2) A shift in either the A curve or the D curve will alter the calculated value of flow reserve at any perfusion pressure, and, therefore, flow reserve measurements must be interpreted in light of such possibilities (34).

Measuring coronary flow in the fetus. Coronary flow is difficult to measure in the fetus because of the small size of the fetal heart and because the heart is less accessible than is the adult heart. In experimental animals, fetal coronary flow is usually measured by the microsphere method as introduced by Rudolph and Heymann (69). This method takes advantage of the fact that small (15 µm) plastic spheres will distribute themselves in proportion to tissue blood flow when injected and thoroughly mixed in the left ventricle. Because the spheres are too large to traverse a capil-
lary, they are trapped in tissue in proportion to the flow to that tissue. The number of spheres trapped in a portion of tissue can be quantified by detecting a sphere label, such as radioactivity, color, or fluorescence.

Coronary flows in the fetal heart can also be studied by installing a cuff-type Doppler sensor around the proximal left main coronary artery or the circumflex coronary artery so that flow velocities can be measured during changing experimental conditions (Fig. 5). The distribution of the circumflex artery includes most of the left ventricular free wall and a small portion of the septum. The output (Doppler shift) of the flow sensor correlates nicely with flow measured by the microsphere method (63). Figure 6 shows the flow-velocity profile of the circumflex artery under resting conditions. The Doppler method suffers from several liabilities. For a Doppler shift to correlate well with flow, the diameter of the vessel must remain constant during the measurement. Furthermore, the Doppler sensor only measures flow velocities for the vessel that it surrounds so that right and left ventricular flows cannot be compared unless a probe is placed on both main coronary arteries. Doppler shifts must also be calibrated to yield true flow. This is usually done by measuring a wide range of flows by the microsphere method while recording the Doppler shift at each flow. Once calibrated, the Doppler sensor allows continuous flow measurement. Right ventricular myocardial flows are measured by the microsphere method while left ventricular flows use both Doppler and microsphere methods.

Myocardial oxygen consumption and coronary flow. The list of determinants of myocardial oxygen consumption is long and includes work load, heart rate, metabolic state of the myocardium, and many other factors. The multifactorial nature of oxygen consumption has made it an ongoing area of intense study in the adult (12, 15, 20, 43, 68, 70). In contrast, few studies on oxygen consumption in the embryonic and fetal hearts have been reported.

Most of the foundational work for our understanding of fetal blood flow and oxygen consumption was carried out during the previous decade by Fisher et al. (24–26). They were the first to sample fetal coronary sinus blood on a chronic basis and to measure changes in left ventricular oxygen consumption, oxygen delivery, and substrate utilization during the perinatal period and in adult life. The significant finding of their work is that myocardial oxygen consumption is similar in fetuses (9 ml·min⁻¹·100 g⁻¹) but with a striking 40% increase in the neonate when oxygen consumption is very high. They also found that carbohydrate (lactate) accounts for a significant portion of the myocardial fuel needs in the fetal sheep, whereas it accounts for about one-third of the fuel needs of the adult heart, the remainder being mostly lipid.
Because of its low partial pressure of oxygen, fetal arterial blood carries about one-half the oxygen found in adult blood. Therefore one might expect that coronary flow would be twice that found in the adult. Fisher et al. (26) used the radiolabeled microsphere method to show that, indeed, resting blood flow to the ventricles of the fetus is roughly twice that of adult levels (Fig. 7). This figure also shows several other features of myocardial flow during the life of an individual. First, right ventricular flow is higher than left ventricular flow in the fetus. This fits with the known differences in wall tension and work load as mentioned above. Second, as work load dominance switches from the fetal right ventricle to the left ventricle after birth, left ventricular flow is increased and right flow decreases (72). Finally, blood flow per unit heart tissue weight decreases from the newborn period to adulthood. Fisher et al. (25) showed that left ventricular myocardial oxygen delivery is similar during the fetal period to levels found in the adult but they found an increase in myocardial oxygen delivery during neonatal life when left ventricular work loads and body oxygen needs are especially high. On the other hand, right ventricular oxygen delivery decreases from fetal levels throughout life (25).

