The Effects of Increasing Mean Arterial Pressure on Left Ventricular Output in Newborn Lambs

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We developed a preparation in which the mean arterial pressure of the newborn lamb is varied with little change in left ventricular preload. Seven lambs underwent balloon atrial septostomy and were instrumented with vascular catheters, an electromagnetic flow transducer on the aorta, and a balloon occluder on the aorta. Four different preload conditions were established by volume infusion or withdrawal. Aortic flow was measured continuously during gradual occlusion of the aorta. Curves relating mean left atrial pressure to stroke volume before balloon inflation showed a plateau, with little increase in stroke volume with increases in atrial pressure above 7 mm Hg. With balloon inflation, stroke volume decreased linearly with increases in mean arterial pressure. Curves relating left atrial pressure to stroke volume constructed at constant mean arterial pressure showed no plateau but showed continued increases in stroke volume as left atrial pressure was increased to 10 mm Hg. When mean arterial pressure is constant, the Starling mechanism is operative in newborn left ventricles. The plateau in newborn lamb function curves is due to increases in mean arterial pressure that accompany volume infusion. (Circulation Research 1990;67:78–83)

Methods

Animals

We studied seven newborn lambs of mixed western breed, aged 3–14 days (mean, 7.9 days). Reference was made to results in a similar preparation in 10 fetuses with gestational ages ranging from 126 to 129 days (0.85–0.9 gestation) as previously reported.7

Surgical Preparation

Prophylactic antibiotics were administered 1 hour before surgical procedures. After fasting for 4–6 hours, the lamb was anesthetized using fluothane, the trachea was intubated, and the lamb was maintained on mechanical ventilation with a mixture of oxygen and 0.75–1.0% fluothane. Polyvinyl catheters (0.076 cm i.d.) were placed by cut-down in the right hind limb artery and vein. A left thoracotomy was performed in the fourth intercostal space, and a polyvinyl catheter (0.076 cm i.d.) was placed in the left internal mammary artery and advanced into the brachiocephalic trunk. The pericardium was then opened wide, avoiding injury to the phrenic nerve. The left hind limb vein was exposed by cut-down, and a 6F Fogarty atrioseptostomy catheter was advanced from the hind limb vein into the right atrium, through the foramen ovale, and into the left atrium. Proper position in the left atrium was visualized directly. Balloon atrioseptostomy was performed three or four times, the catheter was removed, the vein was tied off
proximally and distally, and the skin incision was closed. A left atrial polyvinyl catheter (0.076 cm i.d.) was then placed through a purse string in the left atrial appendage. A previously calibrated electromagnetic flowmeter (C and C Instruments, Culver City, Calif.) was placed around the ascending aorta, above the origin of the coronary arteries and proximal to the brachiocephalic trunk. A balloon occluder, made in our laboratory, was then placed around the descending aorta distal to the ductus arteriosus. An 8F polyvinyl catheter with multiple holes was placed into the pleural space for drainage. The pericardium was left open to prevent any possible constriction of the heart or tamponade effect. The ribs were then approximated and the thoracotomy closed in separate layers. Through a single neck incision, both carotid sheaths were exposed. An 8F catheter was placed into a jugular vein and advanced into the superior vena cava for withdrawal and infusion of blood for the volume studies. A polyvinyl catheter (0.076 cm i.d.) was placed in one of the carotid arteries and advanced into the brachiocephalic trunk. Specially made catheters were placed around both vagus nerve trunks. These catheters consisted of a 1-cm-long 12F polyvinyl sheath that was placed around the vagus nerves. Each sheath was supplied by a polyvinyl catheter (0.038 cm i.d.) for irrigation with lidocaine of the polyvinyl sheath and vagal trunks. The neck incision was closed, and after resumption of normal respirations, the lamb was extubated and allowed to recover with close monitoring of arterial blood gases and continuous water-seal suction to the chest tube. After the lamb recovered completely, all catheters were heparinized, capped, and placed under an elastic chest bandage. Lambs were returned to their mothers and allowed to feed, and they received intravenous penicillin G potassium and kanamycin daily until all studies had been completed.

