Postischemic functional recovery in immature hearts is influenced by performance index and assessment technique

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Torrance, Shona M., and Carin Wittnich. Postischemic functional recovery in immature hearts is influenced by performance index and assessment technique. Am J Physiol Heart Circ Physiol 281: H2446–H2455, 2001.—In the in vivo immature heart, conflicting results are reported for postischemic functional recovery. This study determines whether interpretations of functional recovery are influenced by the contractile performance index (systolic pressure, developed pressure, and maximum rate of systolic pressure increase per unit time) reported or the assessment technique (isovolumetric and variable-volume) utilized. In neonatal pigs (n = 6) on cardiopulmonary bypass, each performance index was examined using both assessment techniques before myocardial ischemia and at 15, 30, and 60 min of reperfusion. With the use of the variable-volume technique, all performance indexes had significantly different recovery. With the use of the variable-volume assessment technique, recovery of systolic pressure was significantly better than the other indexes. When recovery was compared between the two assessment techniques, systolic pressure recovered significantly better when assessed using the variable-volume technique. For each performance index, the correlation between isovolumetric and variable-volume techniques was positive before ischemia but negative during reperfusion, suggesting that the assessment techniques identified conflicting postischemic contractile performances. Thus both the contractile performance index reported and the assessment technique employed are ultimately important in interpreting postischemic functional recovery in the immature heart.

CONTROVERSY PERSISTS regarding the immature heart’s potential for postischemic recovery of contractile performance. Although many studies report moderate postischemic functional recovery, some studies have identified recovery above 85% (1, 11, 28, 30) or below 30% (23, 30, 36). The use of different contractile performance indexes and assessment techniques could contribute to these conflicting findings. For instance, various studies report contractile performance indexes, including peak systolic pressure (9, 10, 12, 15, 20, 23, 35, 36), developed pressure (1, 13, 15, 17, 23, 28, 30, 32, 36), systolic pressure-rate product (10, 20), and maximum rate of systolic pressure increase per unit time (+dP/dt max) (1, 8, 10, 12, 13, 17, 23, 36), but rarely examine more than one or two of these indexes simultaneously. Interestingly, studies (23, 36) that examined two indexes found that peak systolic pressure had two- to threefold greater postischemic functional recovery than either developed pressure or +dP/dt max. This suggests that contractile performance indexes may vary in their susceptibility to ischemic injury. One focus of this study was to simultaneously examine postischemic recovery of three different contractile performance indexes to compare their sensitivity to ischemia-reperfusion in the immature heart.

In the immature heart, myocardial performance is frequently assessed in animal studies using fixed-preload assessment techniques. For example, in the isolated immature heart, common fixed-preload assessment techniques include the working Langendorff model with fixed left atrial pressure (11, 12, 20, 23, 36) or isovolumetric intraventricular balloon (1, 2, 8, 13, 15, 17, 29, 30, 32). These techniques are also commonly used to examine the functional response of the immature heart to stresses such as ischemia (1, 2, 8, 13, 15, 17, 29, 30, 32). In contrast, variable-volume performance assessment techniques, generated by incrementally increasing the end-diastolic volume and determining the intraventricular pressure-volume relationship, are more frequently reported in adult hearts and rarely reported in the immature heart (9, 10). In addition, many aspects of contractile performance are highly sensitive to sarcomere length or ventricular volume (3, 21, 24). Thus isovolumetric and variable-volume performance assessment techniques examine different aspects of myocardial contractile performance, which could yield conflicting results and contribute to existing controversies in the literature. Thus the second focus of this study was to simultaneously examine myocardial performance using both isovolumetric and variable-volume assessment techniques to determine whether they
provide quantitatively different information about postischemic recovery of contractile performance in the immature heart. To achieve these two goals, this study assesses and compares left ventricular (LV) function, as measured by three commonly reported contractile performance indexes (systolic pressure, developed pressure, and +dP/dt\(_{\text{max}}\)) in the same in vivo immature hearts using both isovolumetric and variable-volume performance assessment techniques both before ischemia and during postischemic reperfusion.

