Preserved contractile function despite atrophic remodeling in unloaded rat hearts

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Welsl, Denise C., Konstantina Dipla, Patrick H. McNulty, Anbin Mu, Kaie M. Ojamaa, Irwin Klein, Steven R. Houser, and Kenneth B. Margulies. Preserved contractile function despite atrophic remodeling in unloaded rat hearts. Am J Physiol Heart Circ Physiol 281: H1131–H1136, 2001.—The present study was designed to determine whether myocardial atrophy is necessarily associated with changes in cardiac contractility. Myocardial unloading of normal hearts was produced via heterotopic transplantation in rats. Contraction of isolated myocytes (1.2 mM Ca2+/37°C) were assessed during field stimulation (0.5, 1.0, and 2.0 Hz), and papillary muscle contractions were assessed during direct stimulation (2.0 mM Ca2+/37°C; 0.5 Hz). Hemodynamic unloading was associated with a 41% decrease in median myocyte volume and proportional decreases in myocyte length and width. Nevertheless, atrophic myocytes had normal fractional shortening, time to peak contraction, and relaxation times. Despite decreases in absolute maximal force generation (Fmax), there were no differences in Fmax/area in papillary muscles isolated from unloaded transplanted hearts. Therefore, atrophic remodeling after unloading is associated with intact contractile function in isolated myocytes and papillary muscles when contractile indexes are normalized to account for reductions in cell length and cross-sectional area, respectively. Nevertheless, in the absence of compensatory increases in contractile function, reductions in myocardial mass will lead to impaired overall work capacity.

LVAD support, a recent editorial comment by Soloff (31) has raised the valid concern that reduced myocardial mass might limit or offset the potential benefits of LVAD support. Such concerns are amplified by other recent studies demonstrating that cardiac unloading and atrophy produce activation of gene-expression patterns associated with fetal development and pathological hypertrophy (5).

In this context, it is reasonable to consider whether myocardial unloading of normal myocardium with associated reductions of muscle mass necessarily result in decreased pump performance. For the purpose of this discussion, we use the term “atrophy” to denote reductions in cardiac and/or myocyte size below normal levels. On the basis of previous studies reporting that atrophy of normal hearts is associated with changes in gene expression resembling those observed with pathological hypertrophy (5, 20, 26), we hypothesized that myocardial unloading would produce concordant decrements in both myocardial mass and function. To test this hypothesis, we unloaded normal myocardium via heterotopic transplantation (HT) in the rat, and performed morphological and functional analyses on isolated myocytes and force-mechanics studies on isolated papillary muscles.

METHODS

Animals. Inbred male Lewis rats weighing 275–375 g were used. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Approval for the studies performed was obtained from the institutional animal care and use committee at Temple University School of Medicine and the North Shore University Hospital.

Surgical procedure. Heterotopic heart transplants were performed using a surgical procedure that has been described in detail previously (17, 22). In brief, rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg ip). The chest was opened and the donor heart was exposed. Heparin was administrated and the vena cavae and pulmonary veins were ligated. The heart was then excised and flushed with...
cold heparinized saline via a catheter in the ascending aorta. The heart was kept in a cold, high-potassium, cardioplegic buffer solution (30) until transplantation. Recipient rats were anesthetized and prepared in the same manner as donor rats. The abdomen was entered through a midline incision and the abdominal aorta and inferior vena cava were exposed and clamped. The donor heart was placed in the right abdominal cavity and end-to-side anastomoses were made between the donor’s aorta and the recipient’s abdominal aorta and between the donor’s pulmonary artery and the recipient’s vena cava. Care was taken to keep the heart cold throughout the surgical procedure, and the total duration of cold ischemia ranged from 45 to 60 min for all allografts. This procedure allows the intact, transplanted heart to be adequately perfused while remaining hemodynamically unloaded. Unoperated Lewis rats served as controls.

Myocyte isolation. Myocytes were isolated from seven normal Lewis rat hearts and six heterotopically transplanted hearts 2 wk after heart transplantation. Rats were anesthetized with an injection of pentobarbital sodium (150 mg/kg ip) and a cardiectomy was performed immediately. Each heart was placed on a modified Langendorf perfusion apparatus and perfused with a nonrecirculating, nominally Ca²⁺-free solution containing Krebs-Henseleit buffer (KHB) solution [in mM: 12.5 glucose, 5.4 KCl, 1 lactic acid, 1.2 MgSO₄, 130 NaCl, 1.2 NaH₂PO₄, 25 NaHCO₃, and 2 sodium pyruvate (pH 7.4)] with 10 mM taurine until the coronaries were clear of blood. The heart was then perfused for 10–15 min with a recirculating digestion solution containing type II collagenase (180 U/ml) and 20 mM taurine. The heart was then removed from the cannula and the right ventricular tissue was removed before the digested left ventricle (LV) was minced in the collagenase solution. The resulting cell suspension was filtered and centrifuged at 25 g. Isolated myocytes were resuspended in a KHB solution containing 1% wt/vol bovine albumin, 10 mM taurine, and 0.5 mM CaCl₂. All solutions were equilibrated with 95% O₂-5% CO₂. The temperature was kept at 37°C throughout the isolation procedure.