Myocardial flow with right ventricular systolic load. Figure 8 shows that right ventricular stroke volume decreases with increasing pulmonary arterial pressure as the proximal main pulmonary artery is acutely constricted by an occluder. Stroke volume decreases as pulmonary arterial pressure goes up until the heart generates a pressure where the right ventricle fails to eject and stroke volume falls precipitously (63). This sudden, reproducible drop in function at a particular pressure, designated as the “toleration point,” is a feature of right ventricular function in the fetal sheep. Could oxygen delivery be limiting the function of the right ventricle at the toleration point? An acute increase in right ventricular pressure in the adult circulation is associated with a significant coronary vasodilatory response (27, 77). Would this be true for the fetus? The flow limitation question was addressed by increasing systolic load in increments between the resting pressure and the toleration point in seven fetuses and measuring coronary flow by the microsphere method (32) at each pressure increment. To check whether a maximal work load would use all of the coronary flow reserve that could be theoretically available to the ventricle, adenosine was infused into the left atrium at a rate of 60 µg·min⁻¹·kg⁻¹ (based on dose-response studies) to maximally dilate the coronary bed.

Figure 9 shows that right ventricular flow increased as work load increased. This finding was expected (13, 34). However, there were a number of unexpected findings from these studies. For example, left ventricular flow followed right ventricular flow as a constant proportion, 0.65 ± 0.02 (SD), although loading conditions for the left ventricle and coronary perfusion pressure did not change significantly. The interdependence of coronary flow in the two ventricles has not been studied in the fetus so that the mechanism of left

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Fig. 7. Myocardial flows in fetus, newborn, and adult sheep. [Adapted with permission from Fisher et al. (24).]

Fig. 8. Plot of right ventricular stroke volume (ml/kg)-mean pulmonary arterial pressure relation in a single fetus. Increases in pulmonary arterial pressure were obtained by pulmonary occlusion. [Borrowed with permission from Reller et al. (63).]

Fig. 9. Ventricular myocardial flow at 4 different pressure loads (Control – P4) and during adenosine administration (Ad). Control had a mean pulmonary arterial pressure of 53 mmHg, P2 is 57 mmHg, P3 is 63 mmHg, P4 is 71 mmHg. [Reproduced with permission from Reller et al. (63).]
ventricular flow change with right ventricular loading remains a mystery. However, the mechanical interaction between the ventricles has been investigated in the newborn sheep (51). Also note that the maximal flow that the ventricle could generate at the toleration point (Fig. 9, P4) was significantly less than the flow obtained with chemical dilation. It appears that the ventricle is not able to take advantage of flow reserve even at the point where the ventricle is failing to eject. This suggests, but does not prove, that oxygen delivery is not the limiting factor causing the acute right ventricular failure in the face of maximal systolic load.

This study also showed that flow increased linearly with increases in metabolic activity as judged by the product of heart rate and peak systolic pressure (Fig. 10). This so-called “double-product” or “rate-pressure product” is commonly used as a quick estimate of changes in oxygen consumption for the adult heart (45), although its use is approximate at best. It has been shown that heart rate and pressure equally and independently affect myocardial left ventricular oxygen consumption in the immature heart as well (71) and that the rate-pressure products of the fetus and adult are similar in magnitude (25). Unfortunately, it is not possible to sample venous blood draining the right ventricle to measure oxygen uptake in that ventricle. Therefore testing the correlation of the rate-pressure product and right ventricular oxygen consumption was not possible in this study.

In the adult heart there are regional variations in flow across the free wall of the two chambers. In the mature left ventricle, subendocardial flow decreases more than subepicardial flow during ischemic episodes. Even though the free walls of the near-term fetal heart are on the order of 4 mm in thickness (Table 1), it is possible to separate the inner and outer layers of the myocardium to compare flows (55, 56). Table 2 shows endocardial to epicardial flow ratios with the increases in right ventricular systolic pressure load. Note that no significant changes were found in the ratio even with severe right ventricular systolic pressure loading.

Coronary flow with chronic fetal hypoxemia and hypercapnia. To see whether chronic hypoxemia and hypercapnia would alter coronary flow regulation, four near-term fetuses that were too hypoxic to be considered normal, were studied 7 days after surgery (64). In these fetuses, the carotid arterial blood yielded a pH of 7.33 ± 0.01 (normal is 7.38), P<sub>CO₂</sub> of 49.8 Torr (vs. 43 Torr), a P<sub>O₂</sub> of 16.1 (vs. 20 Torr), and an O<sub>2</sub> content of 5.3 ml/dl (vs. 8 ml/dl). It was assumed that these fetuses had been hypoxic since surgery. When resting coronary flow was measured in these fetuses, it was found that their resting flows were nearly identical to the maximal flows obtained with adenosine in normoxic animals (Fig. 11), even though their coronary perfusion pressure was not changed (49.1 vs. normal 47.4 Torr). Therefore, it appeared that the hypoxemia had exhausted the entire coronary flow reserve. To test this hypothesis, adenosine was infused into the left atrium as mentioned above. However, it was found that, surprisingly, a flow reserve was present. Figure 11 shows that

