Effects of Pulmonary Insufficiency on Biventricular Function in the Developing Heart of Growing Swine

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Background—This study was conducted to determine the effects of chronic pulmonary insufficiency (PI) on right (RV) and left (LV) ventricular function in young growing swine.

Methods and Results—Six PI and 5 control animals were studied. PI was induced by transcatheter placement of stents across the pulmonary valve. Indices of systolic function (ejection fraction, cardiac output, and cardiac functional reserve), diastolic function (compliance), and myocardial contractility (the slope of the relationship of end-systolic pressure versus end-systolic volume \( E_{max} \) and the slope of the \( dP/dt_{max} \)–end-diastolic volume relationship \( M_{dP/dt} \)) were assessed within 2 days of intervention and 3 months later. MRI was used to quantify PI and ventricular volumes. Conductance catheter techniques were used to obtain indices of contractility and diastolic compliance from pressure-volume relations at rest and under dobutamine infusion. In the PI group, pulmonary regurgitant fraction was 49.2 ± 5.9% at 3-month follow-up. RV cardiac functional reserve was limited, diastolic function was preserved, and myocardial contractility was altered (\( E_{max} = 2.6 ± 0.3 \text{ mm Hg/mL for the PI group versus } 3.5 ± 0.4 \text{ mm Hg/mL for control; } P<0.01 \)). LV cardiac functional reserve was limited, ventricular compliance decreased, and myocardial contractility was preserved.

Conclusions—In the young developing heart, chronic PI alters biventricular systolic function, RV myocardial contractility, and LV diastolic performance. (Circulation. 2003;108:2007-2013.)

Key Words: myocardial contraction ■ magnetic resonance imaging ■ heart defects, congenital ■ ventricles ■ valves

Surgical therapy of cyanotic congenital heart disease requiring right ventricular (RV) outflow tract reconstruction often results in pulmonary insufficiency (PI). Over the last decade, it has become apparent that many of these patients can develop severe RV dysfunction, which in some cases is irreversible.¹

Understanding the mechanisms and timing of ventricular dysfunction due to PI is essential to develop clinical strategies to prevent myocardial damage while minimizing intervention. However, the asymmetric morphology of the RV and the complex functional interaction between the RV and left ventricle (LV) make evaluation particularly challenging. In an attempt to address this issue, various techniques have been used to evaluate RV morphology and function. MRI has been useful to evaluate PI and its effects on RV function. Pressure-volume loops acquired with conductance techniques have recently been extended to the RV, providing valuable information about ventricular function.²

A model of chronic PI was developed in the young growing swine to monitor the effects of PI on the developing heart over time. MRI and conductance catheter techniques were used to evaluate the effects of PI on biventricular systolic, myocardial contractile, and diastolic function.

Methods

Animal Model

Eleven pigs were studied (Pork Power, Patterson, Calif). In 6 animals, stents were placed across the pulmonary valve to induce PI. Five animals served as controls. All animals underwent 4 experimental procedures, including a cardiac catheterization study at the day of intervention (experimental study 1) and a cardiac catheterization study performed 12 weeks later (experimental study 3; Figure 1). Each cardiac catheterization study was followed by an MRI study within 2 days (experimental studies 2 and 4). The mean animal weight at the first procedure was 13.9 ± 1.6 kg, with an increase to 31.1 ± 3.8 kg 3 months later.

The protocol was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Procedures were performed under general anesthesia (1% to 2% isoflurane). Heart rate and systemic arterial pressures were monitored continuously during each procedure.

Study Protocol

Cardiac Catheterization: Experimental Studies 1 and 3

Stent Placement

Self-expanding nitinol stents (Memotherm, Angiomed) with a diameter of 18 mm and a length of 20 mm were placed across the pulmonary valve by a transcatheter technique.³ Angiograms in the RV were performed to confirm the presence of PI and to exclude...
tricuspid insufficiency due to RV dilation at the follow-up catheterization.

**Hemodynamic Measurements**

Cardiovascular pressures were measured with catheters connected to a fluid-filled transducer. Pressures were measured in the right atrium, RV, main pulmonary artery, pulmonary artery wedge position, and LV.

**Conductance Catheter Measurements**

A 5F to 7F dual-field combination pressure-conductance catheter (Millar Instruments) was used for RV and LV loops. The catheter tip was positioned in the ventricular apex. It was connected to a Sigma-5 signal-conditioner-processor (Leycom) that computed time-varying segmental conductance and generated analog output. The analog output was digitized at 200 Hz by an analog-to-digital data card (National Instruments) connected to a Power Macintosh computer (Apple) and analyzed with LabView 5.2 software (National Instruments).

Acquisition of pressure and volume data was obtained during muscle relaxation with vecuronium bromide (0.025 mg/kg IV) and with the ventilator held at end expiration. Data were acquired continuously before and during occlusion of the inferior vena cava with a balloon catheter. Measurements were acquired at rest and during dobutamine infusion. Dobutamine was infused at a rate of 3 μg · kg⁻¹ · min⁻¹, and data were obtained after 10 minutes of hemodynamic stability. High-dose dobutamine was avoided to minimize the effects of the interval-strength relationship.

**MRI: Experimental Studies 2 and 4**

MRI was performed with a GE 1.5-T magnetic resonance imager (Sigma-5, General Electric Medical Systems) with a standard body coil.

A velocity-encoded cine (VEC) MRI sequence was used in a plane perpendicular to the dominating flow direction in the main pulmonary artery to measure pulmonary forward and regurgitant flow volumes. Instantaneous flow volumes were summed to give total forward and regurgitant flow per cardiac cycle. The following acquisition parameters were used for VEC MRI: repetition time/echo time = 25/7 ms, slice thickness = 5 mm, flip angle = 30°, field of view = 24×24 cm, matrix = 256×192, and VEC = 200 cm/s.

A cine MRI sequence in the cardiac short-axis plane was used to assess LV and RV chamber volumes. End-systolic and end-diastolic volumes were measured by manually tracing the area of the endocardial surfaces. Ventricular chamber volumes were computed as the sum of LV and RV volumes of all slices containing LV and RV chambers. The following imaging parameters were used: repetition time/echo time = 8/5 ms, slice thickness = 10 mm, spacing = 0, flip angle = 20°, field of view = 24×24 cm, matrix = 256×128, phases = 16. Two independent observers analyzed cine MR and VEC MR images.

**Histopathology**

At the end of the last study, the pigs were killed, and the hearts were excised. The position and patency of the stents in the pulmonary artery were evaluated. The ventricular septum and RV and LV free walls were dissected and weighted. Tissue samples from an apical, middle, and basal section of RV and LV free walls were excised and immediately fixed in 70% formalin solution for histopathology. Masson’s trichrome was used for staining interstitial collagen.

**Calculations**

**Quantification of Pulmonary Regurgitant Fraction**

Pulmonary flow volumes were generated from the VEC MRI sequences. The volume of PI per heartbeat was calculated as the retrograde flow in the main pulmonary artery in diastole. The regurgitant fraction was calculated as the ratio of pulmonary antegrade to retrograde flow volume.

**Systolic Function**

Indices of biventricular systolic function were derived at rest from cine MRI and conductance measurements and during dobutamine infusion from conductance measurements. Stroke volume was calculated as the difference in end-diastolic and end-systolic volumes, and ejection fraction was calculated as stroke volume divided by end-diastolic volume. Cardiac functional reserve was defined as the ability of the ventricles to increase stroke volume during dobutamine infusion.

**Myocardial Contractility**

Indices of RV and LV contractility were derived from the conductance data obtained during inferior vena cava occlusion. The pressure-volume loop data were analyzed as preload decreased, and the slopes of the 2 relationships were calculated. During the pre-ejection period, pressure was differentiated, and the slope of the dP/dtmax-end-diastolic volume relationship was calculated (Mendystole). The end-systolic index of contractility was calculated by defining end systole as the point of maximal elastance by the iterative method. From the end-systolic point derived from each loop, the slope of the relationship of end-systolic pressure versus end-systolic volume was calculated (EESystole).