**Mean Arterial Pressure Studies**

After a minimum of 2 days recovery, the lamb was blindfolded and placed in a sling under a heat lamp. Fluid-filled catheters were connected to Statham P23Db pressure transducers (Statham Instruments, Oxnard, Calif.) that were calibrated to the nearest 1 mm Hg with a static mercury column. Continuous recordings of mean and phasic aortic pressure and mean left atrial pressure were recorded on a Beckman 8-channel direct-writing recorder (Beckman Instruments, San Jose, Calif.). Heart rate was measured by a cardiacotachometer triggered by the phasic aortic pressure signal. Phasic and mean ascending aortic blood flow were measured from the electromagnetic flow probe on a Statham SP2202 flowmeter and continuously recorded. Stroke volume (less coronary blood flow) was calculated by dividing ascending aortic blood flow by heart rate.

Before studies were begun, arterial blood gases were obtained from the carotid artery in each lamb. Only those lambs with pH greater than 7.35, PO$_2$ greater than 80 mm Hg, and $O_2$ saturation greater than 92% were studied. After control hemodynamic measurements were made, vagal blockade was instituted by instilling 0.25 ml of 2% lidocaine into each vagus nerve catheter. Vagus blockade was confirmed by a brief balloon occlusion and by observing the lack of significant heart rate change. A second set of hemodynamic measurements was taken before beginning the actual studies. Lidocaine was reinfused approximately every 20 minutes to ensure continuous blockade of the baroreceptor response and was usually given three times per study.

For ventricular function curves, left atrial pressures were adjusted by withdrawal of blood or infusion of fresh heparinized whole blood obtained from another lamb under sterile conditions or from the mother if lamb blood was unavailable. The experiment was performed four times, once at each target mean left atrial pressure. A baseline left atrial pressure of approximately 4 mm Hg was present. A mean left atrial pressure of 1 mm Hg was obtained by the withdrawal of approximately 50 ml blood. Mean left atrial pressures of 7 and 10 mm Hg were obtained by the infusion of approximately 50 and 100 ml blood respectively. After 5–10 minutes, a steady state was achieved at each target mean left atrial pressure, and a set of resting hemodynamic measurements was obtained before changing aortic pressures. While continuous hemodynamic measurements were obtained, the aortic balloon was gradually inflated over 30–60 seconds and then was deflated. Typically, inflation of the balloon resulted in a 15–20 mm Hg rise in mean aortic pressure, which resulted in five to seven data points for the function curves. Ten to 15 minutes was allowed between trials for the pressures and flows to return to baseline or steady state. Arterial blood gases were monitored, and the experiment did not proceed if the pH dropped more than 0.04 units. After all studies were completed, lambs were killed using intravenous pentobarbital. Postmortem examination was performed to confirm the positions of the catheters, balloon occluder, and flow transducer, as well as the adequacy of the atrial tear after atrioseptostomy.

The study protocol was in accordance with institutional guidelines and was approved by the Committee on Animal Experimentation at our institution.

**Fetal Preparation**

The fetal surgical preparation has been previously reported. Access to the fetus was accomplished after maternal spinal anesthesia, laparotomy, and hysterotomy. Vascular catheters were placed identically to the lambs, and an ascending aortic flow transducer was placed. The balloon occluder, however, was placed around the aortic isthmus, distal to the origin of the common brachiocephalic artery but proximal to the ductus arteriosus, and balloon atrioseptostomy was not performed. All fetal pressures were reported less the simultaneously measured amniotic pressure. We previously have demonstrated that aortic occlusion at this site allows an increase in ascending aortic
pressure without a significant increase in mean left atrial pressures.7

Statistical Analysis
Values are expressed as mean±SD. Comparisons involving measured values such as heart rate, mean left atrial pressures, and stroke volumes were made by parametric tests (paired and unpaired Student’s t test and repeated measures analysis of variance). Comparisons involving derived values such as regression line slopes and points were made by nonparametric tests (Wilcoxon signed rank test and Mann-Whitney U test, and Friedman test). In all of the analyses, differences were considered statistically significant if p<0.05.6,8

Results
The average baseline heart rate after vagal blockade was 226±15.4 beats/min, and the average baseline stroke volume per kilogram was 1.19±0.18 ml. Balloon inflation elicited very small decreases in heart rate—on average, 4.05%—and the change in heart rate with balloon inflation was not significantly different at different mean left atrial pressures (p>0.05, repeated measures analysis of variance).

Mean left atrial pressures averaged 3.64±0.748 mm Hg at the start of the experiment. With volume withdrawal or infusion, mean left atrial pressures were close to the target pressures (Table 1). At peak balloon inflation, the increases in mean left atrial pressure were small (less than 1 mm Hg) but statistically significant (Table 1).