Table 2. Myocardial flow ratio during incremental increases in pulmonary arterial pressure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
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<tbody>
<tr>
<td>Right</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ventricular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inner/outer</td>
<td>1.23±0.21</td>
<td>1.21±0.18</td>
<td>1.14±0.17</td>
<td>1.03±0.15</td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ventricular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inner/outer</td>
<td>1.13±0.10</td>
<td>1.08±0.10</td>
<td>1.10±0.07</td>
<td>1.05±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data from Reller et al. (63). P2-P4, incremental increases in pulmonary arterial pressure.

Fig. 10. RV flow (±SD) as a function of heart rate-peak systolic blood pressure product at 4 levels of systolic work load (r = 0.98). [Reproduced with permission from Reller et al. (63).]

Fig. 11. RV and LV myocardial blood flow using radiolabeled microsphere technique. Myocardial flows were measured in normoxic fetuses at baseline (Control), during acute right ventricular pressure loading (Load), and during adenosine administration (Adenosine; n = 7; Ref. 12). Myocardial blood flow was later measured in a group of chronically hypoxic fetuses (n = 4) at baseline (Control) and with adenosine. Maximal myocardial flow with adenosine in hypoxic fetuses was significantly greater than any other measured flow. Baseline (Control) hypoxic myocardial blood flow was not different from maximal myocardial blood flow in normoxic fetuses. [Borrowed with permission from Reller et al. (64).]
Table 3. Comparison of hemodynamic variables at baseline, during adenosine infusion, with acute hypoxemia, with L-NNA infusion, and with subsequent acute hypoxemia

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 14)</th>
<th>Adenosine (n = 11)</th>
<th>Hypoxemia (n = 6)</th>
<th>L-NNA (n = 10)</th>
<th>Acute Hypoxemia with L-NNA (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>3.0 ± 1.4</td>
<td>4.2 ± 1.9</td>
<td>7.1 ± 1.5††</td>
<td>4.2 ± 2.0</td>
<td>5.2 ± 2.6</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>2.8 ± 1.4</td>
<td>3.5 ± 1.7</td>
<td>7.0 ± 2.9††</td>
<td>3.4 ± 2.4</td>
<td>Not obtained</td>
</tr>
<tr>
<td>Systolic carotid arterial pressure, mmHg</td>
<td>57 ± 6</td>
<td>57 ± 8</td>
<td>66 ± 5††</td>
<td>69 ± 7††</td>
<td>80 ± 13†</td>
</tr>
<tr>
<td>Diastolic carotid arterial pressure, mmHg</td>
<td>39 ± 5</td>
<td>37 ± 5</td>
<td>43 ± 6†</td>
<td>50 ± 4††</td>
<td>54 ± 8†</td>
</tr>
<tr>
<td>Mean carotid arterial pressure, mmHg</td>
<td>47 ± 3</td>
<td>46 ± 4</td>
<td>52 ± 6††</td>
<td>58 ± 5††</td>
<td>62 ± 8†</td>
</tr>
<tr>
<td>Coronary perfusion pressure (mean CA – RA)</td>
<td>44 ± 4</td>
<td>42 ± 4</td>
<td>45 ± 6</td>
<td>53 ± 5††</td>
<td>57 ± 7†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>166 ± 14</td>
<td>173 ± 25</td>
<td>151 ± 17*</td>
<td>140 ± 19††</td>
<td>118 ± 18‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. L-NNA, N-nitro-L-arginine. Coronary perfusion pressure = mean carotid arterial pressure – right atrial pressure. *Different from baseline (P < 0.05); †different from adenosine (P < 0.05); ‡compared with baseline, adenosine, and acute hypoxemia (P < 0.05). Data reproduced from Reller et al. (62). CA – RA, mean carotid artery – mean right atrial pressure.