Myocyte morphometric analysis. freshly isolated cells were fixed in an isoosmotic solution of 1.5% glutaraldehyde in 0.06 M phosphate buffer. This fixative does not alter cell volume or shape as previously shown by Gerdes and colleagues (12). For each fixed-cell suspension, average myocyte volume or shape was measured from each myocyte suspension, and cells were selected based on visible striations and the absence of membrane blebs or abnormal granularity. For each myocyte preparation, average myocyte cross-sectional area was calculated by dividing median myocyte volume by mean myocyte length.

Isolated myocyte function. For analysis of basal myocyte contractility, myocytes were placed in a chamber on the stage of an inverted microscope. The chamber was superfused at 1–2 ml/min with Tyrode solution [containing, in mM: 150 NaCl, 5.4 KCl, 1 CaCl₂, 1.2 MgCl₂, 10 glucose, 2 pyruvate, and 5 HEPES (pH 7.4)] at 37°C. Myocytes were chosen based on the same criteria as for the morphometric analysis as well as the absence of spontaneous contractions in 1 mM Ca²⁺. Contractions were measured during field stimulation at 0.5 Hz using a video edge-detection system (Crescent Electronics; Sandy, UT). Data were stored on a computer for later analysis using Axotape software (Axon Instruments; Foster City, CA). Resting myocyte length, fractional shortening (change in length divided by resting length), time to peak contraction, and time to 50% relaxation were determined in 8–12 cells from each heart. In a subset of 4–6 cells from each heart, the frequency dependence of contraction was assessed by increasing the stimulation frequency from 0.5 to 1.0 to 2.0 Hz. Steady-state fractional shortening was measured at each stimulation frequency.

Papillary muscle function. A septal papillary muscle was dissected from 10 normal Lewis rat hearts and 6 heterotopically transplanted donor hearts 2 wk after transplant surgery. Rats were anesthetized and a cardiectomy was performed as described above. The papillary muscles were mounted horizontally at an arbitrary length between metal clips on an isometric strain-gauge transducer in a Plexiglas bath. The chamber was superfused with KHB containing 2.0 mM CaCl₂ and equilibrated with 95% O₂-5% CO₂ at 37°C. Differences in the CaCl₂ concentrations used for isolated myocytes and papillary muscles reflect established differences in the dose-response curves for Ca²⁺ between the two preparations (3). The papillary muscle was stimulated with a Grass square-wave stimulator (Grass Instruments; Quincy, MA) at a stimulation voltage 25% above threshold with a 5-ms pulse width. After being mounted, each muscle was equilibrated for 30 min while being stimulated at a frequency of 2 stimulations/min. Muscle length was then gradually and incrementally increased until the muscle attained the length at which maximal developed force was achieved (Lmax). The muscle was then stimulated twice per minute for the next 20 min at Lmax to confirm a stable (<10% variation during 20 min) developed force. Twitches from muscles achieving a stable developed force were recorded on a two-channel paper-chart recorder (Gould Instruments; Valley View, OH). For experimental measurements, developed force and time to 50% relaxation were calculated for 5–10 consecutive contractions at a frequency of 0.5 Hz. The diameter of each muscle was then measured in place with a calibrated microscope. Cross-sectional area was calculated by assuming the muscle had a cylindrical shape, and developed force was normalized for the cross-sectional area of the muscle.

Statistical analysis. All data reported in the text and tables are expressed as means ± SE. Comparisons between heterotopically transplanted and control hearts (in both myocytes and papillary muscles) were analyzed by unpaired Student’s t-test using a commercially available software program (StatView version 4.1; Abacus Concepts; Berkeley, CA). A P value <0.05 was considered statistically significant for all hypothesis testing.

RESULTS

Morphometric measurements. The results of organ and cellular morphological analyses are presented in Table 1. Compared with normal hearts from age-matched rats, 2 wk of hemodynamic unloading via HT was associated with a 23% decrease in the absolute heart weight (P < 0.001) and a 26% decrease in the heart weight-to-body weight ratio (P < 0.001). Of note, paired comparisons of the weights of transplanted hearts at the time of transplantation and at explantation 2 wk later demonstrated an average heart mass decrease of 28 ± 1% (P < 0.001).
At the cellular level, 2 wk of hemodynamic unloading produced a 41% decrease in median myocyte volume. This decrease in cell volume reflected both a 21% decrease in average myocyte length (P < 0.001) and a 26% decrease in average myocyte cross-sectional area (P < 0.02). These cardiac and cellular morphological findings demonstrate that 2 wk of myocardial unloading via HT is sufficient to produce marked organ and cellular atrophy of the normal heart.

Function measurements. The contractile function measurements for isolated myocytes and papillary muscles from normal control hearts and heterotopically transplanted hearts are presented in Table 2. As illustrated in Fig. 1 and summarized in Table 2, compared with normal cardiac myocytes, isolated myocytes from unloaded hearts demonstrated no differences in fractional shortening, time to peak contraction, or time to 50% relaxation at 0.5 Hz. Recognizing that physiological differences might be more apparent during increased stimulation frequency, we examined the fractional shortening of 34 myocytes from control hearts and 30 myocytes from unloaded hearts at stimulation frequencies of 0.5, 1.0, and 2.0 Hz. As shown in Fig. 2, equivalent fractional shortening of control and unloaded myocytes was also observed during progressive increases in stimulation frequency.

In isolated papillary muscles, there were no differences in the peak developed force (F\textsubscript{max})/area, time to F\textsubscript{max}, or time to 50% relaxation in papillary muscles isolated from unloaded transplanted hearts compared with papillary muscles from control hearts. However, absolute F\textsubscript{max} in muscles from transplanted hearts was reduced compared with normal controls. As illustrated in Fig. 3, this reduction was roughly proportionate to the decrease in papillary muscle cross-sectional area that was observed in unloaded hearts.

### DISCUSSION

Cardiac unloading of normal myocardium and pathologically hypertrophied myocardium results in atrophic remodeling at both the organ and cellular levels (1, 4, 8, 9, 17, 19, 27, 34). Data from the present studies indicate that the normal myocardium of heterotopically transplanted rat hearts undergoes atrophic remodeling at the organ and cellular levels as would be expected with the hemodynamic unloading created by this model. This rapid remodeling is clearly shown by the reduced heart weight-to-body weight ratio and the decreased myocyte length, width, and volume in our transplanted rat hearts. However, unlike previous studies with this model, these studies demonstrate

### Table 1. Heart and myocyte morphology

<table>
<thead>
<tr>
<th></th>
<th>Control Hearts</th>
<th>Unloaded Hearts</th>
</tr>
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<tbody>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>215 ± 12</td>
<td>330 ± 15</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.12 ± 0.05</td>
<td>0.86 ± 0.02*</td>
</tr>
<tr>
<td>Heart weight/Body weight, g/kg</td>
<td>3.58 ± 0.12</td>
<td>2.63 ± 0.09*</td>
</tr>
<tr>
<td>Median myocyte volume, ( \mu \text{m}^3 )</td>
<td>31,115 ± 1,571</td>
<td>18,375 ± 1,829</td>
</tr>
<tr>
<td>Cell length, ( \mu \text{m} )</td>
<td>140 ± 12</td>
<td>110 ± 5*</td>
</tr>
<tr>
<td>Cell width, ( \mu \text{m} )</td>
<td>33 ± 4</td>
<td>26 ± 3*</td>
</tr>
<tr>
<td>Cell cross-sectional area, ( \mu \text{m}^2 )</td>
<td>225 ± 12</td>
<td>167 ± 11*</td>
</tr>
</tbody>
</table>

Values are means ± SE; *P < 0.05 vs. control hearts. †Donor rat body weight in unloaded hearts.

