Myocyte Recovery After Mechanical Circulatory Support in Humans With End-Stage Heart Failure

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Background—The failing myocardium is characterized by decreased force production, slowed relaxation, and depressed responses to β-adrenergic stimulation. In some heart failure patients, heart function is so poor that a left ventricular assist device (LVAD) is inserted as a bridge to transplantation. In the present research, we investigated whether circulatory support with an LVAD influenced the functional properties of myocytes from the failing heart.

Methods and Results—Myocytes were isolated from human explanted failing hearts (HF-myocytes) and failing hearts with antecedent LVAD support (HF-LVAD-myocytes). Studies of myocyte function indicated that the magnitude of contraction was greater (9.6 ± 0.7% versus 6.9 ± 0.5% shortening), the time to peak contraction was significantly abbreviated (0.37 ± 0.01 versus 0.75 ± 0.04 seconds), and the time to 50% relaxation was reduced (0.55 ± 0.02 versus 1.45 ± 0.11 seconds) in the HF-LVAD-myocytes compared with the HF-myocytes (P < 0.05). The HF-LVAD-myocytes had larger contractions than the HF-myocytes at all frequencies of stimulation tested. The negative force-frequency relationship of the HF-myocytes was improved in HF-LVAD-myocytes but was not reversed. Responses to β-adrenergic stimulation (by isoproterenol) were greater in HF-LVAD-myocytes versus HF-myocytes.

Conclusions—The results of the study strongly support the idea that circulatory support with an LVAD improves myocyte contractile properties and increases β-adrenergic responsiveness. (Circulation. 1998;97:2316-2322.)

Key Words: heart-assist device • myocytes • cardiomyopathy • calcium

Congestive heart failure is a leading cause of death in the United States.1,2 It results from a number of cardiovascular diseases, including coronary artery disease, hypertension, valvular lesions, and primary cardiomyopathies. A common feature of most forms of heart failure is that the disease either involves or eventually produces excessive hemodynamic demands on the LV. The primary disease and the associated hemodynamic burden are thought to be responsible for induction of electrical and mechanical alterations in myocytes, which further exacerbate the condition.

Contractile alterations of myocytes from failing hearts include reduced rates of shortening and lengthening, reduced contraction magnitude, and diminished responsiveness to β-adrenergic agonists.3-8 The response of failing myocytes to increasing beating rates is also abnormal.9 In the normal heart, contraction rate and magnitude increase with beating rate (often termed a positive force-frequency relationship), whereas in the failing myocardium, contractions decrease over the same range of frequencies.10 These cellular abnormalities contribute to the systolic and diastolic defects of the failing heart.

Common medical therapy for HF includes reducing the hemodynamic burden with afterload-reducing agents and increasing the inotropic state.11 Vasodilators increase cardiac output but usually do little or nothing to improve dysfunctional myocyte properties.12,13 Nearly all existing inotropes have tended to increase mortality when used clinically.14 Therefore, current novel HF treatments are aiming to produce permanent improvement in cardiac performance by reversing dysfunctional myocyte properties, optimizing ventricular geometry,15 or reinfiltarting myocardial scars with functional myocytes.16,17

In some patients, CHF can become so severe that survival is unlikely without implantation of an LVAD for hemodynamic support. Currently, these devices are used primarily as a bridge to heart transplantation. Previous studies of LVAD-supported hearts have shown that there is significant myocyte remodeling and reduced myocardial expression of cardiac genes, such as atrial natriuretic peptide and brain natriuretic peptide associated with CHF.18 The working hypothesis of the present study is that LVAD support reduces the hemodynamic demands of the failing LV and results in improved myocyte contractile behavior. The specific objective of the present research was to determine whether LVAD support of failing
human hearts is associated with beneficial changes in myocyte function. Our findings demonstrate that LVAD support reveals functional plasticity even in the most severely failing human hearts and support the concept that mechanical circulatory support may promote improvement in myocardial function.

**Methods**

**Myocyte Isolation From Explanted Hearts**

Human ventricular myocardium was obtained from 22 patients with severe HF at the time of orthotopic cardiac transplantation. HF was secondary to ischemic (n=10) or idiopathic (n=12) cardiomyopathy. Of the 22 patients, 6 underwent LVAD placement (n=3 ischemic, n=3 idiopathic) for an average of 111 days. All of these patients are included in the data presented. Patients’ characteristics and hemodynamic data (before and after LVAD placement) are presented in Tables 1 and 2, respectively.