The experimental protocol included measuring control flows, flows during adenosine administration, flows during fetal hypoxemia, flows with the NO synthase inhibitor N^6^-nitro-L-arginine (L-NNA), and flows with both hypoxemia and L-NNA. Fetuses were made acutely hypoxic by reducing the fraction of inspired oxygen to the ewe. Average fetal arterial blood values during hypoxemia were pH 7.31 ± 0.06; Pco2 45.8 ± 9.3 Torr; Po2 8.8 ± 0.8 Torr; O2 content 1.7 ± 0.2 ml/dl. Table 3 shows the hemodynamic effects in the fetus with these various treatments. It is important to note that mean carotid pressure was increased during hypoxic episodes and during L-NNA administration.

During bouts of severe hypoxemia, the fetal cardiac output fluctuations exceeded chemical dilatation with adenosine (Fig. 12, “Ad” vs. “Hypox”). At first we thought this surprising result could be due to a miscalculation of the dose so that the coronary tree was not fully dilated by the dose of adenosine that we originally determined. However, the maximal dilatation by adenosine was equivalent to that found in previous experiments and, furthermore, increasing the dose even further did not bring about increases in flow. We then thought that the perfusion pressure might have increased enough to make the reserve larger. From the pressure-flow curve generated during chemical dilatation, the small (5 mmHg)
rise in arterial pressure could not account for the powerful coronary dilation during hypoxemia.

Next, in the same study we sought to determine the role of NO in the normal control of coronary flow in the fetus and during arterial hypoxemia. Blockade of NO synthase did reduce myocardial flow by some 30% at baseline ($P_{<0.05}$), with the flow reduction becoming greater as oxygen content is reduced (Fig. 13; Ref. 57). Of great interest is the fact that coronary flow did not exceed that during adenosine administration when NO synthase was antagonized. These findings need to be interpreted in light of recent data indicating that inhibiting NO synthesis attenuates coronary dilation by adenosine (39). From this investigation we learned the following. 1) NO production exerts a basal coronary vasodilatory effect in the fetus, a finding that is quantitatively similar to that seen in the adult circulation (42, 57). 2) The blockade of NO production by the coronary endothelium had an unexpected effect on myocardial oxygen consumption. This is shown in Fig. 14. Although the rate-pressure product was not affected by adenosine or L-NNA administration, nevertheless oxygen consumption was depressed by some 50% with the blockade of NO synthase. This remains unexplained. A similar finding was found by Bernstein et al. (9), but not by Maekawa et al. (48), in adult dogs. 3) Acute hypoxemia in the fetus induces a myocardial blood flow response that exceeds maximal flow obtained by adenosine at a similar perfusion pressure. This flow response would appear to be unique to the fetus and to be at least in part mediated by the NO pathway.

Yet these findings must be interpreted in light of elegant studies of ewes kept at 3,820 m altitude during pregnancy. Kamitomoto et al. (40, 41) showed that fetuses raised at altitude had normal resting coronary flows ($200 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and increases in flow ($150\%$) similar to controls under acute hypoxic stress conditions (fetal arterial $P_{O_2}$ decreased acutely from 19 to 11 Torr). These studies point to our remaining ignorance regarding the role of stage of development and duration of hypoxemia in generating a coronary response in the fetus.

Regulation of atrial myocardial blood flow. Hemodynamic data suggest that atrial function is more important to ventricular filling in the fetus with a greater contribution of atrial “systole” to ventricular end-diastolic volume than for the adult (60). In addition, the fetus appears to rely on a more coordinated atrial contraction to fill its less compliant ventricles (55, 61, 67). Thus inadequate myocardial blood flow to the fetal atria would likely compromise atrial and, therefore, ventricular function. We postulated that atrial myocardial blood flow might be compromised under conditions

![Fig. 13. Relationship between coronary flow as assessed by left circumflex coronary Doppler-flow velocities and arterial $O_2$ content in fetuses made hypoxemic (○) compared with similar degrees of hypoxemia during L-NNA infusion (▲). Points on 2 curves were fitted using a 2nd-degree polynomial. Note that flow is depressed at all oxygen contents when nitric oxide synthase is antagonized. [Borrowed with permission from Reller et al. (62).]](http://ajpregu.physiology.org/)

![Fig. 14. Myocardial oxygen consumption under 3 conditions. Note that administration of adenosine did not affect oxygen uptake. However, $O_2$ consumption was reduced during inhibition of nitric oxide synthase with L-NNA. *Different from baseline ($P_{<0.05}$). [Reproduced with permission from Reller et al. (62).]](http://ajpregu.physiology.org/)

![Fig. 15. Pressure-flow relationships of normal fetal heart. Top line is fetal dilation curve during adenosine administration, determined from experimental data (62) with the adenosine point (A) at resting arterial pressure. H, flow under hypoxic conditions; W, under maximal working conditions; B, under baseline conditions at rest. A hypothetical autoregulation curve is drawn in to suggest a possible flow pressure relationship in fetus. However, such a curve has not yet been constructed in fetus.](http://ajpregu.physiology.org/)
causing increased ventricular blood flow and that this compromise could significantly alter atrial function and ventricular filling in the fetus.

