Myocardial function after fetal cardiac bypass in an ovine model

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Objective: Fetal cardiac surgery might improve the prognosis of certain complex congenital heart defects that have significant associated mortality and morbidity in utero or after birth. An important step in translating fetal cardiac surgery is identifying potential mechanisms leading to myocardial dysfunction after bypass. The hypothesis was that fetal cardiac bypass results in myocardial dysfunction, possibly because of perturbation of calcium cycling and contractile proteins.

Methods: Midterm sheep fetuses (n = 6) underwent 30 minutes of cardiac bypass and 120 minutes of monitoring after bypass. Sonomicrometric and pressure catheters inserted in the left and right ventricles measured myocardial function. Cardiac contractile and calcium cycling proteins, along with calpain, were analyzed by means of immunoblotting.

Results: Preload recruitable stroke work (slope of the regression line) was reduced at 120 minutes after bypass (right ventricle: baseline vs 120 minutes after bypass, 38.6 ± 6.8 vs 20.4 ± 4.8 [$P = .01$]; left ventricle: 37 ± 7.3 vs 20.6 ± 3.9, respectively [$P = .01$]). Tau (in milliseconds), a measure of diastolic relaxation, was increased in both ventricles (right ventricle: baseline vs 120 minutes after bypass, 32.7 ± 4.5 vs 67.8 ± 9.4 [$P < .01$]); left ventricle: 26.1 ± 3.2 vs 63.2 ± 11.2, respectively [$P = .01$]). Cardiac output was lower and end-diastolic pressures were higher in the right ventricle, but not in the left ventricle, after bypass compared with baseline values. Right ventricular troponin I was degraded by increased calpain activity, and protein levels of sarco(endo)plasmic reticulum calcium ATPase were reduced in both ventricles.

Conclusions: Fetal cardiac bypass was associated with myocardial dysfunction and disruption of calcium cycling and contractile proteins. Minimizing myocardial dysfunction after cardiac bypass is important for successful fetal surgery to repair complex congenital heart defects. (J Thorac Cardiovasc Surg 2011;141:961-8)

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Despite impressive medical and surgical advances, certain complex congenital heart defects (eg, hypoplastic left heart syndrome with intact atrial septum) continue to have significant associated mortality and morbidity either in utero or shortly after birth, often at great cost.[1] This is in part due to fetal end-organ injury that has occurred before birth because of altered intracardiac blood flow patterns.[2] Fetal cardiac surgery, alongside other evolving fetal cardiac interventions, has the potential to alter these outcomes.

Early studies examining fetal cardiac surgery focused on developing tools and techniques for extracorporeal circulation or fetal cardiac bypass and then overcoming the detrimental response of the placenta to bypass.[3,4] Many of these technical challenges have been studied and at least partially overcome,[5] but successful clinical translation has yet to be achieved. The ability to perform intracardiac procedures depends on understanding the mechanisms leading to cardiac dysfunction and eventually developing methods to protect the fetal myocardium.

Unlike the postnatal heart, the fetal right ventricle (RV) and left ventricle (LV) pump in parallel, and pressure differences between the chambers are normally minimal.[6] The fetal RV is the main pumping chamber, and output is higher compared with that of the LV, which supplies coronary and upper body circulation. Fetal hearts also have limited reserves to increase cardiac output because the ventricle is operating near the top of its function curve.[7] Increases in blood volume induce only a small increase in fetal cardiac output,[7,8] whereas increases in heart rate and contractility are more important in maintaining fetal cardiac output. The unique requirements of immature circulation and myocardium require directed protection, and understanding the myocardial dysfunction is necessary to develop regimens for cardiac surgery.

Our research group previously demonstrated that cardiopulmonary bypass can result in myocardial dysfunction and

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Abbreviations and Acronyms

LV = left ventricle
PRSW = preload recruitable stroke work
RV = right ventricle
SERCA2a = sarco(endo)plasmic reticulum calcium ATPase
SR = sarcoplasmic reticulum
TnI = troponin I

altered calcium cycling in neonates.[9] However, immature cardiomyocytes differ in morphology and function from adult and even neonatal cardiomyocytes. There are species-specific differences in the prenatal and postnatal development of excitation/contraction coupling and discord regarding the maturation and importance of Ca^{2+}-induced Ca^{2+} release and the sarcoplasmic reticulum (SR) in mediating fetal contraction.[10]

Cardiopulmonary bypass in neonates leads to degradation of contractile proteins, possibly contributing to cardiac dysfunction.[11] Structural proteolysis of troponin I (TnI), the inhibitory subunit of troponin, is associated with myocardial stunning and reduced cardiac contractility.[12] TnI is systematically degraded by the calcium-activated cysteine protease calpain after cardiopulmonary bypass in adults and neonates.[11,13] In addition, inhibition of calpain activation has been shown to be protective for ischemic and hypoxic hearts.[14]

In the current study the hypothesis was that fetal cardiac bypass results in postsurgical myocardial dysfunction for the fetus. We report reduced fetal cardiac function associated with cardiac bypass procedures and present potential mechanisms for the detected dysfunction.

