Changes in Myofibrillar Content and Mg-ATPase Activity in Ventricular Tissues From Patients with Heart Failure Caused by Coronary Artery Disease, Cardiomyopathy, or Mitral Valve Insufficiency

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Force development and shortening by cardiac muscle occur as a result of the interaction between actin and myosin within the myofibrillar lattice. This interaction is dependent upon intracellular ionized calcium and is controlled by the troponin-tropomyosin regulatory proteins situated along the actin filament. In this study, we compared the myofibrillar content and myofibrillar Mg-ATPase activity of normal human ventricular muscle with that of ventricular muscle from patients in end-stage failure caused by coronary artery disease or cardiomyopathy and ventricular muscle from patients with heart failure due to mitral valve insufficiency. The results show that the amount of myofibrillar protein (mg/g wet wt ventricle) in hearts in end-stage failure (coronary artery disease and cardiomyopathy) is significantly lower compared with normal hearts and hearts in failure due to mitral valve insufficiency. However, the Mg-ATPase activity of myofibrils from hearts in both end-stage failure and failure due to mitral valve insufficiency is significantly lower compared with myofibrils from normal hearts. The data suggest that the reduction in the amount of myofibrillar protein in ventricular tissue is a pivotal event that may be responsible for the progression of heart disease to the point of end-stage failure. (Circulation Research 1988;63:380-385)

Two primary cardiac defects are associated with congestive heart failure: the inability of heart muscle to generate ventricular pressure to adequately propel blood through the cardiovascular system and/or the inability of the ventricle to completely relax and allow sufficient filling during diastole. These defects, either together or independently, lead to a reduction in cardiac output to a level that is too low to support the normal physiological function of the body.

Results from animal studies have suggested that abnormalities in cardiac membranes and contractile proteins are present in some models of congestive heart failure. Abnormalities that have been reported include alterations in the structure and function of the contractile proteins, sarcoplasmic reticulum, and sarcolemmal membrane. The induction and extent of alterations in these intracellular systems appear to be influenced by numerous factors such as the way heart failure is produced, the magnitude of the cardiac insult used to initiate failure, or the species of animal used. A crucial question that has not been answered, however, is if the results obtained from experimental models of congestive heart failure are relevant to the mechanism that produces congestive heart failure in humans.

To gain insight into the possible myocardial defects associated with congestive heart failure in humans, we have examined left ventricular tissue from normal human hearts, from human hearts in end-stage failure due to coronary artery disease or cardiomyopathy, and from human hearts in pre-end-stage failure due to valvular insufficiency. In these studies we determined 1) if the enzymatic properties of the myofibrillar proteins are depressed in heart failure; 2) if the content of the myofibrillar proteins is de-
creased in heart failure; and 3) if there is a relation between the severity of heart failure and the extent of defects associated with the contractile proteins.

**Materials and Methods**

**General Procedure**

*Ventral tissue.* Left ventricular tissue was obtained from patients during cardiac transplant surgery (Brigham and Women's Hospital, Boston, Massachusetts). These patients were diagnosed to have end-stage chronic heart failure due to idio-pathic cardiomyopathy or coronary artery disease. Left ventricular tissue was also obtained from accident victims whose hearts were unsuitable for cardiac transplantation. There were no overt signs of cardiac disease upon direct physical examination. Left ventricular tissues from this group were categorized in this report as normal.

Hearts were removed from patients during transplant surgery or after removal of transplant organs. The heart was washed of blood, and the left ventricular free wall was dissected into 2-3-g sections and immediately frozen in liquid nitrogen. The ventricular muscle was stored immersed in liquid nitrogen.

*Left ventricular papillary muscles.* Portions of left ventricular papillary muscles (near the chordae tendineae) were excised from patients during mitral valve replacement surgery (Albany Medical College, Albany, New York). These patients were at advanced stages of heart failure but had not yet reached end-stage heart failure due to mitral valve insufficiency. In this study, hearts of these patients were classified as at pre-end-stage failure. The segments of muscle were stored in ice-cold lactated Ringer's solution and studied within 60 minutes after removal from the patient.