### Table 2. Myocyte and papillary muscle contractions

<table>
<thead>
<tr>
<th></th>
<th>Control Hearts</th>
<th>Unloaded Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated myocyte studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cells</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>8.9 ± 0.6</td>
<td>9.9 ± 0.5</td>
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<tr>
<td>Time to peak contraction, ms</td>
<td>163 ± 4</td>
<td>161 ± 6</td>
</tr>
<tr>
<td>Time to 50% relaxation, ms</td>
<td>242 ± 7</td>
<td>240 ± 11</td>
</tr>
<tr>
<td>Isolated papillary muscle studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of muscles</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Cross-sectional area, ( \mu \text{m}^2 )</td>
<td>0.186 ± 0.024</td>
<td>0.142 ± 0.029*</td>
</tr>
<tr>
<td>F\textsubscript{max}, mg</td>
<td>268 ± 25</td>
<td>184 ± 53*</td>
</tr>
<tr>
<td>F\textsubscript{max}/area, g/mm\textsuperscript{2}</td>
<td>1.70 ± 0.25</td>
<td>1.55 ± 0.30</td>
</tr>
<tr>
<td>Time to F\textsubscript{max}, ms</td>
<td>152 ± 5</td>
<td>164 ± 10</td>
</tr>
<tr>
<td>Time to 50% relaxation, ms</td>
<td>85 ± 2</td>
<td>97 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE. F\textsubscript{max}, maximal force generation. *P < 0.05 vs. control hearts.
intact contractile function at both the cellular and muscular levels when myocyte shortening is normalized to resting cell length and when papillary muscle force generation is normalized to cross-sectional area. These findings of preserved function using normalized indexes of contractility suggest that atrophic remodeling does not adversely affect sarcomere function under basal conditions. Nevertheless, the striking reduction in absolute $F_{\text{max}}$ as a result of atrophic remodeling does demonstrate that a net loss of sarcomeres and myocardial mass will reduce myocardial work capacity even when sarcomere function is preserved.

Similar to previous studies of in vivo cardiac unloading of the normal heart (4, 8, 9, 17–19), the present studies demonstrated morphological atrophy at the organ and tissue levels. Specifically, we observed a 23% reduction in heart weight and a 27% reduction in heart weight-to-body weight ratio at 2 wk after HT. At the cellular level, we observed that average myocyte volume in unloaded hearts was 41% smaller than in normally loaded control hearts. In addition to demonstrating that organ atrophy is a reflection of cellular atrophy, our cellular morphometrics also demonstrate for the first time that unloading of the normal heart produces balanced reductions in both cellular length and cross-sectional area consistent with reductions in both series and parallel sarcomeres. These proportionate decreases in length and cross-sectional area after unloading of the normal heart are similar to the balanced 20% decreases in myocyte length and width observed by Zafeiridis and colleagues (34) after mechanical unloading with a LVAD in severely failing human hearts (34). However, the present finding contrasts with the disproportionately large (14%) decreases in myocyte cross-sectional area compared with relatively smaller (6%) decreases in myocyte length observed by Gerdes and colleagues (11) after closing a chronic aortocaval fistula in rats. These distinctions may reflect the more complete reductions in both ventricular preload and afterload produced by HT and mechanical circulatory support.

The present studies evaluated isolated myocyte contractility in the atrophied heart. These in vitro studies revealed fractional shortening and relaxation responses in myocytes from atrophied hearts that did not differ from those observed in nonatrophied normal controls. By design, functional studies using isolated myocytes assess myocyte contraction and relaxation in the absence of preload or afterload. Our findings demonstrate that load-independent shortening is normal in myocytes with morphological atrophy. As is conventional in such studies, we measured fractional shortening to provide an index of sarcomere shortening. Thus our findings indicate that basic sarcomere function is intact despite morphological atrophy. Because changes in the rates of contraction and relaxation often precede changes in the magnitude of shortening, our findings of normal times to peak contraction and 50% relaxation in unloaded myocytes further attest to preservation of contractile function. Finally, equivalent responses to increasing stimulation frequency also suggest intact contractile function in unloaded myocytes. Beyond the normal cellular contractility that we observed in atrophic myocytes, a recent study by Ritter and colleagues (29) reported supranormal contractility and Ca$^{2+}$ transients in mouse myocytes 5 days after HT. Although distinctions between the present findings and this previous report could reflect species differences or the differences in the duration of unloading, the observations nevertheless support the contention that morphological atrophy need not be associated with impairment of sarcomere function. On the other hand, preliminary observations by Ito and co-workers (15) suggest a slowing of contraction kinetics and decreased contractile reserve in rat myocytes after 5 wk of hemodynamic unloading. Together with the present studies, the studies of Ritter and colleagues (29) and Ito and co-workers (15) suggest possible time-dependent changes in myocyte contractility and contractile reserve in the setting of morphological atrophy with initial increases and subsequent decreases.