MATERIALS AND METHODS

Animal Model

All animals received humane care in compliance with the ''Principles of laboratory animal care'' formulated by the National Society for Medical Research and the ''Guide for the care and use of laboratory animals'' prepared by the Institute of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996). The Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Research Foundation also approved the protocol.

Singleton pregnant ewes from 100 to 114 days of gestation were studied; term was approximately 148 days. Six fetuses (2.4 ± 0.4 kg) underwent sternotomy with 30 minutes of cardiac bypass, and 6 fetuses were killed immediately after sternotomy for collection of baseline tissue samples. Surgical preparation and fetal cardiac bypass were performed as previously described by our group.[15-17] Briefly, ewes were fasted for 24 hours before sedation with ketamine and diazepam, intubated, and maintained on 2% isoflurane and oxygen. Ewes received buprenorphine (Buprenex; 0.3 mg administered intramuscularly) and penicillin G. Catheters were placed in a ewe's femoral artery and vein for collection of blood to measure blood gases and to deliver intravenous fluids. After midline laparotomy and minor hysterotomy, catheters were placed in the fetal femoral artery for collecting blood samples and monitoring arterial blood pressure. Through the same hysterotomy, an umbilical flow probe (4–6 mm; Transonic Systems, Ithaca, NY) was placed to measure placental blood flow.

Fetal Cardiac Bypass

Using methods previously described,[15-17] fetal cannulation was performed with a 10F to 12F Bio-Medicus venous cannula (Medtronic, Minneapolis, Minn) in the jugular vein and a 6F to 8F Bio-Medicus arterial cannula in the carotid artery. Fetal chest sternotomy was performed to visualize cannula placement for optimal drainage and to simulate surgical stress required in future interventional studies in the clinical application. Hemodynamic values were continuously recorded with a PowerLab data acquisition system (AD Instruments, Colorado Springs, Colo). Fetal cardiac bypass was conducted with a roller pump system using normothermia, vacuum-assisted venous drainage with a Baby-RX reservoir (Terumo, Somerset, NJ), and a heat exchanger. The aorta was not crossclamped, and the heart continues to be perfused. The placenta was the sole oxygenator, and the blood prime for the bypass circuit was collected from a nonmaternal adult sheep. Fetal cardiac bypass lasted 30 minutes, with a target flow rate of 200 mL · min^{-1} · kg^{-1} based on our prior studies.[15-17] The fetuses were monitored for 120 minutes after cessation of bypass. Ewes and fetuses were killed by means of pentobarbital overdose for autopsy measurement of fetal morphometrics, confirmation of catheter positions, and tissue sample collection.

Fetal Cardiac Instrumentation and Measurements

Six 2-mm piezoelectric crystals (Sonometrics Corp, London, Ontario, Canada) were glued onto 3 axes of the fetal heart, and pressure catheters (Millar Instruments, Houston, Tex) were inserted through the myocardium into the LV and RV, allowing real-time measurement of fetal cardiac function. Dimensional and functional analysis of fetal ventricular performance over the course of the experiment was performed with Cardiosoft software (Sonometrics Corp). Pressure–volume loops to measure complex contractility parameters were recorded during transient vena caval occlusion. Measured contractility parameters included the following: preload recruitable stroke work (PRSW), the ratio of stroke work to end-diastolic volume and a load-independent measure of systolic function; maximal elastance, the pressure–volume relationship of end-systolic points during vena caval occlusion and an afterload-insensitive indicator of systolic function; tau, the isovolumic relaxation constant and a marker of diastolic function; and end-diastolic pressure–volume relationship, an indicator of ventricular stiffness. Stroke volume was determined by using a 2-axis ellipsoidal model to estimate the shape of the ventricles. Tau was estimated by using a zero asymptote exponential with a sampling interval cutoff that equaled the end-diastolic pressure plus 5 mm Hg.

Blood-Sampling Regimen

Maternal and fetal arterial blood was collected for blood gas measurement, immunoassays, and metabolite analysis immediately on gaining arterial access, just before cardiac bypass, at 30 minutes of bypass, and at 30, 90, and 120 minutes after bypass. Blood gases were measured with an i-STAT clinical analyzer, (i-STAT Corp, Windsor, NJ). Maternal and fetal lactate values were measured with a YSI 2300-STAT analyzer (YSI Corp, Yellow Springs, Ohio).