**MATERIALS AND METHODS**

**Preparation.** Immature male Yorkshire pigs (3 days old, \(n = 6\)) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg), intubated, and mechanically ventilated with medical air. A catheter was inserted into the right carotid artery and advanced to the aortic arch to monitor arterial blood pressure. Another catheter was inserted into the right jugular vein and advanced to the superior vena cava to monitor central venous pressure. These catheters were connected to pressure transducers (COBE; Lakewood, CO) and a physiological recorder (BIOPAC System; Goleta, CA). Arterial blood samples were obtained, and appropriate adjustments were made to ensure normal physiological values for the arterial partial pressures of oxygen (P\(\text{O}_2\)) and carbon dioxide (P\(\text{CO}_2\)) as well as HCO\(_3^-\) and pH (ABL30 Acid-Base Analyzer, Radiometer; Copenhagen, Denmark).

After sternotomy and systemic heparinization (400 IU/kg heparin sulfate), piglets were placed on normoxic normothermic cardiopulmonary bypass (CPB) using a Sarns 9000 Perfusion System (3M Sarns; Ann Arbor, MI). Appropriate adjustments maintained both arterial mean blood pressure (61 ± 1 mmHg) and central venous pressure (< 2 mmHg). The CPB perfusion circuit was primed with fresh porcine blood, gas exchange was via a “Minimax” membrane oxygenator (Medtronic; Anaheim, CA), and gas flows were adjusted to maintain normal physiological blood gases (P\(\text{O}_2\), P\(\text{CO}_2\), HCO\(_3^-\)) and pH (AJP-Heart Circ Physiol).

**Table 1. Arterial physiological parameters before and during CPB**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-CPB</th>
<th>CPB</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(\text{O}_2), mmHg</td>
<td>107.3 ± 7.2</td>
<td>111.3 ± 5.1</td>
<td>0.73</td>
</tr>
<tr>
<td>Oxygen saturation, %</td>
<td>97.5 ± 0.4</td>
<td>97.0 ± 0.4</td>
<td>0.56</td>
</tr>
<tr>
<td>P(\text{CO}_2), mmHg</td>
<td>35.6 ± 2.4</td>
<td>35.5 ± 1.5</td>
<td>0.98</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.02</td>
<td>7.40 ± 0.02</td>
<td>0.82</td>
</tr>
<tr>
<td>HCO(_3^-), mmol/l</td>
<td>22.0 ± 0.8</td>
<td>21.0 ± 0.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Base excess, mmol/l</td>
<td>−1.6 ± 0.8</td>
<td>−2.4 ± 0.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>7.4 ± 0.6</td>
<td>6.9 ± 0.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>25 ± 2</td>
<td>30.6 ± 1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>37.9 ± 0.4</td>
<td>37.7 ± 0.1</td>
<td>0.51</td>
</tr>
<tr>
<td>Myocardial temperature, °C</td>
<td>NA</td>
<td>38.4 ± 0.1</td>
<td>NA</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>56 ± 2</td>
<td>61 ± 1</td>
<td>0.17</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>198 ± 13</td>
<td>184 ± 5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Values are means ± SE. CPB, cardiopulmonary bypass; P\(\text{O}_2\), arterial partial pressure of oxygen; P\(\text{CO}_2\), arterial partial pressure of carbon dioxide; MAP, mean arterial pressure; NA, not applicable.

After CPB was established, stable baseline isovolumetric and variable-volume myocardial performance data were obtained for each heart (\(n = 6\)) before ischemia. An aortic cross clamp (AXC) was then applied just above the aortic valve to initiate global myocardial ischemia. Normothermia was maintained throughout the ischemic in-

\(+dP/dt_{\text{max}}\) was utilized as a measure of contractility. In this study, myocardial function was determined by both isovolumetric (fixed balloon volume) and variable-volume (pressure-volume relationship) assessment techniques. The absolute values for each contractile performance index (systolic pressure, developed pressure, and +dP/dt\(_{\text{max}}\)) and diastolic pressure were recorded for each balloon volume at each study interval.

For isovolumetric assessment, fluid was injected into the balloon to establish a fixed end-diastolic volume. The balloon volume used was that which initially produced normal physiological ventricular peak systolic and diastolic blood pressure. Thus balloon volume varied for each heart depending on its size and the animal’s normal LV pressures. For each heart, once this volume was determined during baseline preischemic assessment, the same volume was used throughout the experiment.