The fetal sheep that were placed in ventricular flow studies were also evaluated to determine whether atrial myocardial blood flow is regulated independently of fetal ventricular myocardial blood flow (47). This investigation indicated several findings of interest. First, at baseline, fetal atrial myocardial blood flows were less than half that measured in the fetal ventricle per gram tissue (−90 ml·min⁻¹·100 g⁻¹ tissue for the right atrium vs. 197 and 253 ml·min⁻¹·100 g⁻¹ for the left ventricle and right ventricle, respectively). However, the crucial finding in this investigation was that during acute pressure loading conditions, the percent increase in atrial myocardial blood flow in response to loading was actually greater than for ventricular myocardial blood flow (a nearly 3-fold increase vs. a doubling of ventricular blood flow). Furthermore, acute ventricular pressure loading was associated with significant increases in right atrial a-wave pressure (active atrial contraction), a finding that likely reflects an increase in atrial wall stress and right atrial myocardial oxygen demand.

In summary, these findings indicate that atrial blood flow in the fetus is regulated independently of ventricular myocardial blood flow and that there is no evidence of any compromise of atrial blood flow with increasing work load. Atrial flow actually exceeded ventricular flow. Finally, the increase in right atrial systolic pressure with right ventricular pressure loading suggests that atrial myocardial blood flow regulation is influenced by atrial work.

CONCLUSIONS

Coronary reserve was found to be dependent on the experimental conditions. We conclude that right ventricular flow is rapidly increased in response to increased systolic load, but we surmise that the entire coronary flow reserve cannot be used in such circumstances. It also appears that right and left ventricular flows are linked even if one ventricle is required to do all the work. We suspect that subtle mechanical cross talk explains this phenomenon. Preliminary experiments indicate that the coronary tree is highly plastic and responds to conditions of chronic arterial hypoxemia by a substantial increase in the cross-sectional area of the resistance portions of the coronary tree. Coronary flows in hearts of fetuses that have been chronically hypoxic are enormous when the coronary bed is fully dilated. Acute hypoxemia stimulates the normal fetal heart to dilate to levels that exceed chemical dilation. This response is blocked by antagonism of NO synthase.

THE FUTURE

It is quite clear that the regulation of coronary flow in the immature heart is so different from that of the adult that it warrants extensive research. Several areas are particularly ripe for study. 1) Coronary flow appears to be linked between the ventricles in unanesthetized fetuses. Further work is required to confirm this finding. If such a link exists, the mechanisms will prove to be important and fascinating. 2) The plasticity of the coronary tree before birth should be of interest to students of cardiac physiology, whether their interest is in the mature or immature myocardium. To what extent is the coronary tree in immature myocardium able to alter its growth? What are the chemical signals for remodeling the coronary tree? How are these chemical signals regulated and how is their effectiveness altered with age of the individual? Is it possible to regain plasticity in the aging heart? 3) The mechanisms that underlie the regulation of coronary flow, on a moment-by-moment basis, have not been studied. To what degree are these mechanisms similar to those of the adult? Are there microdomains in the immature myocardium as postulated for the adult coronary tree (39)? If so, how are they regulated and at what stage of development do they arise? 4) Autoregulation has not been much studied in the immature heart. Although it is clear that the coronary reserve is large in the prenatal sheep heart, the shape and position of the autoregulatory curve (if there is one) have not been described. Figure 15 shows a hypothetical autoregulatory curve and the genuine dilation pressure flow curve in the sheep fetus (compare with Fig. 4). 5) The acute regulation of coronary flow and the chronic regulation of coronary growth are under the influence of a large number of genes that have not been studied during development. How are these genes regulated and can they be manipulated for therapeutic purposes?

These questions are an intellectual gold mine awaiting those with pans, sluice boxes, and a love of discovery.

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