Western Blot Analyses

Fetal LV and RV free wall tissue samples were collected at 120 minutes after bypass, flash-frozen, and stored at –80°C until processed. Tissues from all fetuses (n = 6 per group) were homogenized in 10 mmol · L^{-1} 3-[N-morpholino] propane sulfonic acid buffer with protease and phosphatase inhibitors and stored at –80°C until use. Western blots were performed with 30 to 50 μg of total proteins separated on 4% to 12% acrylamide bis-tris gradient gels (Invitrogen, Carlsbad, Calif) by means of sodium

TABLE 1. Fetal myocardial contractility indices at baseline and after bypass

		After bypass					
	Baseline	30 min	60 min	90 min	120 min	*P* value, ANOVA	*P* value, linear trend
Maximal elastance (slope of regression)							
RV	49.8 ± 7.9	35.3 ± 27.0	44.8 ± 29.6	37.7 ± 30.3	26.8 ± 13.5	.77	.25
LV	47.8 ± 18.2	32.0 ± 25.9	37.0 ± 16.1	35.8 ± 12.7	39.2 ± 12.4	.92	.57
PRSW (slope of regression)							
RV	38.6 ± 6.8	23.3 ± 7.7*	19.7 ± 6.3*	19.4 ± 2.7*	20.4 ± 4.8*	.01	<.01
LV	37.0 ± 7.3	24.5 ± 5.4*	20.4 ± 3.7*	25.1 ± 5.1*	20.6 ± 3.9*	.01	.01
Tau (ms)							
RV	32.7 ± 4.5	44.2 ± 8.4*	54.1 ± 19.2*	56.9 ± 6.3*	67.8 ± 9.4*	<.01	<.01
LV	26.1 ± 3.2	48.3 ± 17.8	55.1 ± 24.1	67.8 ± 22.8*	63.2 ± 11.2*	.01	.01
EDPVR (slope of regression)							
RV	0.04 ± 0.03	0.12 ± 0.13	0.10 ± 0.13	0.10 ± 0.12	0.15 ± 0.17	.77	.34
LV	0.15 ± 0.10	0.23 ± 0.15	0.24 ± 0.22	0.25 ± 0.20	0.27 ± 0.16	.85	.29
dP/dt_{max} (mm Hg · s^{-1})							
RV	942 ± 187	905 ± 221	832 ± 135	819 ± 143	801 ± 140	.70	.18
LV	1121 ± 132	878 ± 190	958 ± 178	896 ± 218	827 ± 134	.14	.04
dP/dt_{min} (mm Hg · s^{-1})							
RV	854 ± 66	681 ± 128*	684 ± 43*	689 ± 78*	638 ± 35*	<.01	<.01
LV	890 ± 75	713 ± 132*	721 ± 64*	704 ± 87*	682 ± 74*	.02	<.01
SW (mL · mm Hg^{-1})							
RV	42.8 ± 16.1	46.2 ± 17.8	44.5 ± 14.8	41.3 ± 15.5	33.2 ± 14.1	.79	.33
LV	49.9 ± 25.3	53.9 ± 29.4	47.0 ± 21.4	46.4 ± 21.5	35.6 ± 14.7	.83	.06

Values are means ± standard deviations of 6 animals per group: in-group analyses with analysis of variance and linear trend analyses. *ANOVA*, Analysis of variance; *RV*, right ventricle; *LV*, left ventricle; *PRSW*, preload recruitable stroke work; *Tau*, time constant of isovolumic relaxation; *EDPVR*, end-diastolic pressure–volume relationship; *dP/dt*, derivative of the change in pressure/change in time; *SW*, stroke work. *$P < .05$ compared with baseline values by means of 1-way analysis of variance for repeated measurements.

dodecylsulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Some membranes were immunoblotted with anti–sarco(endo)plasmic reticulum calcium ATPase (SERCA2a) antibodies (Abcam, Cambridge, Mass) and antibodies for total phospholamban and phospholamban phosphorylated at serine 16 and threonine 17 (Fluorescience Ltd, Leeds, England). Secondary antibodies were alkaline phosphatase–conjugated goat anti-rabbit or anti-mouse IgG. Proteins were visualized with a chemiluminescent detection system according to the manufacturer's instructions (Invitrogen). Immunoblots were also incubated with antibodies to the housekeeping proteins α-sarcomeric actin or glyceraldehyde-3-phosphate dehydrogenase (Abcam) for normalization of the blots. SERCA2a and phospholamban data were presented as the ratio of the densitometry of the target to the housekeeping proteins.

TnI Degradation

Homogenated myofibrils were concentrated from RV and LV tissue collected at baseline or at 120 minutes after bypass. Five micrograms of protein from the myofibril preparation was separated by means of sodium dodecylsulfate–polyacrylamide gel electrophoresis, as previously described.[18] Relative TnI degradation levels were measured by means of immunoblotting with anti-TnI antibodies (Research Diagnostics, Flanders, NJ). Degradation of TnI was measured as the percentage in the degradation bands of the total densitometry for TnI in each lane of the immunoblot.