Gradual increases in mean arterial pressure brought about by balloon inflation produced linear decreases in stroke volume at each mean left atrial pressure in each animal studied (Figure 1). The slopes of these lines were similar to one another at mean left atrial pressures of 4, 7, and 10 mm Hg. The line at a mean left atrial pressure of 1 mm Hg was quite flat, and the slope was significantly different from the slopes at 4, 7, and 10 mm Hg (Z=2.207, p=0.0272, Wilcoxon signed rank test). Although the slopes at 4, 7, and 10 mm Hg were similar, the lines were shifted to the right and upward with increasing mean left atrial pressure (Figure 2), and these positions were significantly different (χ2=14, p<0.001, Friedman’s test).

The function curves relating stroke volume to mean left atrial pressure before balloon inflation showed linear increases in stroke volume with increased mean left atrial pressure, up to an atrial pressure of 7 mm Hg, above which there was little additional increase in stroke volume when aortic pressure was allowed to fluctuate. The stroke volume at a mean left atrial pressure of 10 mm Hg was not significantly higher than at 7 mm Hg for this curve (Wilcoxon signed rank test, Z=1.363, p=0.164).

In each newborn lamb before balloon inflation, increases in mean left atrial pressure by volume infusion were associated with appreciable increases in mean arterial pressure (Figure 3). This means that for points on the graph in Figure 4 relating mean left atrial pressure to stroke volume, the points at the higher left atrial pressures were measured at higher mean arterial pressures than at the lower mean left atrial pressures. By the selection of points for each animal at which the mean arterial pressure fell within the range of 87.5–92.5 mm Hg, function curves were derived relating stroke volume to mean left atrial pressure at this constant mean arterial pressure. Compared with the function curve developed with variable mean arterial pressure, these constant arterial pressure function curves lacked plateaus, but showed continued increases in stroke volume above mean left atrial pressures of 7 mm Hg (Figure 5). The stroke volume at a mean left atrial pressure of 10 mm Hg was significantly higher than at 7 mm Hg for the constant arterial pressure curve (Wilcoxon signed rank test, Z=1.992, p=0.0466, two-tailed) but was not significantly different for the variable arterial pressure curve (Z=1.363, p=0.1738, two-tailed). The average increase in stroke volume with an increase in mean left atrial pressure from 7 and 10 mm Hg was

### Table 1. Changes in Mean Left Atrial Pressures With Peak Balloon Inflation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Target pressure (mm Hg)</th>
<th>Baseline mean left atrial pressure (mm Hg)</th>
<th>Change with peak balloon inflation (mm Hg)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>3.64±0.75</td>
<td>0.93±0.79</td>
<td>0.0205</td>
</tr>
<tr>
<td>−60 ml</td>
<td>1</td>
<td>1.14±0.69</td>
<td>0.64±0.63</td>
<td>0.0349</td>
</tr>
<tr>
<td>+60 ml</td>
<td>7</td>
<td>6.64±0.56</td>
<td>0.71±0.70</td>
<td>0.0353</td>
</tr>
<tr>
<td>+120 ml</td>
<td>10</td>
<td>10.35±0.95</td>
<td>0.71±0.81</td>
<td>0.0582</td>
</tr>
</tbody>
</table>

Values are mean±SD. Significance determined by paired Student’s t test.

**Figure 1.** Representative afterload function curve for one lamb, relating mean arterial pressure and stroke volume at various mean left atrial pressures.
6.4% for the variable arterial pressure curve and 17.5% for the constant arterial pressure curve.

Comparison With Fetal Data

The function curves relating stroke volume to mean arterial pressure were quite similar to curves obtained from fetuses at the same mean left atrial pressure, with linear decreases in stroke volume with increasing mean arterial pressure (Figure 2). The slopes of these lines were not significantly different between fetuses and newborns at each left atrial pressure ($p>0.05$, Mann-Whitney $U$ test); however, the positions of the curves were markedly different for all but the curve at a mean left atrial pressure of 1 mm Hg, with newborn curves being shifted to the right and upward, when compared with fetal curves (Figure 2). The form of the curve relating stroke volume to mean left atrial pressure for newborns, with a plateau at high left atrial pressures, was similar to the curves obtained in fetuses (Figure 4). However, the newborn lamb curve was shifted upward relative to the fetal curve. Newborn stroke volume was significantly greater than fetal stroke volume at each of the four mean left atrial pressures ($p<0.05$, unpaired Student’s $t$ test).
**Discussion**

