Incomplete Recovery of Myocyte Contractile Function Despite Improvement of Myocardial Architecture With Left Ventricular Assist Device Support

Amrut V. Ambardekar, MD; John S. Walker, PhD; Lori A. Walker, PhD; Joseph C. Cleveland, Jr, MD; Brian D. Lowes, MD, PhD; Peter M. Buttrick, MD

Background—Unloading a failing heart with a left ventricular assist device (LVAD) can improve ejection fraction (EF) and LV size; however, recovery with LVAD explantation is rare. We hypothesized that evaluation of myocyte contractility and biochemistry at the sarcomeric level before and after LVAD may explain organ-level changes.

Methods and Results—Paired LV tissue samples were frozen from 8 patients with nonischemic cardiomyopathy at LVAD implantation (before LVAD) and before cardiac transplantation (after LVAD). These were compared with 8 nonfailing hearts. Isolated skinned myocytes were purified and attached to a force transducer, and dimensions, maximum calcium-saturated force, calcium sensitivity, and myofilament cooperativity were assessed. Relative isoform abundance and phosphorylation levels of sarcomeric contractile proteins were measured. With LVAD support, the unloaded EF improved (10.0±1.0% to 25.6±11.0%, P=0.007), LV size decreased (LV internal dimension at end diastole, 7.6±1.2 to 4.9±1.4 cm; P<0.001), and myocyte dimensions decreased (cross-sectional area, 1247±346 to 638±254 μm²; P=0.001). Maximum calcium-saturated force improved after LVAD (3.6±0.9 to 7.3±1.8 mN/mm², P<0.001) implantation but was still lower than in nonfailing hearts (7.3±1.8 versus 17.6±1.8 mN/mm², P<0.001). An increase in troponin I (TnI) phosphorylation after LVAD implantation was noted, but protein kinase C phosphorylation of TnI decreased. Biochemical changes of other sarcomeric proteins were not observed after LVAD.

Conclusions—There is significant improvement in LV and myocyte size with LVAD, but there is only partial recovery of EF and myocyte contractility. LVAD support was associated only with biochemical changes in TnI, suggesting that alternate mechanisms might contribute to contractile changes after LVAD and that additional interventions may be needed to alter biochemical remodeling of the sarcomere to further enhance myofilament and organ-level recovery. (Circ Heart Fail. 2011;4:425-432.)

Key Words: ventricle-assist device ▪ ventricular remodeling ▪ heart failure

The left ventricular assist device (LVAD) is a well-established therapy for patients with end-stage heart failure (HF). The mechanical unloading of the LV with the LVAD and the subsequent restoration of cardiac output result in improvements in HF symptoms, functional status, quality of life, and end-organ perfusion. In addition to these systemic effects, some patients undergoing LVAD support show improved function of the native LV, termed reverse remodeling. Such organ-level improvements include decreased LV chamber size, decreased LV mass, and improved LV ejection fraction (EF) and have been accompanied by systemic effects such as normalization of catecholamine levels, natriuretic peptide levels, and circulating cytokines like tumor necrosis factor-α.

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Some of these clinical markers of reverse remodeling at the organ level also have been demonstrated at the cellular and molecular levels. Previous studies have noted reduction in myocyte hypertrophy as well as improvement in overall cardiac histology after LVAD support. Furthermore, in vitro studies have demonstrated improvements in myocardial contractile properties, restoration of adrenergic receptor density and responsiveness, and improved calcium handling with LVAD support.

Because both clinical and cellular evidence suggest the plausibility of reversing end-stage disease, the concept arose that LVADs may be used as a bridge to recovery in patients with HF. Indeed, this was met with great enthusiasm after a single-center report of the successful explantation of LVADs in 11 of 15 patients with nonischemic cardiomyopathy treated with a combination of unloading with an LVAD followed by conventional HF medical therapy and the selective β2-adrenergic receptor agonist clenbuterol. However, such high rates of recovery were not observed in the multiinstitutional...
tional LVAD Working Group study where only 6 (9%) of 67 patients underwent LVAD explantation for recovery. At this time, the mechanisms of reverse remodeling remain poorly understood, and it appears that restoring myocardial contractility may be more complex than what had been considered initially. Therefore, we postulated that myocyte contractility and biochemistry at the most fundamental contractile level of the heart—the sarcomere—evaluated before and after LVAD placement might reveal an explanation for this phenomenon. Our first goal was to assess sarcomeric contractile properties by measuring direct isometric forces on skinned isolated cell preparations. Second, because the cardiac sarcomeric proteins myosin-binding protein C (MyBPC), troponin T (TnT), TnI, tropomyosin, and regulatory myosin light chain (MLC-2) are known to affect contractility, we evaluated whether posttranslational modifications and other alterations in these proteins were affected by LVAD support.

Methods

Patient Selection and Tissue Acquisition
Paired LV tissue samples were collected from 8 patients with end-stage nonischemic cardiomyopathy at the time of LVAD implantation (before LVAD) and before cardiac transplantation (after LVAD) and flash frozen. Nonfailing control samples were obtained from 8 donor hearts that were harvested for transplantation but then unused for noncardiac reasons. The tissue obtained at the time of LVAD implantation consisted of a core sample from the LV apex, at the in-flow cannula of the LVAD. The tissue collected at the time of cardiac transplantation consisted of an analogously sized fragment excised from the explanted heart. All tissue samples were flash frozen immediately in liquid nitrogen in the operating room (without the use of any cardioplegia solutions) and transported to a freezer where they were stored until use. This storage method has been shown to preserve sarcomeric contractile protein phosphorylation state and to allow for the accurate assessment of the myocyte contractile parameters described herein.14–16 The ischemia time from removal of LV tissue from the patient to freezing in liquid nitrogen for the before LVAD samples was <10 seconds and for the after LVAD and nonfailing samples, <10 minutes. The Colorado Multi-center Institutional Review Board approved the protocol for the collection, storage, and analysis of human tissue.