Calpain Activity Assay

Calpain activity, which is responsible for the proteolysis of TnI, was assessed in fetal RV and LV homogenates by means of fluorogenic assay (Calbiochem, San Diego, Calif) with a SpectraMax Gemini XS spectrafluorometer (Molecular Devices, Sunnyvale, Calif). Cleavage of the fluorescent substrate Ac-Leu-Leu-Tyr-AFC was analyzed in 96-well plates at an excitation of 400 nm and emission at 505 nm. Positive and negative controls were established by the addition of calpain I and calpain inhibitor (Z-Leu-Leu-Tyr-fluoromethyl ketone) to some samples.

Statistical Analyses

All values were expressed as means ± standard deviations. The functional data were analyzed by using 1-way analysis of variance for repeated measurements with Dunnett post-hoc analyses and a post-hoc test for linear trend with GraphPad Prism version 5.0 software for Mac (GraphPad Software, San Diego, Calif). Western blot analyses and activity assays were compared between treatments with analysis of variance.

RESULTS

Contractility Indices

The myocardial contractility parameters are shown in Table 1. PRSW, a load-independent index for systolic function, decreased in both ventricles when compared with baseline values at 30, 60, 90, and 120 minutes after bypass ($P = .01$). In contrast, maximal elastance, an index for systolic function that is preload dependent, did not change during the period of observation. Tau, an index of diastolic function, increased in the RV and LV during the 120-minute observation period ($P < .01$). Both ventricles showed a linear trend for increasing Tau ($P = 0.01$). In addition, dP/dt_{min}, another index of diastolic function, was consistently lower compared with baseline values in the RV ($P < .01$) and LV ($P = .02$) throughout the 120 minutes after bypass.

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TABLE 2. Fetal hemodynamic parameters at baseline and after bypass

		After bypass					
	Baseline	30 min	60 min	90 min	120 min	*P* value, ANOVA	*P* value, linear trend
SV (mL)							
RV	1.1 ± 1	0.9 ± 0.7	0.8 ± 0.6	0.9 ± 0.6	0.7 ± 0.5	.96	.48
LV	1.7 ± 1.3	1.7 ± 0.6	1.7 ± 0.7	1.6 ± 0.7	1.5 ± 0.6	1.00	.71
CO (mL/min)							
RV	199 ± 60	130 ± 87	115 ± 64	111 ± 56	71 ± 27*	.02	.01
LV	346 ± 58	282 ± 13	290 ± 12	297 ± 72	300 ± 84	.95	.57
EDP (mm Hg)							
RV	8.3 ± 1.2	25.0 ± 1.8*	26.3 ± 1.9*	27.7 ± 2.2*	30.4 ± 3.2*	<.01	<.01
LV	13.8 ± 1.6	12.8 ± 2.5	11.6 ± 1.8	11.1 ± 2.0	11.5 ± 2.1	.41	.05
Heart rate (beats · min^{-1})	146 ± 11	173 ± 31	174 ± 12	172 ± 11	175 ± 32	.52	.26
UmbQ (mL · min^{-1})	400 ± 96	389 ± 104	352 ± 82	328 ± 84	316 ± 105	.63	.14
FMAP (mm Hg)	41.5 ± 1.9	37.5 ± 4.7	39.6 ± 7.0	39.7 ± 1.0	33.4 ± 3.5	.26	.11
PVR (mm Hg · mL^{-1} · min^{-1})	0.11 ± 0.03	0.13 ± 0.08	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.04	.97	.96
SVR (mm Hg · mL^{-1} · min^{-1})	67.3 ± 1.7	112.3 ± 56.5	113.3 ± 54.1	103 ± 32.5	93.5 ± 28.9	.70	.92

Values are means ± standard deviations of 6 animals: in-group analyses with analysis of variance and linear trend analyses. *ANOVA*, Analysis of variance; *SV*, stroke volume; *RV*, right ventricle; *LV*, left ventricle; *CO*, cardiac output; *EDP*, end-diastolic pressure; *UmbQ*, placental flow; *FMAP*, fetal mean arterial pressure; *PVR*, placental vascular resistance; *SVR*, systemic vascular resistance. *$P < .05$ compared with baseline values by means of 1-way analysis of variance for repeated measurements.

Myocardial function parameters are not measured during cardiac bypass because of the dependence on extracorporeal support.