**Preparation of homogenate.** Segments of the left ventricle or papillary muscles from individual patients were weighed, finely minced with scissors, and transferred to precooled glass homogenizers. The tissue was suspended in exactly 20 volumes (wt/vol) of ice-cold 30 mM 3-[N-morpholino]propanesulfonic acid (MOPS; pH 7.1 at 30° C) and then homogenized using a Thomas glass homogenizer (Swedesboro, New Jersey) and a motor-driven Teflon pestle revolving at 500 rpm as previously described. The temperature of the cardiac tissue was kept at 0-4° C during the homogenization procedure. The homogenization procedure was carried out for 4-5 minutes to obtain a homogeneous suspension. Protein content of the homogenate was determined using the method of Lowry et al.11

**Preparation of myofibrils.** Tissue homogenates, prepared from papillary muscles or the ventricular free wall, were transferred to 50-ml centrifuge tubes, diluted 1:1 with ice-cold solution A (mM:KCl 60, MOPS 30, MgCl₂ 2, pH 7.10 at 4° C) and centrifuged at 12,000g for 10 minutes using a Sorvall RC-5B centrifuge (Wilmington, Delaware) and SS 34 rotor. The supernatant fraction was discarded and the pellet resuspended in 40 ml of solution A and centrifuged at 12,000g for 10 minutes. This step was repeated twice. The pellet was then homogenized in 40 ml of solution A, which contained 1% Triton X-100, and centrifuged at 3,000g for 10 minutes. This pellet was suspended in 40 ml of solution A, which contained 1.0 mM EGTA, and centrifuged at 3,000g for 15 minutes. For the final step, the pellet was resuspended in solution B and centrifuged at 15,000g for 15 minutes. All of the above procedures were carried out at 0-4° C. Myofibrils were suspended in solution B and the protein concentration was determined using the method of Lowry et al.11 The volume of the myofibrillar suspension was adjusted with solution B (pH 7.1 at 30° C) to give a final concentration of 4 mg myofibrillar protein/ml and myofibrils were assayed for Mg-ATPase activity within 24 hours of isolation as described below.

**Assay of myofibrillar Mg-ATPase activity.** The Mg-ATPase activity of myofibrils was measured in a 0.5 ml volume of a solution that contained 60 mM KCl, 30 mM MOPS, 7.5 mM MgCl₂, 5.0 mM Na₂ ATP, 0-1 mM CaCl₂, 1-2 mM EGTA (to obtain ionized calcium concentrations from pCa 8–5.09), and 0.4 mg myofibrillar protein at pH 7.10 and 30° C. The concentration of ionized calcium in the reaction mixture was determined using a computer program that solves multiple-binding equilibria for metals and ligands in solution. The binding constants used in this study were similar to those described by Fabiato and Fabiato.12 The Ca-EGTA solutions were calibrated using the method of Mio-sescu and Thieleczek.13 Myofibrillar proteins were incubated in the reaction solution in the absence of Na₂ATP for 10 minutes at 30° C. The Mg-ATPase was activated by the addition of 25 µl of 100 mM Na₂ATP and stopped after 10 minutes by the addition of 0.5 ml of ice-cold 10% trichloracetic acid. Each preparation was assayed in triplicate. The tubes were immediately placed on ice, centrifuged for 10 minutes at 3,000g, and the supernatant fraction was assayed for inorganic phosphate (Pi) using the method of King.14 The total amount of ATP hydrolyzed was less than 15% of the initial concentration. Data are presented as nanomoles Pi per milligram myofibrillar protein per minute.

**Statistical Analysis**

Statistical comparisons between mean values were made using analysis of variance or the Dunnett's t test. In this study, p < 0.05 was considered statistically significant.

**Results**

**Cardiac Catheterization**

Before cardiac transplant surgery, patients were evaluated by cardiac catheterization. These data for
the various cardiovascular parameters are listed in Table 1. In general, most of the values for the patients with heart failure due to coronary artery disease or cardiomyopathy fall outside of the range of normal values. Cardiac pump performance was severely compromised as suggested by ejection fraction of approximately 13% for both groups of transplant patients compared with the normal range of 60-70%.