Hearts received cold, blood-containing, high-potassium cardioplegic solution in vivo. Explanted hearts were transported from the operating suite to the laboratory in cold KHB solution (12.5 mmol/L glucose, 5.4 mmol/L KCl, 1 mmol/L lactic acid, 1.2 mmol/L MgSO₄, 130 mmol/L NaCl, 1.2 mmol/L NaH₂PO₄, 25 mmol/L NaHCO₃, and 2 mmol/L Na pyruvate, pH 7.4) in <5 minutes.

Myocytes were disaggregated by use of a modification of isolation techniques developed in this laboratory. Briefly, the heart was weighed and rinsed in KHB, and a small catheter was placed into the lumen of an artery or a vein, which supplied a noninfarcted free wall region of the LV. The area perfused via this cannulated vessel was removed from the heart and rinsed with a nonrecirculating Ca²⁺-free solution (1000 mL KHB, 10 mmol/L taurine) for 30 minutes. Then, 200 mL of KHB containing 180 U/mL collagenase, 20 mmol/L BDM, 20 mmol/L taurine, and 0.05 mmol/L CaCl₂ was recirculated for 30 minutes. The tissue was rinsed for 10 minutes with KHB containing 10 mmol/L taurine, 20 mmol/L BDM, and 0.2 mmol/L CaCl₂. The tissue was then removed from the cannula, and midmyocardium tissue was minced in the rinse solution.

**Selected Abbreviations and Acronyms**

- BDM = 2,3-butanedione monoxime
- CHF = congestive heart failure
- HF = heart failure
- KHB = Krebs-Henseleit solution
- LV = left ventricle/ventricular
- LVAD = LV assist device
- RCL = resting cell length
- SR = sarcoplasmic reticulum

**TABLE 1. Patient Characteristics**

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Average ± SEM: 57.87 ± 2.94

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Average ± SEM: 54.3 ± 4

M indicates milrinone; ACE, ACE inhibitor; LOS, losartan (angiotensin II blocker); BB, β-blocker medication; CaB, calcium blockers; N, nitrate; DG, digoxin; Hy, hydralazine; D, dobutamine; AM, amiodarone; DU, diuretic; ISC, ischemic cardiomyopathy; and IDIO, idiopathic cardiomyopathy.
TABLE 2. Hemodynamic Data

<table>
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<th>Measurements</th>
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<th>Post-LVAD</th>
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<td>PA systolic</td>
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<td>29±2‡§</td>
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<tr>
<td>PCWP</td>
<td>20±2</td>
<td>30±1†</td>
<td>13±2‡</td>
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<tr>
<td>Systolic BP</td>
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<td>99±6</td>
<td>134±11‡§</td>
</tr>
<tr>
<td>Diastolic BP</td>
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<td>MAP</td>
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<td>Cardiac index</td>
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<td>LVEF</td>
<td>14.2±2.0</td>
<td>12.5±1.3</td>
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RAP indicates right atrial pressure; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; BP, blood pressure; MAP, mean arterial pressure; and LVEF, LV ejection fraction.

*Five of six HF-LVAD patients were on intra-aortic balloon pump support when these hemodynamics were measured.
†Significant difference between HF and pre-LVAD at P<0.05.
‡Significant difference between pre- and post-LVAD at P<0.05.
§Significant difference between HF and post-LVAD at P<0.05.

Myocyte Functional Measurements

Myocytes were plated in a chamber on the stage of an inverted microscope. The chamber was superfused at 1 to 2 mL/min with Tyrode’s solution (130 mmol/L NaCl, 5.4 mmol/L KCl, 1 mmol/L CaCl, 1.2 mmol/L MgCl, 10 mmol/L glucose, 2 mmol/L pyruvate, and 5 mmol/L HEPES, pH 7.4) at 35°C. Myocytes were chosen on the basis of their morphological appearance (rod shape, no hypercontracted areas, no membrane blebs) and the absence of spontaneous contractions in 1 mmol/L Ca²⁺. Contractions were measured by use of an edge-detection (Crescent Electronics) technique as described previously. Data were stored on computer for later analysis.

Biochemical Co. Indo-1 (acetoxyethyl ester) was obtained from Calbiochem.