Important changes in intrinsic contractile state are thought to occur between fetal and newborn life. Left ventricular output increases two- to threefold at birth.10-12 In vivo comparisons of contractile force in fetuses and newborns, using postextrasystolic potentiation,2 dP/dt maximum, and velocity of circumferential fiber shortening,12 have shown greater contractile force in the postnatal ventricle. The mechanisms responsible for these changes are uncertain. Electron microscopy shows that myofibrils in the fetal heart are sparse and randomly organized compared with the newborn heart and have a higher water content.13,14 Fetal myocardium is less compliant than adult myocardium,15 exhibiting properties of increased series elasticity and decreased contractile element content.16 In the lamb, sympathetic innervation is detectable at 75-85 days gestation and proceeds through gestation but is largely complete by term.17 The development of β-adrenergic receptors in the fetal ventricle may be an important factor and may be under the influence of thyroid hormone.18 Whereas plasma catecholamine levels increase at birth,19 propranolol administration only slightly blunts this increase in left ventricular output.20

Cardiac output in fetuses and newborns has been thought to be principally determined by heart rate, based on prior studies that have demonstrated little increase in stroke volume with volume infusions that raised mean left atrial pressures significantly.4,6,20 These studies have not taken into account the increases in afterload that accompany volume infusions. As in our studies of fetuses,7 such volume infusions were associated with significant rises in mean arterial pressure. When curves relating stroke volume per kilogram versus mean left atrial pressure are constructed at constant mean arterial pressures, stroke volume continues to increase when preload increases above resting levels in both fetuses and newborns. Therefore, the Starling mechanism is operative at even these higher filling pressures. It is likely that the plateaus observed in function curves at filling pressures above resting levels are actually due to the effects of the increases in mean arterial pressure that accompany rapid volume infusion.

Sudden increases in afterload in a beating heart are normally associated with a fall in output with a rise in end-systolic volume, and a subsequent rise in end-diastolic volume that increases ejection on the next beat by the Starling mechanism. This increase in ejection compensates for the fall in ejection caused by increased afterload and is known as preload reserve.21 As we have previously reported,7 when preload is allowed to rise with afterload in the fetal left ventricle, little change in ejection occurs until high arterial pressures are reached, at which point preload reserve is presumably exceeded and stroke volume falls. The assessment of the independent effect of afterload on performance is hampered if preload is not controlled. Although studies of isolated beating hearts have been reported in which preload is kept constant by servomechanisms,22 our preparations are unique in that we are able to vary mean arterial pressure and left atrial pressure independently in chronically instrumented, closed-chest, unsedated fetal and newborn lambs.

Meaningful comparisons between our findings in fetuses and newborns may not be possible. The function curves clearly occupy different positions, but the different positions may be explainable by unmeasured differences in preload or afterload, in addition to intrinsic differences in cardiac contractility. End-diastolic fiber length is determined by the ventricular compliance and the transmural pressure. Ventricular compliance is likely to be different between fetuses and newborns. We did not measure transmural pressure. If fetal intrathoracic pressure is positive and newborn intrathoracic pressure is mostly negative, however, equivalent mean left atrial pressures may represent quite different transmural pressures and, therefore, fiber length. The use of mean arterial pressure as an index of afterload for comparisons between fetuses and newborns is also problematic. Afterload is perhaps best characterized by some measure of wall stress,23,24 which in turn depends on pressure, wall thickness, and chamber diameter and geometry, all of which may be very different in fetuses and newborns. Therefore, equivalent mean arterial pressures likely represent quite different wall stress.

Heart rate and contractile force are also closely related. Increases in heart rate are associated with immediate increases in force of contraction, known as the Bowditch effect.25 Because of local instillation of lidocaine around the vagus nerves in our preparation, heart rate did not change despite great increases in arterial pressure; however, baseline heart rates in our newborn lambs were significantly higher than in our fetal lambs. The positive effect of heart rate on contractile force, measured by poststimulation potentiation of dP/dt, has been shown to be operative in the fetal26 and newborn lamb27 in vivo and in vitro.25 It is possible that our results suggesting higher newborn stroke vol-
umes are at least partially explained by heart rate effects on contractile force.

The late fetal development of myocardial contractile capability may be important in the transition to the postnatal circulation, in which the left ventricle is exposed to a sharply increased afterload because of the loss of the placental circulation. The sensitivity of the fetal and newborn left ventricle to increases in mean arterial pressure has important implications for the clinical management of premature newborn infants.

References

Key Words • afterload • preload • ventricular function
The effects of increasing mean arterial pressure on left ventricular output in newborn lambs.
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Circ Res. 1990;67:78-83
doi: 10.1161/01.RES.67.1.78

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