Hemodynamic and Fetal Blood Gas Parameters

The fetal hemodynamic parameters before and after bypass are shown in Table 2. Hemodynamic parameters during the bypass period were similar to those previously reported by our research group.[16] Stroke volume, heart rate, umbilical blood flow, fetal arterial blood pressure, and placental vascular resistance did not change after fetal bypass. Cardiac output decreased in the RV after fetal cardiac bypass; however, LV cardiac output was constant during the 120-minute period of observation. A similar trend was observed for end-diastolic ventricular pressure (ie, RV pressure increased after bypass with steady values in the LV compared with baseline values). Fetal P_{CO_2} and lactate levels increased whereas P_{O_2} and pH levels decreased after bypass (see Table E1), as previously reported by our group and others.[3,4,15-17]

Contractile Protein Degradation

TnI degradation from the 29-kd intact protein to the 26-kd degradation product was evident after fetal bypass in the RV (Figure 1). In the RV the densitometry of the TnI degradation band at 26 kd at baseline was 6.1% ± 0.7% of the total detectable TnI and increased to 10.3% ± 1.8% at 120 minutes after fetal bypass ($P < .001$). In the LV no difference was detected between baseline at 6.4% ± 1.2% and 7.9% ± 2.5% at 120 minutes after bypass ($P = .41$). Activity of calpain I and II, the calcium-activated proteases that systematically degrade cardiac TnI, increased in fetal RV and LV homogenates after cardiac bypass compared with activity at baseline. Calpain activity (in fluorescence units per milligram of protein per minute) in the fetal LV at baseline was 461 ± 94 and increased to 955 ± 365 at 120 minutes after bypass ($P = .006$). The calpain activity in the RV was 393 ± 73 at baseline and increased to 775 ± 250 at 120 minutes after fetal cardiac bypass ($P = .01$).

Calcium Cycling Proteins

Levels of SERCA2a protein, the SR ATPase that regulates calcium reuptake from the cytosol, were reduced after fetal bypass in both the LV and RV (Figure 2). The ratio of SERCA2a to α-sarcomeric actin in the LV was 0.82 ± 0.16 at baseline and 0.49 ± 0.23 at 120 minutes after bypass ($P = .02$), and in the RV the ratio was 1.04 ± 0.26 at baseline and 0.37 ± 0.23 after 120 minutes ($P = .002$). In addition, phosphorylation of phospholamban at serine 16 decreased in the LV and RV after fetal bypass compared with baseline values (Figure 3). The ratio of phosphorylated phospholamban at serine 16 to glyceraldehyde-3-phosphate dehydrogenase in the LV was 0.81 ± 0.16 at baseline and 0.52 ± 0.17 at 120 minutes after bypass ($P = .02$), and in the RV the ratio was 0.36 ± 0.07 at baseline and 0.18 ± 0.1 after 120 minutes ($P = .01$). There was no change in the level of total phospholamban protein in either ventricle compared with baseline values. Phosphorylation of SERCA2a at threonine 17 was not detectable by means of immunoblotting at any time point.

DISCUSSION

This is the first study that shows the hemodynamic response of the fetal RV and LV after fetal cardiac bypass and offers potential mechanisms for further investigation

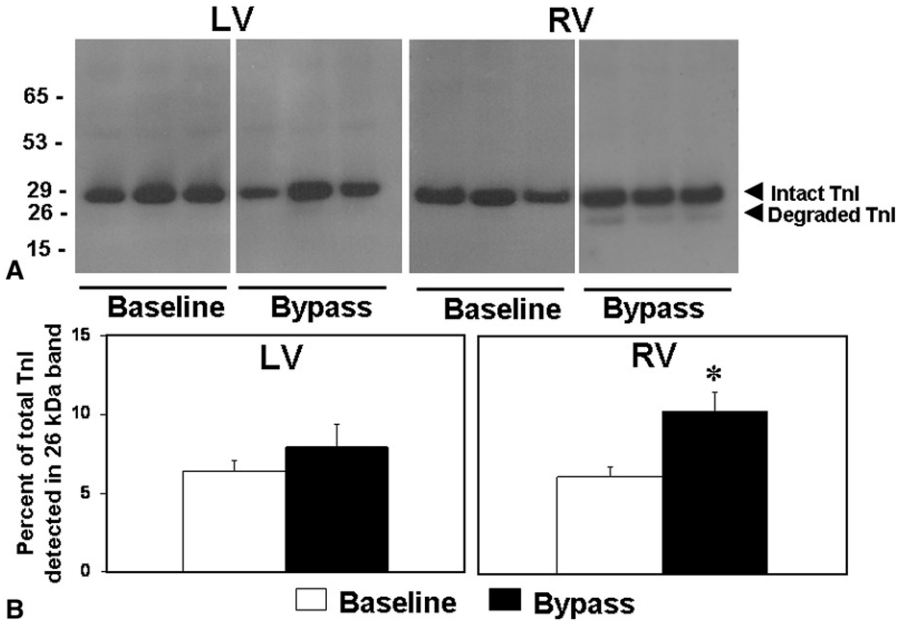

FIGURE 1. Troponin I degradation in the right ventricle *(RV)* and left ventricle *(LV)* after fetal bypass. A, Representative immunoblots of troponin I *(TnI)* in RV and LV tissue at baseline and 120 minutes after fetal bypass. B, TnI degraded after 120 minutes of fetal bypass expressed as a percentage of total TnI detected in each lane (n = 6 per group). *$P < .05$ compared with baseline controls. All lanes from the same tissues are from the same immunoblot, but nonapplicable lanes from the center of the image have been digitally removed.

of the associated cardiac dysfunction. The importance of these findings is in understanding the response to fetal bypass in an acute and stressful surgical situation for future translational studies.

Fetal Myocardial Function

The present study used a combination of sonomicrometry and micromanometers to measure cardiac function, myocardial contractility parameters, and hemodynamics of the

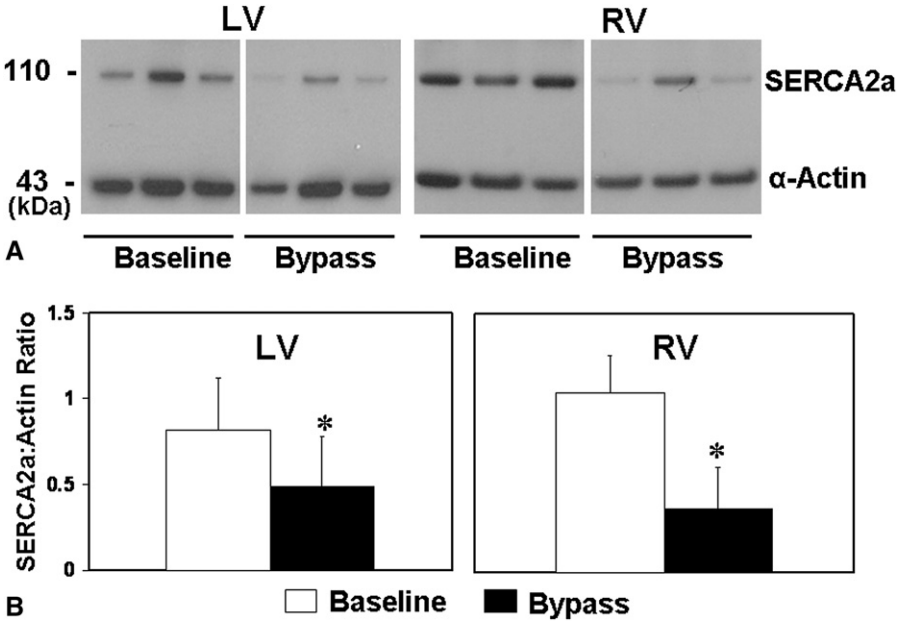

FIGURE 2. Sarco(endo)plasmic reticulum calcium ATPase *(SERCA2a)* protein levels in the right ventricle *(RV)* and left ventricle *(LV)* after fetal bypass. A, Representative immunoblots of SERCA2a in RV and LV tissue at baseline and 120 minutes after fetal bypass *(Bypass)*. B, SERCA2a detected and expressed as the ratio of SERCA2a to α-sarcomeric actin in each lane (n = 6 per group). *$P < .05$ compared with baseline controls. All lanes from the same tissues are from the same immunoblot, but nonapplicable lanes from the center of the image have been digitally removed.

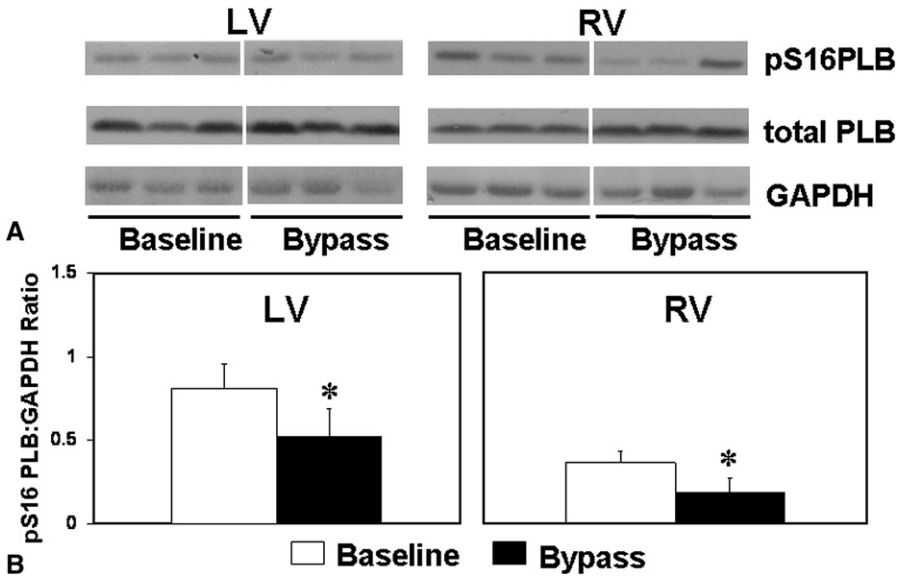

FIGURE 3. Total and phosphorylated phospholamban in the right ventricle *(RV)* and left ventricle *(LV)* after fetal bypass. A, Representative immunoblots of phospholamban *(PLB)* phosphorylated at serine 16 *(pS16)* and total PLB protein. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase. B, Phosphorylated PLB detected expressed as the ratio of PLB to GAPDH in each lane (n = 6 per group). $*P < .05$ compared with baseline controls. All lanes from the same tissues are from the same immunoblot, but nonapplicable lanes from the center of the image have been digitally removed.

fetal RV and LV with fetal cardiac bypass. This methodology is useful to assess myocardial contractility using indices such as PRSW, which is the load-independent linear relationship between stroke work and end-diastolic volume. Fetal systolic dysfunction was evident in the present study, as demonstrated by lower PRSW after bypass in both ventricles. The decreased PRSW after bypass indicated a continual loss of myocardial inotropism. Aside from the decrease in systolic parameters, we also observed diastolic dysfunction in the fetal heart after cardiac bypass, with increased Tau and lower dP/dt_{min} values in both ventricles. These decreases in diastolic function are often indicative of calcium cycle disruption and typically signal a loss of sufficient myocardial relaxation between beats. Insufficient myocardial relaxation leads to lower stroke volume and eventually to lower cardiac output. The reduced fetal RV output translates to less umbilical blood flow and, ultimately, to placental dysfunction. The same impairment of diastolic relaxation was reported for children undergoing cardiopulmonary bypass for repair of congenital heart defects.[19]

Our in vivo findings share some similarities with previous studies in the ex vivo fetal sheep heart, in which biventricular PRSW was assessed as an index of contractility after various methods of cardiac arrest.[20] In these studies by Malhotra and colleagues,[20] neither RV nor LV PRSW recovered to prearrest baseline values with fibrillatory or normocalcemic cardioplegic arrest. Of note, the findings from these in vitro models were generated by using single-axis sonomicrometry, providing pressure–dimension relationship data as opposed to pressure–volume relationship data. Furthermore, this in vitro model used very short prebypass and postbypass assessment periods of 15 minutes. The current study uses 3-axis sonomicrometric measurements in vivo to evaluate myocardial function based on biventricular pressure–volume relationships for an extended 120-minute postbypass period.

In the present study contractility was altered in both ventricles after bypass, but Tau, the diastolic relaxation index, along with cardiac output and end-diastolic pressure, was altered earlier and more profoundly in the RV, the systemic pump for the fetal system. Thornburg and Morton[8] demonstrated that the fetal RV, in particular, works near the top of the Frank–Starling curves, and an additional increase in arterial pressure resulted in decreased RV stroke work and cardiac output. Zhou and associates[21] determined that there was both LV and RV myocardial dysfunction after bypass in a late third-trimester fetal goat model. Using echocardiographic measurements, they reported that the Tei index, which is an indication of global cardiac dysfunction, was increased 1 hour after cardiac bypass.

The combined RV and LV cardiac output from the present study was equivalent to the output reported elsewhere.[6] However, the individual contribution of RV and LV cardiac output after fetal bypass in the present study differed from previously reported values. Of note, previous studies used chronically instrumented animals with little fetal stress at the time of the measurements. In contrast, the present acute study induces a significant stress response, producing an increase in serum concentrations of cortisol, β-endorphin, and vasopressin, a potent vasoconstrictor.[16] The stress response leads to increased afterload and potentially to decreased cardiac output. Systemic vascular resistance has a key

role in determining fetal cardiac output, with several studies showing an inverse relationship between afterload and cardiac output.[8] Again, the RV cardiac output might also be more sensitive to these changes because the circumferential radius/wall thickness ratio is greater for the RV than the LV.[22] Therefore the wall stress in the RV at similar transmural systolic pressures is greater than in the LV and might contribute to the lower RV cardiac output detected in the present study. The more pronounced decrease in RV compared with LV function after cardiopulmonary bypass also occurs in children when there is increased risk of abnormal RV diastolic dysfunction.[19]

We did not observe increased placental vascular resistance, as previously described in the fetal sheep bypass model,[3,5] but our group has not routinely detected significant increases in placental vascular resistance in prior studies with this model.[15,17] We have previously reported stress-induced disruption of vasoactive mediators, such as vasopressin and nitric oxide, after fetal cardiac bypass.[16,17] The stable placental vascular resistance highlights the fact that the effects of fetal cardiac bypass on myocardial function are multifactorial and not solely due to alterations in placental hemodynamics.