Statistical Analysis

All data in the text and tables are reported as mean±SEM. Differences between the two groups were assessed by t tests for independent samples. Differences among multiple measurements were assessed by ANOVA. Differences in the isoproterenol response of HF-myocytes and LVAD-supported myocytes (HF-LVAD-myocytes) were assessed by calculating the changes in contractile parameters observed in the presence of isoproterenol minus that at baseline and performing a t test for independent samples on the differences between the two groups.

Results

Basic Contractile Properties

Contractions at 0.2 Hz were measured in 57 HF-myocytes and 35 HF-LVAD-myocytes. Figure 1a shows steady-state twitches in representative myocytes from the two groups, and Figure 1b shows the corresponding rates of shortening and relengthening. Summary data of all contractile parameters measured are presented in Table 3. These experiments showed that the shortening magnitude, as a percentage of RCL, the rate of shortening, and the rate of relengthening were significantly greater in the HF-LVAD-myocytes than in the HF-myocytes. The time to peak shortening and the time to

TABLE 3. Contractile Characteristics of HF-Myocytes and HF-LVAD-Myocytes at 0.2 Hz

<table>
<thead>
<tr>
<th>Contractile Characteristic</th>
<th>HF-Myocytes</th>
<th>HF-LVAD-Myocytes</th>
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<tr>
<td>Magnitude of shortening, % RCL</td>
<td>6.9±0.5</td>
<td>9.6±0.7*</td>
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<td>Time to peak contraction, s</td>
<td>0.75±0.04</td>
<td>0.37±0.01*</td>
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<tr>
<td>Time to 50% relaxation, s</td>
<td>1.45±0.11</td>
<td>0.55±0.02*</td>
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<tr>
<td>Rate of shortening, % RCL/s</td>
<td>38.0±4.9</td>
<td>109.9±12.1*</td>
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<tr>
<td>Rate of relengthening, % RCL/s</td>
<td>36.5±5.4</td>
<td>99.08±12.32*</td>
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*Significant difference between the HF- and the HF-LVAD-myocytes at P<0.05.
50% relaxation were significantly shorter in HF-LVAD-myocytes versus HF-myocytes. These findings suggest that there are significant improvements in contractile properties of failing myocytes after LVAD support.

**Stimulation Rate Dependence of Contraction**

To examine the rate dependence of myocyte contractile function, we studied the effects of increasing stimulation frequency on the magnitude of shortening in HF-myocytes and HF-LVAD-myocytes. Representative tracings of steady-state twitch contractions at 0.2, 0.5, and 1.0 Hz are shown in Figure 2. Most HF-LVAD-myocytes were able to follow stimulation rates up to 2.0 Hz. However, HF-myocytes were often unstable at rates >1.0 Hz. In the present analysis, we included only myocytes that were capable of beating at rates up to 1.0 Hz. Average data from 13 HF-myocytes and 13 HF-LVAD-myocytes are shown in Table 4. As expected, increased stimulation frequency resulted in a decrease in shortening magnitude in HF-myocytes ($p<0.05$). Although HF-LVAD-myocytes also exhibited a negative force-frequency relationship, the HF-LVAD-myocytes had significantly larger shortening magnitudes than HF-myocytes at all frequencies tested ($p<0.05$). In addition, the HF-LVAD-myocytes tended to require higher stimulation frequencies than HF-myocytes before exhibiting a decline in shortening.

**Isoproterenol Effects**

To determine whether LVAD support leads to improved myocyte adrenergic responsiveness, 18 HF-myocytes and 12 HF-LVAD-myocytes were exposed to $10^{-6}$ mol/L isoproterenol during repetitive stimulation at 0.2 Hz. Preliminary studies showed that this concentration caused maximal inotropic effects in both groups. In these experiments, myocytes with similar baseline contractile properties (contraction magnitude, 7.63% RCL in HF-LVAD-myocytes versus 6.43% RCL in HF-myocytes; $p=0.323$) were chosen. With this approach, subsequent results with isoproterenol would not be biased by different starting conditions. Representative results are shown in Figure 3, and average data are listed in Table 5. Isoproterenol produced a greater absolute increase in the magnitude of contraction in the HF-LVAD-myocyte than in the HF-myocytes. Myocytes with similar RCLs (170 μm) were chosen here.

**Indo-1 Fluorescence**

To examine whether alterations in contractile function of HF-LVAD-myocytes are associated with changes in [Ca^{2+}]_i homeostasis, we examined the cytosolic free Ca^{2+} transients of seven HF-LVAD-myocytes (from one HF-LVAD patient) and five HF myocytes (from one HF patient randomly selected). Representative raw data are shown in Figure 4. These preliminary findings suggest that HF-LVAD-myocytes...
had Ca\(^{2+}\) transients with greater peak systolic ratio and faster rate of decay than HF-myocytes.