For variable-volume assessment, fluid was incrementally injected into the balloon to progressively increase the end-diastolic volume. During the initial baseline assessment, this volume ranged from 0.1 ml to a maximum of 2 ml but did not exceed an LV peak diastolic pressure of 10 mmHg. After this original set of data was obtained, the developed pressure-vs.-volume relationship was generated to assess the appropriateness of this volume range. If there were several data points beyond the linear portion of this curve (higher volume), the maximum volume for all subsequent performance assessments was reduced accordingly. For each heart, once the appropriate volume range was determined during this initial assessment, this same volume range was used to generate performance index-vs.-volume curves at each study interval (baseline and reperfusion). Ultimately, the absolute values for each contractile performance index (systolic pressure, developed pressure, and +dP/dt\(_{\text{max}}\)) and diastolic pressure were recorded for each volume, thus generating a performance index-vs.-volume relationship. Several of these indexes have complex preload-dependent modulation, but variable-volume performance was quantified and expressed as the slope of the linear portion of this performance index-vs.-volume relationship.

**Experimental protocol.** After CPB was established, stable baseline isovolumetric and variable-volume myocardial performance data were obtained for each heart (\(n = 6\)) before ischemia. An aortic cross clamp (AXC) was then applied just above the aortic valve to initiate global myocardial ischemia. Normothermia was maintained throughout the ischemic in-

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ASSESSMENT OF POSTISCHEMIC RECOVERY IN IMMATURE HEARTS

Table 2. Absolute isovolumetric myocardial performance

<table>
<thead>
<tr>
<th>Performance Index, units</th>
<th>Baseline</th>
<th>15 Min</th>
<th>30 Min</th>
<th>60 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure, mmHg</td>
<td>99.1 ± 8.0</td>
<td>70.1 ± 7.8</td>
<td>74.6 ± 6.9</td>
<td>68.5 ± 8.8</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>-2.5 ± 1.0</td>
<td>2.9 ± 2.9</td>
<td>1.1 ± 2.4</td>
<td>1.7 ± 1.8</td>
</tr>
<tr>
<td>Developed pressure, mmHg</td>
<td>102.6 ± 6.0</td>
<td>67.2 ± 5.0</td>
<td>73.5 ± 7.3</td>
<td>71.3 ± 5.4</td>
</tr>
<tr>
<td>+dP/dt_{max}, mmHg/s</td>
<td>1,804.2 ± 176.7</td>
<td>1,255.7 ± 180.2</td>
<td>1,539.8 ± 187.7</td>
<td>1,368.8 ± 178.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. +dP/dt_{max}, maximum rate of systolic pressure increase per unit time.

...interval. Immediately after AXC placement, the LV balloon was filled to generate a pressure of 10 mmHg. The time to ischemic contracture onset (pressure increase of 2 mmHg in the balloon) was measured and recorded for each heart. After the ischemic contracture onset was reached (33.3 ± 3.1 min), the fluid was withdrawn from the balloon, the AXC was removed, reperfusion was initiated, and the “isovolumetric volume” was reinjected into the balloon. Repeat isovolumetric and variable-volume performances were assessed at 15, 30, and 60 min of reperfusion.

Myocardic contracture can be associated with cell swelling, edema, and capillary collapse or compression (18, 19), all of which potentially impede coronary reperfusion (4, 18, 19, 22). For each heart in this study, reperfusion was confirmed by coronary distension and the return of normal arterialized color. Although coronary flow might have varied somewhat between hearts, both isovolumetric and variable-volume performances were assessed in the same hearts; thus any differences in recovery between assessment techniques would not be due to differences in coronary perfusion.

After 60 min of reperfusion, CPB was terminated, and the animals were euthanized by pentobarbital overdose. All experimental procedures and protocols used in this investigation were reviewed and approved by the University of Toronto Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 96-03, Revised 1996) and the Canadian Council on Animal Care guidelines.