Medical records were retrospectively reviewed by a trained physician to obtain demographic and clinical data. Echocardiographic and hemodynamic data were obtained from the medical record at the time closest to before LVAD implantation (to ensure conditions reflecting the time the before-LVAD tissue was obtained) followed by the time closest to cardiac transplantation (to ensure conditions reflecting the time the after-LVAD tissue was obtained). The data obtained from patients being supported with an LVAD reflect the device settings that were clinically indicated at the time for the patients and represent the combined effects of native LV function as well as LVAD-related unloading.

Myocyte Contractility Measurements
Details of the myocyte isolation and experimentation protocols have been described previously.14–17 Briefly, myocytes were purified from the frozen LV samples by mechanical homogenization and subsequently permeabilized with 0.3% Triton X-100. The resulting suspension was placed on the stage of an inverted microscope, and to ensure adequate tissue quality, myocyte fragments with an organized myofibril pattern with clear sarcomeric striations were chosen for the contractility experiments.15 These isolated skinned myocyte fragments were attached to a force transducer and motor in relaxing solution for mechanical contractile experiments. The visible sarcomere striations were set at 2.1 μm for all myocyte experiments.

The force transducer in relaxing solution, and a side-view mirror was used to measure the thickness. Cross-sectional area was calculated using an elliptical approximation. The isolated myocytes were stored on ice and used within 20 hours of isolation. All experiments were performed at 15°C, and because sarcomere length can affect force measurements, the sarcomere length was set to 2.1 μm for all experiments.18

Myocytes were exposed in random order to 6 different activation solutions containing varying concentrations of calcium (pCa, 9.0 to 4.5), and the developed force for each pCa was recorded as shown in Figure 2A. The developed force at the maximal pCa was measured before and after obtaining the measurements of force at the other 6 concentrations. If there was a >15% decline in force between the first and last force measurement at the maximal pCa, we considered this as evidence of cell fragment degradation, and these data were excluded from the analysis. Using these measurements, a force-pCa curve was plotted (Figure 2B), and the Hill equation was fit to derive the following contractile parameters: Fmax (the maximal calcium-saturated developed force normalized to the cross-sectional area of the myocyte); pCa50 (the pCa at which the force is half maximal, a measure of calcium sensitivity); and the Hill coefficient (the slope of the calcium-force relation, an index of myofilament cooperative activation). To minimize measurement variations for any single isolated myocyte, the individual patient contractile data reflect the average of measurement recordings for 3 to 5 myocytes per patient.

Sarcomeric Protein Phosphorylation Assessment
The remaining myocyte preparations not used for mechanical study were acetone precipitated in order to clamp their phosphorylation state and homogenized in 8 mol/L urea, 2.5 mol/L thiourea, 4% CHAPS, 10 mmol/L EDTA, and a mixture of protease and phosphatase inhibitors as previously described.20 Protein concentration of these samples was measured using the BCA Protein Assay Kit (Thermo Scientific).

Phosphorylation levels of the cardiac sarcomeric proteins were determined by separating the proteins with 12% 1D SDS-PAGE and fixing and staining with a phosphoprotein stain (Pro-Q Diamond phosphoprotein gel stain; Invitrogen). After imaging, the same gels were rinsed and stained with a total protein stain (BioSafe Coomassie Blue; BioRad). All gels were imaged using the Typhoon 9410 gel imager (GE Lifesciences), and protein optical densities were measured using ImageJ version 1.42 (National Institutes of Health). To adjust for subtle differences in protein loading, the phosphorylation levels were calculated by dividing the optical density of each sarcomeric protein on the Pro-Q Diamond gel by the optical density of the same protein on the Coomassie gel. In the case of MyBPC, the total protein stain optical density merges with the abundant protein myosin, so the phosphorylation level of MyBPC on the phosphoprotein stain was normalized to essential MLC (MLC-1) as prior studies have reported.20

Figure 1. Photomicrograph example of an isolated skinned myocyte fragment attached to the force transducer and motor in relaxing solution for mechanical contractile experiments. The visible sarcomere striations were set at 2.1 μm for all myocyte experiments.
Additional Assessment of Sarcomeric Proteins

Site-specific phosphorylation of TnI was assessed by Western blots. Proteins were separated with 12% 1D SDS-PAGE as previously described and transferred to PVDF membranes. After the membranes were blocked in 5% BSA and rinsed with TBST, they were incubated overnight at 4°C with a phosphospecific primary antibody to TnI phosphorylated at the putative protein kinase A site serine 22 and 23 (cell signaling, 1:1000) or to TnI phosphorylated at the putative protein kinase C (PKC) site serine 43 using an epitope-specific phosphoserine antibody (Abcam, 1:1000) as previously described.20 The blots then were washed and incubated with secondary antibody (antimouse from Sigma, 1:10 000) for 1 hour at room temperature, washed, and visualized using enhanced chemiluminescence. