Contractile Function, Myosin ATPase Activity and Isozymes in the Hypertrophied Pig Left Ventricle after a Chronic Progressive Pressure Overload

Thomas Wisenbaugh, Paul Allen, George Cooper IV, Henry Holzgrefe, George Beller, and Blase Carabello

From the Divisions of Cardiology, University of Virginia Hospital, Charlottesville, Virginia, and Temple University Hospital, Philadelphia, Pennsylvania 19140 and the Department of Anesthesia, Brigham and Women's Hospital, Boston, Massachusetts

SUMMARY. Experimental right ventricular pressure-overload hypertrophy in small mammals is associated with early muscle dysfunction, even before the onset of overt pump failure. Experimental results are quite heterogeneous regarding muscle function of the pressure hypertrophied left ventricle. Muscle dysfunction of the right or left ventricle, when found, may be causally related to alterations of myosin ATPase activity and isozyme type. However, the effect of a gradual pressure overload, analogous to that which occurs in human aortic stenosis, on myocardial contractile function and myosin ATPase activity has not been studied in a large animal whose normal myosin isozyme pattern resembles that of man. We therefore studied pump performance, myocardial contractile function, and myosin ATPase activity and isozyme pattern in pigs with severe, gradually applied left ventricular pressure overload. Thirteen weeks after supravalvular aortic banding, 10 pigs grew more than 7-fold in body weight and were found to have an aortic stenosis area of 0.5 ± 0.1 cm² with a gradient of 93 ± 12 mm Hg. Compared with nine control animals, the banded animals had a 67% increase in left ventricular mass relative to body weight without overt pump failure as measured by cardiac index and pulmonary artery wedge pressure. Left ventricular ejection performance, measured as shortening fraction, was maintained except in three animals with extreme hypertrophy, in which depressed ejection performance may have been due to an afterload mismatch, myocardial dysfunction, or both. Myocardial contractile function, determined from the end-systolic stress-diameter relationship, was normal except in two pigs in which ejection performance was depressed and left ventricular mass was more than doubled. Only the slow V3 isozyme of myosin ATPase was found in both normal and hypertrophied pig myocardium, and the ATPase activity was normal in pigs with all degrees of hypertrophy. Thus, in a large animal model of severe, gradual left ventricular pressure overload, in which myosin isozyme pattern remains apparently unaltered, moderate hypertrophy can be associated with normal myosin ATPase activity and contractile function that is normal by current methods of evaluation. (Circ Res 53: 332–341, 1983)

CONTRACTILE performance of isolated papillary muscles removed from the pressure-overloaded right ventricles of small mammals with moderate to severe hypertrophy is depressed (Spann et al., 1967; Bassett and Gelband, 1973; Cooper et al., 1973; Hamrell and Alpert, 1977), even when the overload is applied gradually (Cooper et al., 1981). This muscle dysfunction may precede overt right ventricular dysfunction in moderate right ventricular hypertrophy (Cooper et al., 1981). However, in the case of severe hypertrophy (Spann et al., 1972), contractile dysfunction of the papillary muscle has been correlated with depressed pump performance of the right ventricle of which it forms a constituent part. Although a few studies have shown normal contractile function in right ventricular pressure overload (Pannier, 1971; Julian et al., 1981), most studies have shown a progression from moderate hypertrophy with well-preserved pump function despite depressed contractile function per unit mass of myocardium, to severe hypertrophy with depressed muscle and pump performance.

Contractile performance of the hypertrophied left ventricle with severe pressure overload has been less extensively studied on either the muscle or pump level, and the experimental results are quite heterogeneous. Papillary muscles from moderately hypertrophied left ventricles of rats with chronic pressure overload, induced abruptly by acute aortic banding (Bing et al., 1971) or gradually in a hypertensive model (Capasso et al., 1982), shorten with depressed velocity but maintain normal, or even supernormal (Capasso et al., 1981) levels of isometric tension. Papillary muscles from more extensively hypertrophied left ventricles of rats subjected to acute aortic banding develop less tension than normal (Meerson and Kapelko, 1972). The contractile function of the intact left ventricular pump in terms of either the end-systolic stress-diameter relationship (Sasayama et al., 1977) or the more usual...
Our hypothesis was that contractile function and myosin ATPase activity could be normal in such a model of left ventricular hypertrophy. Contractile function was measured using the end-systolic stress-diameter relationship; this measure of contractile performance appears to be independent of loading conditions (Suga et al., 1973; Weber et al., 1976) and correlates with the inotropic state of the intact left ventricle (Borow et al., 1982). Further, since alterations in myosin isoforms and ATPase activity have been postulated as explanations for the depressed myocardial function found in small mammals whose normal myosin isozyme pattern differs from that of man (Lompre et al., 1981), we investigated the ATPase activity and isozyme pattern of myosin in this unique model of supravalvular aortic stenosis which produced severe, gradually imposed pressure overload in the pig.

Methods

Preparation of the Experimental Model

Four- to six-week-old Yorkshire pigs weighing 5–10 kg were sedated with ketamine (20 mg/kg, im) and zylazine (2 mg/kg, im), and then anesthetized with halothane and nitrous oxide. A right thoracotomy was performed in the 2nd intercostal space, with endotracheal ventilation controlled by a Harvard respirator. Through a small pericardiotomy, a strip of polytetrafluoroethylene (courtesy of W. L. Gortex & Assoc., Inc.) precut to give a band circumference of 3 cm was tied around the aortic root just above the origin of the coronary arteries. This circumference was chosen to match the undeformed external circumference of the aortic root just above the coronary arteries in 7-kg pigs, determined postmortem, and gives a cross-sectional area of about 0.7 cm². In all cases, the band was tied tightly snugly around the aorta but did not visibly deform the vessel or create a palpable thrill. The small pericardiotomy was left open, the pneumothorax evacuated with a chest tube, and the chest wall and skin incisions were closed. The animals were returned to the farm to grow for an average of 13 weeks before study. Non-operated pigs served as controls.

Instrumentation and Data Acquisition

The pigs were studied 13 ± 1 weeks after banding. After sedation with ketamine (20 mg/kg, im) and zylazine (2 mg/kg, im), the animals were anesthetized with α-chloralose (100 mg/kg, iv) and morphine sulfate (1 mg/kg, im). Through a midline neck incision, a tracheostomy tube was inserted for ventilation with room air or supplemental oxygen sufficient to keep blood gases in the physiological range, as determined periodically throughout the procedure. Right heart catheterization was performed via the right internal jugular vein with a triple-lumen thermodilution catheter for measurement of right heart pressures and thermodilution cardiac output with a cardiac output computer. Left heart catheterization was performed via the right carotid artery with a manometer-tip catheter. A pullback gradient across the stenosis was obtained, and the stenosis area was estimated with a modification of the Gorlin equation (Hakki et al., 1981). This catheter tip was placed proximal to the stenosis for measurement of pressure during hemodynamic studies. A second, fluid-filled
catheter was used to monitor pressure distal to the stenosis. Fluids were given intravenously prior to mechanical studies as needed to keep the pulmonary wedge pressure at 5–10 mm Hg. A left thoracotomy was performed in the 5th intercostal space. A snare was placed around the descending thoracic aorta. The heart was exposed through a pericardiotomy. Left ventricular minor axis, major axis, and wall thickness were measured with 5 MHz piezoelectric crystals and ultrasonic dimension gauge using methods described previously (Rankin et al., 1976). Briefly, as shown in Figure 1, a pair of 5 mm crystals was sutured to the epicardium of the anterior and posterior walls, respectively, to measure minor axis at the equator. Major axis was determined from another pair of crystals, of which one was positioned at the apex and the other at the base between the aortic root and left atrium. Anterior wall thickness above the papillary muscle was measured between a 2-mm crystal placed at the endocardium through a diagonal tract and a 5-mm crystal sutured to the epicardium directly opposite the endocardial crystal; this position was determined ultrasonically as the shortest distance between the two crystals. Pacing at a rate of 100/min was achieved with wires attached to the left atrium. Propranolol (1 mg/kg) and atropine (0.01 mg/kg) were given intravenously to prevent reflex changes in AV conduction and inotropic state. Baseline pressures and dimensions were recorded. Incremental elevation of proximal aortic pressure was produced by tightening the snare, and repeat measurements of end-systolic thickness and dimensions were obtained at each new end-systolic pressure; the pressure in each case stabilized after 20–30 beats. After release of the aortic snare and hemodynamic equilibration, the reaction was repeated. From four to 12 (mean ± SE = 7 ± 1) aortic constrictions of varying thickness and dimension measurements from each of four to 12 (mean ± SE = 7 ± 1) sets of measurements were obtained for each animal. The experiment was terminated by fibrillating the heart electrically, then quickly removing the heart from the chest. After crystal placement had been carefully documented, the atria, right ventricle, valves, pericardial fat, and any adhesions present were removed from the left ventricle, which was then weighed and quick-frozen in liquid nitrogen. Since proper crystal placement was critical to the measurement of wall stress, any heart in which the endocardial crystal of the wall thickness pair was greater than 1 mm from the endocardium was excluded from the study.

Evaluation of Ventricular Function

We used a modification of the stress-diameter method described previously by Sasayama et al., (1977). This method is based on the end-systolic pressure-volume relationship popularized by Sagawa (1978). This relationship has been found to be linear in both animals and humans, and its slope, $E_{max}$, provides an index of contractile function that is independent of loading conditions (Suga et al., 1973; 1979) and sensitive to inotropic stimulants (Suga and Sagawa, 1974; Suga et al., 1973; Grossman et al., 1977). In comparisons of ventricles of different sizes, wall stress is preferable to pressure, since it normalizes for differences in ventricular wall thickness and chamber dimensions (Weber et al., 1976; Marsh et al., 1979). We calculated circumferential stress ($S_c$) for a thick-walled ellipse (Falsetti et al., 1970), as shown in Figure 1. End-systolic stress was plotted against end-systolic midwall diameter, $b_0$. $S_c$ was determined ultrasonically as the shortest distance between a 2-mm crystal placed at the endocardium through a diagonal tract and a 5-mm crystal sutured to the epicardium directly opposite the endocardial crystal; this position was determined ultrasonically as the shortest distance between the two crystals. Pacing at a rate of 100/min was achieved with wires attached to the left atrium. Propranolol (1 mg/kg) and atropine (0.01 mg/kg) were given intravenously to prevent reflex changes in AV conduction and inotropic state. Baseline pressures and dimensions were recorded. Incremental elevation of proximal aortic pressure was produced by tightening the snare, and repeat measurements of end-systolic thickness and dimensions were obtained at each new end-systolic pressure; the pressure in each case stabilized after 20–30 beats. After release of the aortic snare and hemodynamic equilibration, this process was repeated. From four to 12 (mean ± SE = 7 ± 1) sets of measurements at just as many pressures were obtained for each animal. The experiment was terminated by fibrillating the heart electrically, then quickly removing the heart from the chest. After crystal placement had been carefully documented, the atria, right ventricle, valves, pericardial fat, and any adhesions present were removed from the left ventricle, which was then weighed and quick-frozen in liquid nitrogen. Since proper crystal placement was critical to the measurement of wall stress, any heart in which the endocardial crystal of the wall thickness pair was greater than 1 mm from the endocardium was excluded from the study.

Indices of Hypertrophy

Two independent indices of hypertrophy were examined. To normalize for differences in body size, left ventricular wet weight and the weight of the pig are expressed as LVW:BW ratio in grams/kilogram. As an internal index of concentric hypertrophy, the ultrasonically determined wall thickness and endocardial semimajor axis at end diastole in situ under maximum loading conditions are expressed as $h:R$ ratio.

Myosin ATPase

After preparation of myosin (Murakami et al., 1976), the final product was dialyzed overnight against 5 mM borate buffer at pH 8.0 containing 0.5 mM KCl at 4°C. Protein content was determined using a microbiuret method. The ATPase activity was measured (Barany et al., 1967) as noted below at 25°C in a 2-ml reaction mixture containing:

1. K+EDTA activated: 50 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.5 mM KCl, 2.5 mM ATP, 12.5 μg myosin/ml.

2. Ca++ activated: 50 mM Tris-HCl, (pH 7.6), 10 mM Tris-HCl (pH 7.6), 0.025 mM KCl, 10 mM CaCl2, 2.5 mM ATP, 12.5 μg myosin/ml.

3. Myofibrillar ATPase: 20 mM tris-acetate (pH 7.0), 0.025 mM KCl, 0.05 mM CaCl2, 1.0 mM MgCl2, 1.0 mM ATP, 15 μg myofibrils (an aliquot of myofibrils was taken before the myosin extraction in the procedure above). The reaction was started by the addition of ATP and stopped after 5 minutes with 1.67 N H2SO4. Sodium dodecyl sulfate (SDS) 0.4% was added to the 1.67 N H2SO4 to prevent precipitation of actomyosin in the myofibrillar
ATPase. The liberated P, was determined in each case by a modification of the method of Fiske and Subbarow (1925). This method is simplified because of the low protein concentration, and does not require centrifugation prior to color development. Because the entire sample, rather than a small aliquot, was used, P, determination was enhanced.

**Gel Electrophoresis**

Myosin was examined by both one-dimensional SDS and pyrophosphate (native) gel electrophoresis to examine the myosin purity (SDS) and to determine the number of isoenzymic forms (pyrophosphate).

SDS gels were run according to the method of Laemmli (1970), using a 4.5% stacking and a 13% running gel. Pyrophosphate (native) gel electrophoresis was done by the method of Höh (1976, 1978). These 3% acrylamide gels were run at 2-4°C for 16 hours, with recirculation of the buffer between the anodic and cathodic reservoirs.

**Statistical Analysis**

The end-systolic stress-diameter data for each animal was fit to a linear regression equation (method of least squares) having a regression coefficient (slope) defined as E\(_{max}\), and a diameter intercept defined as b\(_0\) (Mehmel et al., 1981; Suga et al., 1979; Marsh et al., 1979). Variation within groups (control and hypertrophy) was determined by analysis of covariance (Biomedical Computer Programs, 1979). Comparisons for both E\(_{max}\) and b\(_0\) between the two groups were made using the Mann-Whitney rank sum test (Biomedical Computer Programs, 1979). The relationship between b\(_0\) and b\(_{a0}\) within each group was studied, and a comparison of this relationship between the two groups was performed, using multiple linear regression (Biomedical Computer Programs, 1979).

Comparisons between groups were made for the other mechanical, hemodynamic, morphological, and biochemical data using the unpaired two-tailed t-test.

When a value of p < 0.05 was found, the difference was considered to be statistically significant. The data are reported as means ± se.

**Results**

Thirteen ± 1 weeks after banding, piglets initially weighing 7 ± 1 kg grew to 53 ± 5 kg, and had developed severe supravalvular aortic stenosis as demonstrated in Table 1. The hemodynamic data, analogous to those in humans with severe aortic stenosis, reveal an increase in left ventricular end-diastolic pressure in these concentrically hypertrophied ventricles, but normal pump function as assessed by cardiac index and wedge pressure.

The data in Table 2 reveal the significant degree of concentric left ventricular hypertrophy found in the banded animals at the time of study. Relative to body mass, left ventricular mass in the banded animals was 67% greater than that of controls.

The ventricular mechanical data are presented in Table 3 and in Figure 2. Afterload, expressed as end-systolic circumferential stress (Sc), tended to be elevated and shortening fraction tended to be depressed in the hypertrophied animals, although these differences did not reach statistical signifi-

---

**Table 1**

<table>
<thead>
<tr>
<th>Hemodynamic Characteristics of Experimental Pigs</th>
<th>Control</th>
<th>Hypertrophied</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>112 ± 9</td>
<td>191 ± 14†</td>
</tr>
<tr>
<td>Stenosis gradient (mm Hg)</td>
<td>93 ± 12</td>
<td></td>
</tr>
<tr>
<td>Stenosis area (cm(^2))</td>
<td>0.5 ± 1</td>
<td></td>
</tr>
<tr>
<td>Cardiac index (liters/kg per min)</td>
<td>0.11 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Pulmonary artery wedge pressure (mm Hg)</td>
<td>8 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>12 ± 2</td>
<td>20 ± 3*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. In this table and in Table 2, there were nine control animals and 10 hypertrophied animals. Stenosis gradient is the peak systolic pressure gradient across the stenosis. Stenosis area is derived from stenosis gradient and cardiac index using the modified Gorlin equation (Hakki et al., 1981).

\*P < 0.025 compared with control; † P < 0.001 compared with control.

---

**Table 2**

<table>
<thead>
<tr>
<th>Morphological Characteristics of Experimental Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>Hypertrophied</td>
</tr>
<tr>
<td>LV weight (g)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>LV-to-body weight ratio (g/kg)</td>
</tr>
<tr>
<td>h/R ratio</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. Left ventricular (LV) end-diastolic wall thickness (h) and internal radius (R) were measured ultrasonically at end diastole.

\*P < 0.025; † P < 0.005; † P < 0.001.
TABLE 3

Left Ventricular Mechanics

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Hypertrophied</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>0.33 ± 0.01</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Sc (g/cm²)</td>
<td>85 ± 6</td>
<td>116 ± 14</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (g/cm²)</td>
<td>428 ± 21</td>
<td>470 ± 22</td>
</tr>
<tr>
<td>$b_0$ (cm)</td>
<td>4.22 ± 0.21</td>
<td>5.05 ± 0.16</td>
</tr>
</tbody>
</table>

Results are mean ± se. Ejection performance, measured as the shortening fraction (SF), is the ratio of endocardial minor axis shortening during systole to end-diastolic endocardial diameter. End-systolic circumferential stress (Sc) is the afterload prior to acute manipulation of aortic pressure. Contractile performance of the myocardium is measured from the end-systolic stress, end-systolic diameter relationship. This relationship is linear ($r = 0.96 ± 0.01$ for controls, $r = 0.97 ± 0.01$ for hypertrophied animals), with a slope represented as $E_{\text{max}}$ and an intercept on the abscissa represented by $b_0$ (see Methods and Figure 5).

Table 4 shows the mechanical data for each banded animal. Seven animals with shortening fractions $\geq 0.25$ comprise group A, while three animals with shortening fractions $<0.25$ comprise group B. The number of animals in each group is too small to allow statistical comparisons of the two groups. However, the depressed ejection performance in the three group B animals could be explained by the very high afterload (Sc) found in one animal and by the lower contractile function ($E_{\text{max}}$) found in the other two. It is noteworthy that the two animals with the most severe hypertrophy had the lowest $E_{\text{max}}$. Figure 3 shows that these two animals fall below the 95% confidence limit of the shortening fraction-afterload relationship. This suggests that excessive afterload does not alone account for depressed shortening fraction, and that muscle dysfunction was present when hypertrophy exceeded 100% in terms of LV:BW ratio.

The mean myosin ATPase activities using Ca++ or K+EDTA stimulation and the myofibrillar ATPase activity all are shown in Table 5. There were no

FIGURE 2. The end-systolic stress-diameter relationship is shown for each individual control (C) and hypertrophied (H) animal in panels A and B, respectively. In each experiment, stress was manipulated over a wide range by acutely constructing the descending thoracic aorta. Alternating open and closed circles for the data points are used, going from left to right, for visual clarity only. The numbers appearing at the upper and lower end of each plot are end-diastolic midwall diameter (cm) and body weight (kg) to illustrate their influence on the diameter intercept, $b_0$ (see Methods, Evaluation of Ventricular Function). The highest and lowest values of stress (mean ± se), and resulting strain (mean ± se), for C and H animal groups are shown in panel C. Definitions of Sc, bmid, and $b_0$ are in Methods.

FIGURE 3. Relationship between baseline endocardial shortening fraction (SF) and end-systolic circumferential stress (Sc) before acute manipulation of aortic pressure. The best fit through data points from the hypertrophied animals ($P < 0.01$, $r = -0.83$) demonstrates decreasing ejection performance with increasing afterload. Of the three group B hypertrophied animals, (solid squares), one falls within the 95% confidence band, suggesting impaired ejection performance despite normal $E_{\text{max}}$, as a result of excessive afterload. The other two group B animals fall outside the 95% confidence limits, suggesting impaired contractile function beyond that explained by excess afterload. These two animals had the most extreme hypertrophy. Mean ± se for SF and Sc of the control group is represented by the closed circle and cross bars.
Table 4
Left Ventricular Mechanics of the Animals with Hypertrophy

<table>
<thead>
<tr>
<th>LVW/BW</th>
<th>SF</th>
<th>Sc</th>
<th>Emax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>0.25</td>
<td>134</td>
<td>482</td>
</tr>
<tr>
<td>3.2</td>
<td>0.31</td>
<td>141</td>
<td>449</td>
</tr>
<tr>
<td>3.3</td>
<td>0.29</td>
<td>132</td>
<td>693</td>
</tr>
<tr>
<td>3.1</td>
<td>0.31</td>
<td>135</td>
<td>510</td>
</tr>
<tr>
<td>3.3</td>
<td>0.42</td>
<td>53</td>
<td>363</td>
</tr>
<tr>
<td>4.8</td>
<td>0.36</td>
<td>61</td>
<td>417</td>
</tr>
<tr>
<td>3.5</td>
<td>0.29</td>
<td>68</td>
<td>444</td>
</tr>
</tbody>
</table>

Group B

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>0.17</td>
<td>97</td>
<td>340</td>
</tr>
<tr>
<td>4.3</td>
<td>0.19</td>
<td>194</td>
<td>687</td>
</tr>
<tr>
<td>5.6</td>
<td>0.18</td>
<td>144</td>
<td>335</td>
</tr>
</tbody>
</table>

The hypertrophied animals with impaired ejection performance (SF < 0.25) comprise group B and include two animals with the highest left ventricular weight-to-body weight ratio (LVW:BW). The hypertrophied animals with preserved shortening fraction (SF ≥ 0.25) comprise group A. The abbreviations are defined in the legend of Table 3.

Discussion

The Model

This study employed an animal model resembling aortic stenosis in man with regard to body and heart size, abruptness and severity of pressure overload, and myocardial isozyme pattern. The major new findings of this study are that substantial hypertrophy of the left ventricle is associated with normal myosin ATPase activity, unaltered myosin isozyme patterns, and, except with marked hypertrophy, normal indexes of ventricular contractile function. Thus, our hypothesis has been confirmed.

Most of the variability in the contractile performance of pressure-hypertrophied myocardium reported from various laboratories may be explained in large part by differences in: (1) the ventricle studied, i.e., right or left, (2) the species, (3) the abruptness and severity of the pressure overload, and (4) the degree of hypertrophy produced (Grossman, 1980; Wikman-Coffelt et al., 1979). Although depressed contractile function has been found in myocardium isolated from hypertrophied right ventricles with severe, gradual pressure overload (Cooper et al., 1981), the right ventricle normally pumps an identical volume of blood at a much lower pressure than the left ventricle. Therefore, the left ventricle might tolerate a greater degree of pressure-induced hypertrophy compared with the right ventricle before the onset of contractile dysfunction. Various alterations in contractile performance in response to pressure overload have been found in small species. Depressed tension development (Carey et al., 1978), as well as depressed shortening velocity (Maughan et al., 1979; Capasso et al., 1982) in hypertrophied myocardium of smaller mammals, is associated with depressed myosin ATPase activity; however, the rodent myosin isozyme patterns differ from that of man. The pig, whose overall size, heart-to-body weight ratio (Schaper et al., 1971), and coronary artery distribution (Lumb and Hardy, 1963) resemble those of man, may be a more appropriate model for pressure overload hypertrophy. In adult pig as well as adult human left ventricle (Lompre et al., 1981), there is only one isozyme seen after native gel electrophoresis, and that isomyosin co-migrates with the V5 form of the rat, has the lowest ATPase activity of three known myosin isozymes (Hoh, 1976; Rupp, 1981).

Table 5
Left Ventricular Myosin and Myofibrillar ATPase Activity

<table>
<thead>
<tr>
<th>K⁺EDTA</th>
<th>Ca⁺⁺</th>
<th>Myofibrillar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.21 ± 0.16</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>1.25 ± 0.14</td>
<td>0.50 ± 0.04</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. Units of ATPase activity are μmols P/mg protein/min at 25°C.
Assessment of Contractile Performance

A satisfactory index of contractile function for the intact ventricle has been even more elusive (Sonnenblick, 1974) than that for simple preparations of isolated myocardium. Nevertheless, functional study of the intact hypertrophied ventricle is both desirable and necessary, since it has not yet been possible to fully characterize myocardium isolated from humans or other large species.

An ideal index of contractile function should be insensitive to loading conditions and sensitive to inotropic interventions. Although many of the indices of contractile performance are load-dependent (Mahler et al., 1975), or are extrapolated (i.e., V<sub>max</sub>), the linear relationship between end-systolic pressure and volume is neither (Suga and Sagawa, 1974; Suga et al., 1973; Sagawa et al., 1977; Sagawa, 1978). The slopes of the pressure-volume (Suga and Sagawa, 1974; Borow et al., 1982a) and stress-length (Weber et al., 1976) relationships are sensitive to inotropic interventions. These slopes are depressed, not only in humans with overt myocardial disease (Grossman et al., 1977), but also, in patients with myocardial disease that is not detectable by the more usual ejection phase indices of performance (Takahashi et al., 1980; Borow et al., 1982b). Whether this index applied to the intact pump is as sensitive to alterations of contractile function as force measurements in isolated muscle is unknown. Subtle alterations in contractile function might not have been detected by E<sub>max</sub> in the present study. Indeed, one recent study suggests that the zero stress extrapolated diameter, b<sub>0</sub>, in our study, might be more sensitive to inotropic interventions than the slope, E<sub>max</sub> (Borow et al., 1982c). However, in most other studies using either the pressure-volume or stress-diameter relationship (Borow et al., 1982a; Mehmel et al., 1981; Sagawa et al., 1977; Suga et al., 1973; Takahashi et al., 1980), slope appears to be more sensitive to inotropic state than intercept. In our pigs, the diameter intercept, b<sub>0</sub>, was highly correlated with body and heart size among control animals, as shown in Figure 2. When referenced to end-diastolic mid-wall diameter to cancel out this size dependence, we found that only two hypertrophied pigs had intercepts above the 95% confidence interval for controls (Fig. 5). These same two pigs had the greatest degree of hypertrophy with more than dou-

---

**Figure 4.** Pyrophosphate gel electrophoresis of cardiac myosin from control and hypertrophied pig myocardium demonstrating only one isozymic form which co-migrates with the V<sub>3</sub> isozymic form of the rat (see Methods).

**Figure 5.** The theoretical end-systolic midwall diameter extrapolated to zero stress, b<sub>c</sub>, was normalized to b<sub>d</sub>, the end-diastolic midwall diameter at maximum load, to cancel out size dependence. This dimensionless value, b<sub>c</sub>/b<sub>d</sub>, is illustrated by closed circles with 95% confidence band (dotted lines) for the control pigs (C), and by open circles for the hypertrophied pigs. Only the two pigs with the most extreme hypertrophy exceed the estimated 95% confidence limit of normal.
ble the normal LV mass, and were also the only two pigs identified by the shortening fraction-stress relationship as having depressed contractile function (Fig. 3). In the present investigation of myocardial hypertrophy, the use of stress, which normalizes for differences in ventricular thickness and dimensions (Marsh et al., 1979; Weber et al., 1976; Reichek et al., 1982), was preferred to the use of pressure alone. Circumferential stress for a thick-walled ellipse, which accounts for chamber eccentricity (Janz, 1980), and may be preferable to other models of wall stress (Burns et al., 1971; McHale and Greenfield, 1973), was used. End-systolic diameter was substituted for end-systolic volume in our study, but this should not significantly affect the results (Borow et al., 1982a).

Application of the end-systolic stress-diameter relationship to our model of severe, gradual pressure overload has allowed us to separate the effects of afterload mismatch (Ross, 1976) on left ventricular ejection performance from those of myocardial contractile dysfunction (Carabello et al., 1980). The inverse correlation between ejection performance and afterload described in humans with aortic stenosis (Gunther and Grossman, 1979) was apparent in our hypertrophied animals (Fig. 3). Two of the three animals comprising group B with depressed ejection performance fell below the 95% confidence limits of this relationship. This suggests that depressed contractile function was responsible for the depressed ejection performance, since the depressed ejection performance could not be ascribed merely to excessive afterload. This situation has also been described in humans with aortic stenosis (Carabello et al., 1980).

Myosin ATPase activity and isoenzyme pattern may be altered in hypertrophic myocardium (Carey et al., 1978; Maughan et al., 1979; Litten et al., 1982). Alterations in contractile function that occur following a pressure overload seem to depend, in part, on changes in myosin ATPase activity (Wikman-Coffelt et al., 1982). Pressure-induced hypertrophy of the left ventricle has been studied using the rat (Bing et al., 1971; Meerson and Kapelko, 1972; Pfeffer et al., 1979). However, in rats (Rupp, 1981), as well as in rabbits (Litten et al., 1982), the partitioning between the fast V1 isozyme and the slow V2 isozyme is altered by changes in loading conditions, and this redistribution of isoenzymes may, in part, account for the changes in myosin ATPase activity and myocardial contractile function that follow a pressure overload in these species. In contrast, the ventricular myocardium of adult humans, and other adult large species such as the pig, does not have this polymorphic isozyxme pattern (Lompre et al., 1981); only a single isomyosin is found, and that isomyosin comigrates with V3 of the rat (Fig. 4). The absence of V1 in the adult pig provides a plausible explanation for the unaltered myosin ATPase activity in our model, compared with that of the rodent models of pressure-induced hypertrophy. A potential limitation of the electrophoretic technique we used is that native isomyosins are separable only if they differ in regard to charged amino acids. Using monoclonal antibodies, Clark et al. (1982) have identified in the guinea pig two distinct isomyosins, immunologically resembling V1 and V3, which were not separable on pyrophosphate gel. However, only V3 was found in the adult pig. Thus, while it is known that the neonatal pig myocardium contains a V1 isomyosin that is separable from V2 by pyrophosphate gel (Lompre et al., 1981), V3 is the only isomyosin found in the post-neonatal pig by either the electrophoretic or immunologic technique (Clark et al., 1982). Although the presence in hypertrophied pig myocardium of a heretofore undiscovered isomyosin, "V4," with the same electrophoretic mobility as V3, cannot be excluded on the basis of pyrophosphate gels, the unaltered myosin ATPase activity we found with hypertrophy suggests that a functionally important isoenzyme shift did not occur.

In summary, left ventricular hypertrophy caused by a gradual, severe pressure overload, as with aortic stenosis in man, is associated with normal myosin ATPase activity, unaltered isomyosin pattern, and, except when hypertrophy is extreme, contractile performance that is normal by current methods of evaluation.

References

Borow KM, Green LH, Grossman W, Braunwald E (1982c) Left ventricular end-systolic stress-shortening and stress-length relations in humans. Normal values and sensitivity to motropic...
York, American Elsevier
Suga H, Kitabatake A, Sagawa K (1979) End-systolic pressure determined stroke volume from fixed end diastolic volume in the isolated canine left ventricle under a constant contractile state. Circ Res 44: 238–249

INDEX TERMS: Contractile function • Myosin ATPase and isozymes • Left ventricular hypertrophy • Pressure overload
Contractile function, myosin ATPase activity and isozymes in the hypertrophied pig left ventricle after a chronic progressive pressure overload.

T Wisenbaugh, P Allen, G Cooper, 4th, H Holzgrefe, G Beller and B Carabello

Circ Res. 1983;53:332-341
doi: 10.1161/01.RES.53.3.332

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/53/3/332

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/