Data analysis. In all study animals, the absolute value for each performance index was determined in each heart using both assessment techniques. The “baseline” value for each performance index was the mean value obtained from multiple baseline performance assessments conducted before ischemia. At each reperfusion interval, the absolute value for each performance index was again determined, but postischemic recovery of each contractile performance index was also expressed as a percentage of the baseline value. All three time points were used to calculate the mean percent recovery throughout reperfusion. Paired t-test (31) was used to analyze both differences in recovery between contractile performance indexes within each assessment technique and differences in recovery of each performance index between assessment techniques. To further explore the relationship between performance assessment techniques for individual hearts, the correlation between isovolumetric and variable-volume assessment techniques was determined and quantified using the Pearson correlation coefficient (31). Correlation analysis before ischemia (baseline) was performed on the absolute data, whereas correlation analysis during reperfusion was performed on both the absolute data and the percent recovery data for all three reperfusion intervals. Positive correlations indicate that isovolumetric and variable-volume assessment techniques had a direct relationship and would yield consistent conclusions. In contrast, no correlation indicates dissociation between the assessment techniques, whereas negative correlation indicates that these two performance assessment techniques had an inverse relationship and thus would yield contradictory findings. Values are expressed as means ± SE. Statistical trends were accepted for 0.05 < P < 0.10, and statistical significance was accepted at P < 0.05 (31).

RESULTS

Model perfusion parameters and baseline myocardial performance. Arterial blood gases, acid-base parameters (Pao_{2}, oxygen saturation, PaCO_{2}, pH, HCO_{3}^{-}, and base excess), and hemodynamic parameters (mean arterial pressure and heart rate) were monitored during ventilation before CPB and were maintained within these limits throughout CPB (Table 1). These data confirm that this normothermic normoxic CPB model provided normal physiological and hemodynamic parameters. The absolute baseline values for systolic pressure, developed pressure, and +dP/dt_{max}, as well as diastolic pressure, obtained using both the isovolumetric (Table 2) and variable-volume assessment techniques (Table 3), are shown. The negative isovolumetric diastolic pressures obtained in this experiment are consistent with other animal preparations (5, 27) as well as a study (25) in humans undergoing mitral valvuloplasty. These studies all generated conditions of LV volume clamping; thus the negative

Table 3. Absolute variable-volume myocardial performance (slope)

<table>
<thead>
<tr>
<th>Performance Index, units</th>
<th>Baseline</th>
<th>15 Min</th>
<th>30 Min</th>
<th>60 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure, mmHg/ml</td>
<td>34.9 ± 2.6</td>
<td>28.7 ± 4.2</td>
<td>26.3 ± 4.3</td>
<td>27.9 ± 3.5</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg/ml</td>
<td>4.8 ± 0.9</td>
<td>10.5 ± 1.5</td>
<td>9.4 ± 1.3</td>
<td>10.4 ± 2.3</td>
</tr>
<tr>
<td>Developed pressure, mmHg/ml</td>
<td>31.6 ± 3.0</td>
<td>18.3 ± 3.4</td>
<td>15.4 ± 4.5</td>
<td>17.7 ± 2.0</td>
</tr>
<tr>
<td>+dP/dt_{max}, mmHg/s/ml^{-1}</td>
<td>576.0 ± 62.6</td>
<td>319.8 ± 44.5</td>
<td>302.7 ± 55.9</td>
<td>327.5 ± 39.2</td>
</tr>
</tbody>
</table>

Slope values are means ± SE.

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ventricular pressures produced by the restoring forces of titin (16) became apparent under these experimental conditions. The other baseline isovolumetric performance data are consistent with similar animal models and confirm appropriate baseline physiological performance, whereas the variable-volume performance data delineate the corresponding variable-volume performance. Tables 2 and 3 also contain the absolute performance data at 15, 30, and 60 min of reperfusion.