### Myofibrillar ATPase Activity in Normal and Diseased Ventricular Tissues

Mg-ATPase was measured for myofibrils prepared from the left ventricular free wall of normal hearts and hearts in end-stage failure due to coronary artery disease or idiopathic cardiomyopathy. Ca\(^{2+}\)-dependent Mg-ATPase activities of the diseased groups were not statistically different from those of the normal group at pCa 6.73, 6.28, and 6.16. However, from pCa 5.92-5.09, the Mg-ATPase activity of myofibrils from both diseased groups was significantly lower than those of the normal group (Figure 1). The Ca\(^{2+}\)-independent Mg-ATPase activity of myofibrils from both diseased groups was significantly lower than those of the normal group (Figure 1). The Ca\(^{2+}\)-independent Mg-ATPase activity of myofibrils (pCa 8.00) of the diseased groups and normal group was not different. The data also indicate that there were no statistically significant differences in the mean Mg-ATPase activities of myofibrils from hearts of patients with coronary artery disease and idiopathic cardiomyopathy at all calcium concentrations examined (pCa 6.73-5.09). The Ca\(^{2+}\)-independent Mg-ATPase measured at pCa 8 was also not significantly different between these two diseased groups.

Myofibrils were prepared from individual segments of fresh papillary muscles removed from patients undergoing mitral valve replacement surgery. Only small segments of papillary muscle (approximately 300 mg) were removed from patients during valve replacement surgery. The amount of myofibrillar protein recovered from a single segment of muscle enabled us to evaluate Mg-ATPase activity at only six concentrations of ionized calcium. Data for myofibrillar Mg-ATPase activity are shown in Figure 2. The Mg-ATPase activity of myofibrils from hearts of patients with mitral valve insufficiency was consistently lower when compared with the Mg-ATPase activity of myofibrils prepared from normal hearts, but in many cases not statistically different. The myofibrillar Mg-ATPase activity was similar to the myofibrillar Mg-ATPase activity obtained for hearts of patients with cardiomyopathy or coronary artery disease.

### Recovery of Myofibrillar Proteins From Normal and Diseased Ventricular Tissues

The total amount of tissue protein and the recovery of myofibrillar proteins per gram of ventricular tissue were determined for normal hearts and hearts of patients with mitral valve insufficiency, coronary artery disease, or cardiomyopathy (Figure 3). Total

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**Table 1. Cardiac Catheterization Data of Heart Transplant Patients**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Cardiomyopathy (n=8)</th>
<th>Coronary artery disease (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP (mm Hg)</td>
<td>2-8</td>
<td>13±2</td>
<td>17±5</td>
</tr>
<tr>
<td>RVP (mm Hg)</td>
<td>15-30/2-8</td>
<td>46±6/14±3</td>
<td>46±8/16±5</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>15-30/4-12</td>
<td>51±5/30±2</td>
<td>37±2/25±2</td>
</tr>
<tr>
<td>PCW (mm Hg)</td>
<td>2-12</td>
<td>23±3</td>
<td>24±3</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>100-140</td>
<td>92±479±10</td>
<td>93±2/23±2</td>
</tr>
<tr>
<td>AOP (mm Hg)</td>
<td>100-140/60-90</td>
<td>91±565±3</td>
<td>90±3/62±3</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>2.6-4.2</td>
<td>2.0±0.3</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>EF (%)</td>
<td>60-70</td>
<td>13±4</td>
<td>13±5</td>
</tr>
<tr>
<td>SVR (dyn/cm²s⁻¹)</td>
<td>700-1,600</td>
<td>1,499±79</td>
<td>1,545±156</td>
</tr>
<tr>
<td>PVR (dyn/cm²s⁻³)</td>
<td>20-130</td>
<td>294±58</td>
<td>143±10</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

RAP, right atrial pressure; RVP, right ventricular pressure; PAP, pulmonary artery pressure; PCW, pulmonary capillary wedge pressure; LVP, left ventricular pressure; AOP, aortic pressure; CI, cardiac index; EF, ejection fraction; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance.
protein content per gram wet weight of tissue was not significantly different between the normal group and the three diseased groups. Compared with the normal group, the total recovery of myofibrillar proteins expressed as milligrams protein per gram wet tissue weight was significantly lower for patients with coronary artery disease and cardiomyopathy, but was not significantly different for the patients with mitral valve insufficiency. When the myofibrillar protein recovery was expressed as milligrams myofibrillar protein per 100 mg ventricular protein, recovery of myofibrillar protein was significantly lower than the normal group only in patients with cardiomyopathy.

**Discussion**

The segments of left ventricular tissue were obtained from human hearts at different stages of failure and compared with left ventricular tissue from normal hearts. The data suggest that there is a significant reduction in myofibrillar content and/or myofibrillar Mg-ATPase activity in hearts that show physiological signs of failure. In an attempt to minimize the potential for biological variability in our myofibrillar preparations, segments of the left ventricular wall were selected from approximately the same anatomical location of the heart. Wall stress and blood flow, as well as a number of other factors are known to vary in different regions of the left ventricle and these factors could possibly induce alterations in the biochemical and physiological state of individual muscle cells independent of disease processes. Segments of heart muscle obtained from patients with coronary artery disease were taken from areas of the left ventricular free wall that did not appear to be a focal point of cell death due to infarction. The purpose of choosing segments of the ventricle in this manner was to obtain an area of the ventricular wall that consisted mainly of working muscle cells and not dead cells and scar tissue.

While papillary muscles were used within 60 minutes of excising from patients, all of the ventricular free-wall segments used in this study were frozen in liquid nitrogen; both normal and diseased heart muscle were frozen and stored in an identical manner. In a separate set of experiments, we tested the effect of liquid nitrogen freezing on normal canine ventricle and found that the structure and range of values. The significantly depressed pump performance of hearts (Table 1) in end-stage failure is reflected in a mean ejection fraction of <20%. This is compared with a mean cardiac ejection fraction of 60-70% for individuals with no evidence of cardiovascular disease.15 In light of a mean ejection fraction of <20%, moderately elevated systemic and pulmonary vascular resistances, and a normal heart rate, the mean cardiac index (cardiac output per square meter body surface area) for patients with end-stage heart failure was approximately 2.0 l/min, which falls below the normal range by about 30-40%. It is likely that cardiac stroke volume was influenced by the ventricular dimensions. It should be kept in mind that patients with end-stage heart failure were prescribed a variety of drugs (e.g., cardiotonics, vasodilators, diuretics) to help support the cardiovascular system and raise cardiac output. Therefore, the catheterization data for patients with heart failure may not reflect the true functional state of the heart.

No significant correlations were obtained in this study when values of in situ heart function (i.e., left ventricular pressure development, ejection fraction, cardiac index) were compared with cardiac myofibrillar Mg-ATPase activity obtained for individual patients. Significant correlations have been observed between contractile protein ATPase activity and cardiac muscle performance for isolated heart muscle preparations.16,17 Under these laboratory conditions, parameters that influence force development (e.g., sarcomere length) and the rate of shortening of cardiac muscle (e.g., afterload) can be rigorously controlled. These parameters cannot be rigorously controlled or easily monitored clinically.

In general, cardiac catheterization data obtained from patients before surgery offer some insight into the pathophysiological condition of their cardiovascular system. Most of the mean values for the various indexes of cardiovascular function obtained for the patients in this study fall outside the normal
enzymatic properties of myofibrillar proteins prepared from frozen ventricle are not different from fresh ventricle (E.D. Pagani and A.A. Alousi, unpublished observation). It has also been reported by Price et al,18 who studied normal and diseased human ventricular tissue, and by Litten et al,19 who studied rabbit ventricular tissue, that the enzymatic activity of myosin isolated from fresh or frozen ventricular muscle is not different.

Morphological analyses of normal and hypertrophied human ventricle muscle by electron microscopy20,21 have indicated that there is a reduction in ventricular myofibrillar protein from hearts that show physiological signs of failure. Light microscopic analysis of human ventricle from patients with hypertrophic cardiomyopathy, cardiac hypertrophy due to volume overload or pressure overload, and dilated cardiomyopathy has indicated that the amount of nonmuscle tissue present in hypertrophied hearts increased by approximately 20% compared with normal hearts.22 We were unable to use electron microscopy to evaluate the segments of ventricular muscle from patients with heart failure due to cardiomyopathy or coronary artery disease because the tissue was frozen. In the present study, significantly less myofibrillar protein was found in ventricular muscle from patients with end-stage failure caused by coronary artery disease or cardiomyopathy relative to normal ventricular muscle. However, the myofibrillar protein content in papillary muscles of patients with mitral valve insufficiency was found to be normal (Figure 3). The reduction in myofibrillar protein by 30–40% in hearts in end-stage failure could be due to an increase in nonmuscle cells or to a loss of myofibrillar protein in muscle cells. In either case, an expected consequence of the net loss of myofibrillar protein from ventricular tissue would be a reduction in cardiac contractile force and possibly a decrease in the speed of circumferential cardiac fiber shortening. Hammond et al20 studied biopsy specimens collected over a period of time from individual patients with heart failure caused by idiopathic cardiomyopathy. They found a significant positive correlation between mortality risk and loss of cardiac myofilaments as estimated by electron microscopic analysis. Our data are consistent with the results of Hammond et al20 and could be interpreted to suggest that the loss of myofibrillar protein is an event associated with the progression of heart failure.

In the present study, we found that the mean Mg-ATPase activity of myofibrils isolated from hearts in end-stage failure due to either idiopathic cardiomyopathy or coronary artery disease was significantly reduced relative to values obtained from normal hearts. This finding offers a biochemical basis for one of the defects that could account for the depressed contractile state of the failed human heart. Others have reported that the Mg-ATPase of myofibrils isolated from severely hypertrophied human ventricle is reduced.23,24 Recently, Schwartz et al25 reported that skeletal muscle α-actin messenger RNA accumulates in hypertrophied adult rat hearts. It is possible that a similar process involving the accumulation of skeletal muscle α-actin messenger RNA also occurs in failed human ventricle, and the replacement of cardiac actin by skeletal muscle actin could be a factor responsible for the reduction in myofibrillar Mg-ATPase.

Takeda et al26 reported that there are no enzymatic differences in the Ca2+-activated Mg-ATPase activity of myofibrils of nonhypertrophied and hypertrophied papillary muscles removed from patients during mitral valve surgery. The absolute values for myofibrillar Mg-ATPase activity obtained in our study and those reported by Takeda et al26 are somewhat comparable since the isolation of myofibrillar proteins and the composition and temperature of the reaction mixture used in the assay of Mg-ATPase activity were very similar in the two studies. The values we reported for myofibrillar Mg-ATPase activity for ventricular myofibrils of normal human hearts are considerably higher than values obtained for hearts of patients in end-stage failure and values obtained for hearts of patients with mitral valve insufficiency or aortic stenosis reported by Takeda et al.26 These results imply that the myofibrillar Mg-ATPase activity is as depressed in ventricles of patients who are candidates for valve replacement surgery (pre–end-stage failure) as has been found for hearts of transplant candidates (end-stage failure).

The Ca-ATPase and actin-activated Mg-ATPase of purified myosin isolated from hypertrophied human ventricular muscle have been reported to be not significantly different from values obtained from normal human ventricle.27,28 Ca-ATPase of myosin isolated from human hearts in end-stage failure due to cardiomyopathy or coronary artery disease is also not different compared with that of normal human heart (P.D. Allen, unpublished observation). Our finding that the Mg-ATPase activity of myofibrils is depressed in end-stage failure suggests that the interaction between the thick and thin filaments within the myofibrillar lattice may be abnormal even though the enzymatic activity of myosin is normal. Moreover, we did not find any evidence to suggest that the calcium sensitivity of myofibrils from human hearts in end-stage failure is different from normal human heart; the curves describing the relation between ionized calcium and normalized Mg-ATPase activity are the same.

In conclusion, our study has shown that myofibrillar Mg-ATPase activity is significantly depressed in left ventricular muscle of patients with end-stage heart failure due to cardiomyopathy or coronary artery disease and in patients in advanced stages of heart failure due to mitral valve insufficiency. However, in addition, ventricular myofibrillar protein content appears to be significantly reduced only in end-stage failure but normal in hearts of the patients with mitral valve insufficiency.
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References

KEY WORDS: human heart • myofibrillar ATPase • heart failure
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