**Diastolic Function**

The rate of active ventricular relaxation (τ) cannot be calculated in this model because there are no isovolumic data points in the RV in the presence of PI. Passive ventricular compliance was derived from the end-diastolic pressure-volume relationship. End diastole was defined as the point of maximal volume in association with the lowest pressure. From the end-diastolic points, an exponential fit was constructed and k, the stiffness coefficient, was calculated.

\[
\kappa = \frac{\ln(\text{EDP} - 3.6)}{0.036 - \text{EDV}}
\]

where EDP is end-diastolic pressure and EDV is end-diastolic volume. Antegrade pulmonary forward flow during late diastole from the VEC MRI measurements was used as an indirect estimate of restrictive RV diastolic function.

**Statistical Analysis**

Data measured at rest and during dobutamine infusion (conductance catheter–derived indices of ventricular function) were analyzed by ANCOVA. Variables were coded for animal numbers (PI or control group) and either presence or absence of dobutamine. Data obtained only in the resting state (MRI, hemodynamic data, and weights) were analyzed by either unpaired or paired Student’s t test with Bonferroni correction for multiple analysis. A value of P<0.05 was considered significant. Data are expressed as mean±SD.

**Results**

Stent placement across the pulmonary valve resulted in severe PI (Table 1). RV angiograms revealed no significant tricuspid insufficiency, and pressure measurements showed no pulmonary stenosis.
**Pulmonary Regurgitant Fraction and Global Cardiovascular Effects**

Pulmonary regurgitant fraction in the PI group 2 days after stent implantation was $33.3 \pm 7.2\%$. At 3 months, the regurgitant fraction increased to $49.2 \pm 7.2\%$ ($P<0.001$). In the control group, there was no evidence of pulmonary regurgitant flow in either study. RV end-diastolic and end-systolic volumes were significantly greater in the PI group than in the control group at both MRI studies ($P<0.001$; Table 1). Conversely, LV end-diastolic and end-systolic volumes were significantly decreased in the PI group compared with control at both MRI studies ($P<0.001$).

At both initial and follow-up cardiac catheterizations, all cardiovascular pressures except pulmonary artery pressures were similar in the 2 groups (Table 1). In the PI group, diastolic pulmonary artery pressures were decreased as a result of unrestricted PI ($P<0.05$).

**Systolic Function**

After 3 months, total RV stroke volume was increased significantly in the PI group ($P<0.005$; Tables 1 and 2). Conversely, RV effective cardiac output was decreased significantly because much of the stroke volume returned to the RV through the insufficient pulmonary valve ($P<0.005$). LV stroke volume was decreased significantly in the PI group compared with control ($P<0.001$; Tables 1 and 2).

During infusion of dobutamine, total RV stroke volume did not increase significantly in the PI group (3.3 ± 3.0%; $P<0.001$; Table 2). Similarly, LV stroke volume did not increase significantly in the PI group (8.5 ± 4.6%) but did in the control group (22.9 ± 4.0%; $P<0.001$).

At rest, RV ejection fraction was significantly decreased in the PI group and did not increase significantly during infusion of dobutamine (4.4 ± 2.6%; Table 2). Conversely, RV ejection...
fraction increased significantly in the control group (22±12.3%; P<0.01). At rest, LV ejection fraction was at control levels in the PI group but did not increase during infusion of dobutamine (5.6±3.9%), whereas it increased significantly in the control group (20.3±3.0%; P<0.01; Table 2).

Myocardial Contractility
A set of representative RV pressure-volume loops are shown in Figures 2 and 3. At rest, RV $M_{\text{ipv}}$ was not significantly different in the PI group compared with control (P=0.71), but the response to dobutamine was significantly blunted (P<0.01; Table 3; Figure 4). At rest, LV $M_{\text{ipv}}$ was not significantly different in the PI group compared with control (P=0.89). The increase in response to dobutamine was also similar in both groups (P=0.72; Table 3; Figure 4).

At rest, RV $E_{\text{max}}$ was not significantly decreased in the PI group compared with control (P=0.67), but the response to dobutamine was significantly blunted (P<0.01; Table 3; Figure 4). At rest, LV $E_{\text{max}}$ in the PI group was similar to control (P=0.85), with a normal response to dobutamine (P=0.86; Table 3; Figure 4).