Comparison of postischemic recovery between contractile performance indexes within each assessment technique. Postischemic recovery of systolic pressure, developed pressure, and \( +\frac{dP}{dt_{max}} \) was assessed by both isovolumetric and variable-volume performance assessment techniques. Although the focus of this study is myocardial contractile performance, the diastolic component of myocardial performance may be important for interpretation and thus is also reported. After ischemia-reperfusion, these hearts had an increase of 5.4 ± 0.4 mmHg in the isovolumetric diastolic pressure and an increase of 5.3 ± 0.7 mmHg/ml in the slope of the diastolic pressure-vs.-volume relationship. Thus, in this study, these immature hearts had evidence of quantitatively similar moderate diastolic dysfunction with ischemia-reperfusion. Postischemic myocardial contractile performance was examined in the setting of this moderate diastolic dysfunction. Differences in recovery between contractile performance indexes using the isovolumetric (Fig. 1) and variable-volume (Fig. 2) assessment techniques are both shown.

With the use of the isovolumetric technique, the 72% recovery of systolic pressure was significantly and consistently greater than the 67% recovery of developed pressure (Fig. 1A; \( P < 0.0001 \)) but had a trend to worse recovery than \( +\frac{dP}{dt_{max}} \) (Fig. 1B; 77%, \( P = 0.08 \)). It is interesting to note that hearts with the best recovery of systolic pressure had consistently better recovery of \( +\frac{dP}{dt_{max}} \), whereas hearts with poor recovery of systolic pressure had highly variable recovery of \( +\frac{dP}{dt_{max}} \). The 67% recovery of developed pressure was also significantly lower than the 77% recovery of \( +\frac{dP}{dt_{max}} \) (Fig. 1C; \( P = 0.0015 \)); however, the magnitude of this difference was also dependent on the level of recovery. Specifically, hearts with the best recovery of developed pressure had over 20% higher recovery of \( +\frac{dP}{dt_{max}} \), but hearts with poor recovery of developed pressure had similar or only slightly better recovery of \( +\frac{dP}{dt_{max}} \).

With the use of the variable-volume assessment technique, the 88% recovery of systolic pressure was significantly (\( P < 0.0001 \)) higher than recovery of both developed pressure (Fig. 2A; 65%) and \( +\frac{dP}{dt_{max}} \) (Fig. 2B; 62%). Interestingly, the magnitude of this difference in recovery was also variable. For instance, hearts with the greatest recovery of systolic pressure had

![Fig. 1. Differences in postischemic recovery between contractile performance indexes using the isovolumetric assessment technique. A: systolic vs. developed pressure; B: systolic pressure vs. maximum rate of systolic pressure increase per unit time (+dP/dt_{max}); C: developed pressure vs. +dP/dt_{max}. The data shown include the individual data points for each heart at 15 ( ●), 30 ( ■), and 60 min ( ▲) of reperfusion, the overall means ± SE for each performance index, and the \( P \) values for the paired \( t \)-test.](http://ajpheart.physiology.org/)

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<10% worse recovery of the other indexes, whereas hearts with poor recovery of systolic pressure had as much as 40% lower recovery of developed pressure and $+\frac{dP}{dt_{\text{max}}}$.

Finally, recovery of developed pressure (65%) and $+\frac{dP}{dt_{\text{max}}}$ (62%) were not statistically different (Fig. 2C). Thus, within each assessment technique, the extent of postischemic recovery is determined by the performance index examined.

Comparison of postischemic recovery of each contractile performance index between assessment techniques. Differences in postischemic recovery of each contractile performance index between the isovolumetric and variable-volume assessment techniques were also examined. Specifically, recovery of systolic pressure was 15% lower using the isovolumetric compared with the variable-volume assessment technique (Fig. 3A; $P =$...
In contrast, recovery of developed pressure was not different between the two assessment techniques (Fig. 3B). Additionally, although it did not achieve statistical significance, recovery of +dP/dt max was 15% higher using the isovolumetric compared with the variable-volume assessment technique (Fig. 3C; \( P = 0.12 \)). Thus postischemic recovery of contractile performance is also influenced by the assessment technique utilized.

Although these data examine overall performance index- and assessment technique-specific differences, they do not address the relationship between isovolumetric and variable-volume assessment techniques within the same heart. This relationship can be most accurately assessed by determining the correlation between isovolumetric and variable-volume assessment techniques. Before ischemia (baseline), all three contractile performance indexes produced relatively strong positive correlations between isovolumetric and variable-volume assessment techniques. These correlations were statistically significant and quantitatively similar for systolic pressure (Fig. 4A; \( r = 0.77, P = 0.04 \)) and developed pressure (Fig. 4B; \( r = 0.78, P = 0.04 \)), whereas +dP/dt max yielded a weaker statistical trend (Fig. 4C; \( r = 0.68, P = 0.09 \)). Generally, under baseline conditions, isovolumetric and variable-volume assessment techniques yielded consistent findings for each contractile performance index. With reperfusion after moderate ischemic injury, the significant positive correlations previously identified for all three contractile performance indexes were lost. The absolute data during reperfusion did not yield significant correlations for systolic pressure (Fig. 5A; \( r = 0.39, P = 0.11 \)), developed pressure (Fig. 5B; \( r = 0.14, P = 0.59 \)), or +dP/dt max (Fig. 5C; \( r = 0.23, P = 0.36 \)). Interestingly, one animal had substantially higher baseline performance than all other animals. When the three data points contributed by this outlier animal were removed from the analysis of the absolute data during reperfusion, all three contractile performance indexes yielded negative correlations of varying strength. This shift to a negative correlation with ischemia-reperfusion was confirmed when the reperfusion data for all animals, including the animal with high baseline performance, were expressed as a percentage of baseline. Specifically, hearts had similar significant negative correlations for postischemic recovery of systolic pressure (Fig. 6A; \( r = -0.52, P = 0.03 \)) and developed pressure (Fig. 6B; \( r = -0.53, P = 0.02 \)), whereas +dP/dt max yielded a highly significant strong negative correlation (Fig. 6C; \( r = -0.76, P = 0.0002 \)). Interestingly, the outlier animal identified by examining the absolute data had a percent recovery of performance consistent with all other animals. Thus, after ischemia-reperfusion, the strong positive correlations between isovolumetric and variable-volume assessment techniques for all three contractile performance indexes were lost and actually became negative. This suggests that isovolumetric and variable-volume assessment techniques could yield contradictory findings about recovery after moderate ischemic injury.

**DISCUSSION**

Over the past few years, studies have examined postischemic functional recovery in the immature heart. Although most clinical studies document moderate to severe postoperative myocardial dysfunction...
(6, 7) and low output syndrome (8a) after repair of congenital heart defects, many studies (1, 11, 15, 28) in experimental animals report minimal postischemic functional impairment. Because most clinical outcomes reflect preload-dependent myocardial performance assessment, whereas most experimental studies utilize preload-independent assessment techniques, these contradictory conclusions could be due to study-specific differences in the performance assessment techniques examined. Although the fetal heart has limited pre-
load-dependent modulation of ventricular output (33), the immature heart has more extensive, but not yet fully developed, preload-dependent modulation of contractile performance. Isovolumetric assessment techniques examine the functional response of the heart to a fixed ventricular volume and only examine preload-independent aspects of ventricular performance, whereas variable-volume assessment techniques examine the functional response of the heart to a specific range of ventricular volumes (variable preload), which explores both preload-dependent and preload-independent performance modulation. Thus variable-volume performance assessment techniques could provide more physiologically relevant information than those obtained by isovolumetric analysis alone. Despite this knowledge, experimental studies in immature hearts primarily utilize isovolumetric, not variable-volume, assessment techniques.

In addition, because each performance index (systolic pressure, developed pressure, and $+dP/dt_{\text{max}}$) depends on different cellular mechanisms, which have different susceptibility to ischemic injury, each performance index potentially yields a different magnitude of postischemic dysfunction. For example, using the isovolumetric assessment technique, the reduction in systolic pressure is consistent with the 60% elevation in lactate content observed in these hearts (34), which would indicate the presence of intracellular acidosis. In addition, the 23% reduction in $+dP/dt_{\text{max}}$ was much more variable but generally less than that seen in either systolic (28%) or developed pressure (33%). The reduced postischemic recovery of $+dP/dt_{\text{max}}$ observed in these hearts was likely due to the combined effects of the 25% reduction in ATP levels (34) and intracellular acidosis, which would reduce myosin ATPase activity. The current study confirmed that each contractile performance index and assessment technique yielded different degrees of postischemic dysfunction and that isovolumetric and variable-volume assessment techniques yielded contradictory findings about postischemic recovery of contractile performance. Thus both the performance index and the assessment technique must be considered when interpreting functional recovery in the immature heart.

**Isovolumetric vs. variable-volume assessment techniques.** The importance of the assessment technique itself was investigated by determining whether postischemic recovery of each performance index was quantitatively different when assessed using isovolumetric and variable-volume techniques. Interestingly, recovery of systolic pressure was significantly lower using the isovolumetric compared with the variable-volume assessment technique. Thus despite the overall longer sarcomere length for myofilament deactivation that was identified by the isovolumetric technique, preload-dependent modulation of myofilament deactivation was maintained with ischemia-reperfusion. In contrast, postischemic recovery of developed pressure was quantitatively similar using both the isovolumetric and variable-volume assessment techniques, suggesting quantitatively similar injury to preload-dependent and preload-independent mechanisms. Finally, postischemic recovery of $+dP/dt_{\text{max}}$ was 15% worse using the variable-volume assessment technique, indicating that there was more extensive injury to the preload-dependent mechanisms that regulate $+dP/dt_{\text{max}}$.

The precise relationship between isovolumetric and variable-volume assessment techniques is best investigated by analyzing the correlation using data from individual hearts. At baseline, systolic pressure, developed pressure, and $+dP/dt_{\text{max}}$ all produced positive correlations between isovolumetric and variable-volume assessment techniques. This indicates that these two assessment techniques would yield consistent findings in the unstressed immature heart. These same hearts were then reperfused after moderate ischemic injury, and the previously identified significant positive correlations were lost. In fact, when expressed as a percentage of baseline, all three contractile performance indexes yielded negative correlations during reperfusion. This suggests that the isovolumetric and variable-volume assessment techniques would yield contradictory conclusions about postischemic functional recovery and could yield paradoxical conclusions about the susceptibility to ischemic injury. For example, hearts that had poor postischemic functional recovery using the isovolumetric assessment technique likely had reduced peak cytosolic Ca$^{2+}$ levels during systole. It is interesting to note that these hearts also had the highest functional recovery using the variable-volume assessment technique, indicating that they maintained sarcoplasmic reticulum Ca$^{2+}$-handling capacity and preload-dependent regulation of myofilament Ca$^{2+}$ sensitivity and troponin C Ca$^{2+}$ affinity. These hearts would be most capable of responding to varying loading conditions, and thus would likely maintain heart function in vivo. In contrast, hearts that exhibited good recovery using the isovolumetric assessment technique likely had higher cytosolic Ca$^{2+}$ levels and had optimal troponin C Ca$^{2+}$ binding and myofilament Ca$^{2+}$ sensitivity at fixed ventricular volume. However, these hearts also had poor recovery using the variable-volume assessment technique, indicating that they had impaired preload-dependent performance regulation. This likely occurred due to impaired sarcoplasmic reticulum Ca$^{2+}$ handling, which would profoundly compromise troponin C Ca$^{2+}$ binding and preload-dependent modulation of myofilament Ca$^{2+}$ sensitivity. These hearts would be unable to adequately respond to varying loading conditions, and thus would be less able to sustain heart function in vivo.

In summary, in the in vivo immature pig heart, both the contractile performance index reported and the assessment technique employed are ultimately important in interpreting postischemic functional recovery. With the use of the isovolumetric technique, postischemic recovery of all three performance indexes were significantly different. With the use of the variable-volume technique, systolic pressure had significantly higher recovery then either developed pressure or $+dP/dt_{\text{max}}$. When recovery between the two assessment
techniques was compared, systolic pressure recovered significantly better with the variable-volume assessment technique. In addition, the positive correlation between isovolumetric and variable-volume assessment techniques before ischemia was lost entirely during reperfusion and even became negative when expressed as percent recovery. This indicates that the two assessment techniques would yield contradictory conclusions about posts ischemic functional recovery and could yield paradoxical conclusions about the susceptibility to ischemic injury. Thus both the contractile performance index and the assessment technique are ultimately important in interpreting posts ischemic functional recovery in the in vivo immature heart.

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