**Discussion**

The objective of the present research was to determine whether hemodynamic unloading and reduced neurohormonal activation resulting from LVAD support causes beneficial functional remodeling at the cellular level. The present study compared contractile characteristics of LV myocytes from failing human hearts with those from failing hearts that had been supported with an LVAD. Consistent with previous reports, characteristic features of hypertrophied/failing myocytes included reduced shortening magnitude, slow rates of shortening and relengthening, and prolongation of contraction duration.\(^{20-24}\) The major finding of the present study is that LVAD support of the failing hearts causes improvement of dysfunctional myocyte contractile properties. Functional changes included significant improvements in the contraction magnitude, the rates of shortening and relengthening, and an augmented \(\beta\)-adrenergic responsiveness. A tendency toward improvements in frequency-dependent contractile derangements was also observed. Based on their pre-LVAD hemodynamics, LVAD patients were probably in a more advanced stage of failure than unsupported HF patients; the function of their myocytes would have been expected to be even worse than myocytes from failing hearts not requiring mechanical assistance if the LVAD treatment had no effect on myocyte function. Thus, these findings indicate that LVAD support allows for at least partial recovery of normal myocyte contractile properties and demonstrate that failing cardiac myocytes are not irreversibly damaged and are capable of beneficial phenotypic changes.

**LVAD Support Causes Ventricular Remodeling**

Previous studies have shown that the Heartmate LVAD is an effective means of circulatory support for human with CHF.\(^{25,26}\) These prior studies have shown that LVAD support produces significant structural changes in the failing heart, including reduction of LV dimensions, reduced cardiac mass, and improvement of the passive end-diastolic pressure-volume relationships.\(^{18,27,28}\) Reducing the size of the dilated LV should decrease wall stress and thereby enhance systolic performance in vivo. Recent studies also suggest that remodeling of cardiac myocytes occurs in parallel with changes in cardiac morphology after LVAD support in advanced dilated cardiomyopathy.\(^{29}\) In the present experiments, we show that LVAD support promotes improvement in the contractile properties of the LV myocytes in vitro, further supporting the idea that the pump function of the failing heart may be improved after mechanical circulatory support.

The effects of LVAD support on myocyte function are not easily predicted from previous studies. Along these lines, a previous study in cats by Tomanek and Cooper\(^{30}\) demonstrated that unloading of the right ventricular papillary muscle by transection of the chorda tendonea caused muscle atrophy and reduced functional capabilities. This would suggest that LVAD support might produce substantial additional defects in the failing myocyte. However, there are several fundamental distinctions between these previous studies and LVAD support of the failing human heart. First, the studies of Tomanek and Cooper\(^{30}\) involved normal myocardium, unlikely to improve under any circumstances. In contrast, LVAD support was used in the setting of hemodynamic overload and marked neurohormonal derangements, which may contribute to abnormal myocardial function. In addition, the mechanical unloading is complete in the chordal transection model but partial with LVAD support. These distinctions likely account for the divergent results observed in the past and present studies of myocardial unloading.

**Cellular Basis of Contractile Improvements**

Alterations in cellular Ca\(^{2+}\) homeostasis are thought to underlie many of the contractile abnormalities of the failing heart.\(^{20,31-33}\) Previous studies suggest that significant reductions in peak systolic Ca\(^{2+}\) and a slow rate of decay of the

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**TABLE 5. Isoproterenol Response on Contractile Parameters**

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<td>0.70±0.07(†)</td>
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\(\*\)Significant difference (\(P<0.0125\)) between baseline and isoproterenol (paired \(t\) test).

\(\†\)Significant difference (\(P<0.0125\)) between HF- and HF-LVAD-myocytes (\(t\) test for independent samples).