TnI Degradation

Multiple isoforms of many contractile proteins, such as myosin heavy chain, titan, and TnI, are developmentally regulated in the heart. This study indicated that the cardiac TnI isoform is detectable by means of immunoblotting in the midterm fetal sheep. The shift of TnI isoforms is linked to a decrease in extracellular calcium sensitivity, activation of the calcium-induced calcium release cycle, and greater dependence on SR cycling for maintenance of intracellular calcium homeostasis.[23] Zhou and associates[21] associated increased plasma TnI levels with decreased myocardial function in a fetal goat model. In support of these findings, the present study detected TnI degradation in the fetal heart by means of immunoblotting of myocardial homogenates.

TnI is systematically degraded by the calcium-activated cysteine proteases calpain I and II after cardiopulmonary bypass in adults[13] and neonates.[11] Our study indicated that calpain was activated in the fetal myocardium after cardiac bypass in association with degradation of troponin contractile proteins and myocardial dysfunction after fetal cardiac bypass.

Calcium Cycling

The changes in myocardial contractility might also be due, at least in part, to alterations in calcium cycling proteins. In immature myocytes the SR plays a crucial role in regulating intracellular calcium levels because systolic contraction occurs with the increase in free cytosolic calcium and diastolic relaxation occurs with the active removal of free calcium. The movement of free calcium from the cytosol into the SR is controlled by cardiac SERCA2a. Calcium reuptake by the SR can be increased by increasing the levels of SERCA2a[24] or by enhancing the affinity of SERCA2a for calcium, a step mediated by phospholamban. Phospholamban phosphorylated at serine 16 and threonine 17 relieves the intrinsic inhibition of SERCA2a and stimulates calcium cycling through the SR.[25] In this study of fetal myocardium, lower levels of serine 16 phosphorylated phospholamban were detected along with a decrease in SERCA2a protein levels after fetal cardiac bypass. This preliminary findings indicate that alterations in the calcium reuptake proteins might be responsible, at least in part, for the loss of myocardial contractility after bypass. Although we did not examine additional mediators of the calcium cycle through the SR, such as the ryanodine receptors, or investigate changes in extracellular calcium-channel regulators, SERCA2a alterations are associated with cardiac dysfunction in many forms of heart failure.[24] Although the SR response is still maturing in the fetal sheep hearts, it appears in this study that alterations in proteins that regulate cardiac calcium cycling might be one potential mechanism leading to myocardial dysfunction after cardiac bypass. This mechanism requires further investigation of calcium movement within fetal cardiac myocytes.

Summary

The development of safe, efficient fetal cardiac bypass is essential for successful translation of fetal heart surgery for patients with complex congenital heart disease. This will require a greater understanding of the mechanisms that underlie the associated myocardial dysfunction. This study, as a first step, points toward altered calcium cycling and contractile protein disruption as potential underlying mechanisms of fetal myocardial dysfunction. Further investigations are necessary to determine whether these mechanisms are primary initiators of myocardial dysfunction. Although the focus of fetal bypass studies has previously been on correcting placental insufficiency after bypass, protecting the fetal myocardium from cardiac bypass–induced injury can be equally as important for fetal survival and subsequent development.

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TABLE E1. Fetal blood gas parameters at baseline and after bypass

		After bypass					
	Baseline	30 min	60 min	90 min	120 min	*P* value, ANOVA	*P* value, linear trend
pH	7.28 ± 0.04	7.26 ± 0.10	7.21 ± 0.10	7.17 ± 0.14	7.13 ± 0.14	.26	.03
P_{CO_2} (mm Hg)	58.2 ± 3.4	71.0 ± 9.8	71.9 ± 11.0	78.4 ± 19.1	89.7 ± 15.6*	.02	.002
P_{O_2} (mm Hg)	23.5 ± 1.3	23.4 ± 4.3	23.8 ± 7.2	20.6 ± 5.6	20.6 ± 5.6	.76	.28
Hematocrit (%)	27.8 ± 4.1	30.4 ± 4.5	29.8 ± 4.3	30.4 ± 5.9	31.4 ± 6.1	.87	.34
Glucose (mg · dL^{-1})	19.5 ± 6.2	14.4 ± 12.6	15.6 ± 13.1	13.8 ± 10.9	10.8 ± 9.9	.83	.28
Lactate (mmol · L^{-1})	1.9 ± .4	2.8 ± .7	2.9 ± .7	3.1 ± .9	3.9 ± .9*	.01	<.001

Values are means ± standard deviations of 6 animals. *ANOVA*, Analysis of variance. *$P < .05$ compared with baseline values by means of 1-way analysis of variance for repeated measurements.