Diastolic Function
After 3 months of PI, RV compliance was increased in the PI group compared with control. The stiffness coefficient $\kappa$ was slightly but significantly smaller (P<0.05; Table 3). Conversely, LV compliance was significantly decreased in the PI group, as evidenced by a greater $\kappa$ than in the control group (P<0.01; Table 3).

After only 2 days of cardiac adaptation to RV volume overload, there was notable pulmonary antegrade flow during late diastole with VEC MRI. However, after 3 months, no significant pulmonary antegrade flow was measured, indicative of a transition from a subacutely noncompliant RV to a compliant nonrestrictive RV after 3 months of cardiac adaptation.

**Histopathology**
Gross examination of the implant site of the stents revealed unobstructed stent lumen with only minimal intimal hyperplasia. Total heart weight and the weight of the RV free wall were increased significantly in the PI group (P<0.001), whereas the LV free wall was slightly but not significantly decreased, and the septum was unchanged (Table 1). The ratio of RV to LV free wall weights was about 1:2 in the control and 1:1 in the PI group, indicative of RV hypertrophy in the PI group. Masson’s trichrome staining revealed no evidence for connective tissue proliferation in either ventricle of either experimental group.
Discussion

In this study, a model of PI was created in the developing heart of growing swine. Over 3 months, the animals increased their weight from 13.9 to 31.1 kg. MRI and conductance catheter techniques allowed for careful assessment of biventricular function and geometry.

We found that chronic PI causes significant dilation of the RV that in turn compresses the LV. The changes in geometry go along with global cardiac dysfunction. In this setting, RV functional reserve and myocardial contractility were impaired, whereas diastolic function was preserved. LV functional reserve was also limited. However, in contrast to the RV, myocardial contractility was preserved and ventricular compliance decreased.

Our data from the dP/dtmax end-diastolic volume and end-systolic pressure-volume relation suggest that the RV systolic dysfunction is caused, at least in part, by an altered contractile state at the level of the myocyte itself. The adaptive cardiac response to pathological loading conditions is time dependent and differs between the RV and LV. To the best of our knowledge, this is the first study that provides evidence that in the developing heart, chronic RV volume overload causes changes in RV contractility long before signs of right heart failure become evident. In fact, in clinical work, the optimal timing of repair of PI remains questionable. The data of the present study suggest that sometime between initial RV dilation and significant RV failure with symptoms, subclinical RV dysfunction develops that could be detected by appropriate methods.

The β-adrenergic response, to increase myocardial contractility, has been reported to be more pronounced in the LV than in the RV.8 One possible explanation is a higher density of β-adrenergic receptors in the LV than in the RV.9 Interestingly, in the present study, a significant differential response to β-adrenergic stimulation was found between LV and RV in both experimental groups and between the normal and volume-loaded RV. Teitel et al10 reported a limited ability to increase myocardial contractility in the newborn lamb. The newborn lamb heart functions under a high resting β-adrenergic state and under a much higher level of performance than the adult sheep heart.11 In the present study, RV functional reserve was limited in the PI group.1 These findings imply that the volume-overloaded RV might function already in the resting state at a high level of performance. Thus, any further functional increase is not achievable, either by means of the Frank-Starling mechanism or by increased myocardial contractility due to β-adrenergic stimulation.

Another important determinant of ventricular performance is ventricular-arterial coupling. In the present study, pulmonary artery elastance (Ea) of the PI animals increased during dobutamine infusion, whereas the increase in RV elastance (Emax) was blunted. Thus, RV Ea/Emax was slightly elevated compared with measurements made at rest. This specific coupling has to be taken into account when data of RV myocardial contractility are interpreted. However, in the