Despite morphological atrophy evidenced by reduced cross-sectional area, isolated papillary muscles from unloaded hearts exhibited isometric force generation which was equivalent to that observed in normal hearts when $F_{\text{max}}$ was normalized by cross-sectional area. Because muscle cross-sectional area reflects the number of sarcomeres in parallel, our findings in papillary muscles suggest that basic sarcomere function is intact despite morphological atrophy. In this regard, our findings in isolated papillary muscles are analogous to our findings in isolated myocytes. As in the myocyte studies, the normal time to $F_{\text{max}}$ and the normal time to 50% relaxation in atrophied papillary muscles further attest to intact contractile performance. Our finding of normal $F_{\text{max}}$/area in atrophied papillary muscles under basal conditions is consistent with previous studies by Korecky and co-workers (21) who studied rat isografts 8 wk after HT. A relevant clinical example of the same phenomenon are studies in which LV ejection fraction remained normal while parallel reductions in LV afterload and LV mass were observed in women with anorexia nervosa (32). As in the present study, these previous findings demonstrate that reductions in cardiac workload trigger reductions...
in cardiac mass without affecting the basic force-to-mass ratio. However, our findings differ from previous studies by Cooper and Tomanek (4) who observed depressed magnitudes and rates of contraction in papillary muscles that developed atrophy after chordal transection. The more profound unloading produced by chordal transection likely accounts for the difference between our findings and those of Cooper and Tomanek (4). Indeed, chordal disruption produces marked ultrastructural disarray and severe deterioration of contractile function beyond 3 days of unloading, whereas cardiac isografts beat reliably for 8 wk after HT (21).

Reductions in absolute $F_{\text{max}}$ in atrophied papillary muscles despite intact $F_{\text{max}}$/area values (compared with normal hearts) suggest that, other things being equal, reduced myocardial mass will be associated with reduced organ work capacity. This observation helps explain the reductions in intact heart mechanics observed in several previous studies of unloaded hearts after HT. For example, using an indwelling intraventricular balloon, Galinanes and co-workers (8, 9) reported a stepwise reduction of LV developed pressure during the first 7 days after cardiac unloading via HT. Of note, when transplant-induced reductions in LV mass were attenuated by partially loading the allograft, there was a proportional improvement in the LV developed-pressure measurements (9). Together, these previous studies and the present study indicate that measures of cardiac performance that are not normalized for morphological considerations are likely to be depressed in atrophic hearts, whereas normalized parameters suggest intact or enhanced (29) contractile function under basal conditions. Factors that might alter this relationship between organ mass and pump function are improvements in basic sarcomere function [as observed after LVAD support in failing human hearts (6)] or improvements in chamber geometry, which promote reductions in wall stress.

Previous studies examining atrophic myocardium from heterotopically transplanted rat hearts have reported reductions in the relative abundance of the V1 myosin isoenzyme containing α-myosin heavy chain (MHC) with reciprocal increases in the abundance of the V3 myosin isoform containing β-MHC (5, 9, 10, 17, 20, 24). After HT, previous studies have also reported striking shifts in transporters that mediate glucose uptake and utilization (5) and regulators of glycolysis (23) within cardiac myocytes. Given our findings that atrophic myocardium after HT has preserved contractile function, it is tempting to speculate that the previously observed changes in myosin isoforms and glucose metabolism are not functionally important in the regulation of basal contractility. Alternatively, myosin isoform shifts or other contractile protein abnormalities, which have been associated with reduced Ca$^{2+}$ sensitivity (25), might be functionally balanced by changes in Ca$^{2+}$ cycling that help preserve contractile function. Such balanced adaptations have been well demonstrated in recent studies by Perez and colleagues (28) in spontaneously hypertensive heart failure-prone rats with heart failure. It is also possible that previously reported changes in myosin isoform and glucose metabolism could affect responses to physiological stress without altering basal contractile responses as assessed in the present studies. Ultimately, new studies will be required to determine the conditions under which molecular changes associated with myocardial atrophy are of functional significance.

The present studies show that despite morphological atrophy, myocardial unloading via HT is associated with intact contractile function in isolated myocytes and papillary muscles when contractile indexes are normalized to account for differences in cell length and cross-sectional area, respectively. Based on our findings of reduced absolute $F_{\text{max}}$, it is likely that previously reported reductions in global cardiac function after HT (8, 9) could simply be a reflection of the reduced cardiac mass. These observations have potential implications for recent observations in diseased human hearts unloaded via mechanical circulatory support. On one hand, improved fractional shortening observed in isolated myocytes (6) should improve overall cardiac contractile performance. On the other hand, the findings in the present study suggest that reductions in organ and cellular mass after mechanical circulatory support (1, 34) may tend to offset gains resulting from improvements in contractile function. Consideration of such potential trade-offs involved in reverse remodeling during mechanical cardiac support may help optimize contractile improvements in failing hearts at both the organ and cellular levels.

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