\(\‡\)Significant difference (\(P<0.05\)) between HF- and HF-LVAD-myocytes on the delta scores (\(t\) test for independent samples).
Ca$^{2+}$ transient are largely responsible for the diminished force of contraction and slowed relaxation in the failing myocyte. Our preliminary findings, as illustrated in Figure 4, support the speculation that alterations in calcium homeostasis contribute to the augmented extent of shortening and the faster rate of relaxation observed in the myocytes from LVAD-supported patients. Activator Ca$^{2+}$ is derived primarily from the SR in large mammals, and a reduced SR function in HF could account for both the slow rate of decay of the Ca$^{2+}$ transient, and reduced peak systolic Ca$^{2+}$. Alterations in Ca$^{2+}$ homeostasis in general and impaired SR Ca$^{2+}$ reuptake in particular could also contribute to the abnormal frequency-dependent contractile responses reported previously and observed in the present studies. From this perspective, it is possible that contractile improvements after LVAD support might reflect changes in intracellular Ca$^{2+}$ handling via SR Ca$^{2+}$ ATPase or other mechanisms.

Speculations about the mechanisms of contractile improvement after LVAD support parallel current theories about the progression of myocardial dysfunction after an initiating insult. A number of extracardiac factors have been implicated in the progression of myocardial dysfunction after an initial cardiac insult, including cytokine-mediated toxicity, excessive neurohormonal stimulation, excessive hemodynamic loading conditions, and distorted cardiac geometry with consequent increases in wall stress and mismatch of myocardial oxygen supply and demand. Because these mechanisms all have the potential to depress contractile function and promote myocyte loss through either ischemia or apoptosis, they also represent potential contributors to contractile improvement after LVAD support. Although the present studies do not permit conclusions about whether processes resulting in cardiac myocyte loss have been altered by LVAD support, our findings do indicate that LVAD support produces improvements in the contractile performance of the remaining cardiac myocytes. A variety of intracellular changes might contribute to the improvements in cellular contraction and relaxation observed in myocytes with antecedent LVAD support. Here again, possible mechanisms for improvements in contractile function after LVAD support involve membrane ion channels, calcium regulatory proteins, calcium homeostasis, myofilament calcium sensitivity, and/or myocyte internal load. Further studies will be required to examine these candidate mechanisms of improved contractile function.

**β-Adrenergic Stimulation**

One of the well-known features of the failing human heart is diminished responsiveness to β-adrenergic stimulation. This behavior is thought to result from the long-term high exposure to sympathetic agonists in HF patients. In vitro studies have shown that continuous exposure of myocytes to β-adrenergic agonists can uncouple and downregulate β-adrenergic receptors. The total density of β-adrenoceptors appears to be decreased in HF. However, there is heterogeneity with respect to changes in myocardial β-receptor subpopulations. The present study was designed to study whether there is an overall change in β-adrenergic responsiveness of failing LV myocytes after LVAD support. Isoproterenol was used as the β-adrenergic agonist because it is a nonselective β-agonist. Our findings of augmented contractile responses to isoproterenol after LVAD support indicate improved adrenergic responsiveness. Possible explanations include reversal of receptor downregulation owing to reduced activation of the sympathetic nervous system or the reduced use of exogenous β-agonists (dobutamine, milrinone) in the LVAD-supported patients (Table 1; pre-LVAD versus post-LVAD medication). Alternatively, changes in adrenergic signal transduction (ie, phospholamban and G-stimulatory or G-inhibitory proteins) are also possible.

The time course of contractile improvement after LVAD support could not be accurately determined with the relatively small number of patients included in this report. However, it is clear that the beneficial effects on contractile properties can occur within 3 months. This conclusion is based on the fact that contractile properties of myocytes from six hearts with LVAD support of 75 to 160 days were all similar and significantly better than those of their failing counterparts. Future studies, including larger number of LVAD-supported patients, are needed to address this issue in more detail.

**Conclusions**

This study shows that LVAD support of the failing human heart causes significant improvement in myocyte contractility and β-adrenergic responsiveness. These results clearly demonstrate that failing human ventricular myocytes have the capability to undergo beneficial functional changes in the presence of hemodynamic unloading and improved neurohormonal and circulatory derangements. The ability of LVAD support to promote improvements in cellular contractile performance suggests a unique opportunity for future investigations to elucidate basic molecular mechanisms contributing to contractile defects in the failing human heart. These results also suggest that LVAD support could be useful as a potential means of promoting myocardial recovery. In this regard, the challenges of the future will include determination of (1) which patients are most likely to benefit from this type of therapy, (2) how to optimize myocardial recovery, (3) how to recognize recovery, and (4) how to wean patients from LVAD support. It will also be of critical importance to determine whether LVAD support produces enduring or transient improvements in myocardial function.

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**References**

Cardiomyocyte Recovery After LVAD Support


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