TABLE 3. Indices of Myocardial Contractility and Ventricular Compliance

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Dobutamine</th>
<th>Rest</th>
<th>Dobutamine</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M dP/dt max, mm Hg/mL</td>
<td></td>
<td>Emax, mm Hg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>20.3±3.5</td>
<td>32.8±5.5</td>
<td>2.2±0.4</td>
<td>2.6±0.3</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>LV</td>
<td>33.3±9.6</td>
<td>88.3±18.1</td>
<td>3.9±0.3</td>
<td>5.8±1.2</td>
<td>0.14±0.06</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>25.8±5.9</td>
<td>52.8±12.3*</td>
<td>2.6±0.3</td>
<td>3.5±0.4*</td>
<td>0.17±0.04†</td>
</tr>
<tr>
<td>LV</td>
<td>35.4±8.1</td>
<td>105.6±14.9</td>
<td>4.2±0.4</td>
<td>6.1±1.0</td>
<td>0.10±0.03*</td>
</tr>
</tbody>
</table>

Data of RV and LV for comparison between PI and control group in experimental states of rest and dobutamine. All measurements were performed at 3 months’ follow-up.

*P<0.01, †P<0.05 (ANCOVA).
setting of this animal study, interpretation of the functional impact of Ea/Emax is of particular difficulty. First, PI is per se a major determinant of Ea, and second, the use of stents for creation of PI further complicates the accurate assessment of coupling.

In patients after Mustard operation, Derrik et al.\textsuperscript{12} reported a reduced stroke volume response to exercise and dobutamine stress despite appropriate response in load-independent indices of contraction and relaxation and no evidence of abnormalities of myocardial mechanical performance during diastole. This was attributed to impaired RV filling rate through the intra-atrial pathways. In the present study, a limited effective RV forward flow in conjunction with a decreased LV compliance was present. Thus, LV diastolic function was influenced by means of impaired LV filling due to a limited effective RV forward flow and decreased LV compliance. LV compliance was reduced possibly due to configurational changes induced by RV dilation, because histopathology revealed no evidence for connective tissue proliferation. The resulting net impaired LV diastolic performance might be one explanation for the noted decrease in LV functional reserve despite appropriate LV response in load-independent indices of contraction.

RV diastolic physiology has been investigated in several clinical series in the postoperative assessment of children with tetralogy of Fallot.\textsuperscript{7,13} In such patients, the manifestation of RV restrictive physiology was described early, in the immediate postoperative period, and late.\textsuperscript{7} The origin of the early onset was related to the repair type and intraoperative myocardial injury. Speculation persists regarding the presence of a specific anatomic or physiological substrate for restrictive physiology before operation. In the present study, restrictive RV physiology, as indicated by pulmonary antegrade flow during late diastole, was present in all animals 2 days after induction of PI. At this time, RV volumes were only moderately increased, and pulmonary regurgitant fraction was 33%. After 3 months’ follow-up, the RV was largely dilated, pulmonary regurgitant fraction increased to 49%, RV restrictive physiology was not present, and a small RV stiffness coefficient ($\kappa$) was indicative of a compliant RV. This specific RV diastolic function went along with reduced systolic performance, including contractility. In terms of defining the origin of restrictive physiology, the present data suggest that a transition from a subacute noncompliant to a compliant nonrestrictive RV is, at least in the presence of sudden onset of free PI, a natural course of RV adaption to acute volume overload.

\textbf{Study Limitations}

Because the conductance catheter was for RV measurements aligned in the present study along the axis from the tricuspid valve through the apex, the degree of field inhomogeneity achieved throughout the RV infundibulum is uncertain. However, in the presence of pulmonary regurgitation, the functional response of the RV sinus might differ from that of the infundibulum.\textsuperscript{14}

The amount of pulmonary regurgitation present in this animal study was higher than in most clinical series. In addition, surgical implantation of prosthetic material in the RV has a potential role in ventricular remodeling. More research is needed to illuminate the complex ventricular modeling with growth over prolonged periods of time, to identify the potential mechanisms of the noted decreased contractility, and to prove its potential reversibility.

\textbf{Summary}

Using MRI and conductance catheter techniques, we were able to demonstrate that chronic PI impairs biventricular systolic function, myocardial contractility of the RV, and diastolic performance of the LV. This investigative approach may provide additional insights on the timing and mechanisms of RV dysfunction and failure due to PI.

\textbf{References}


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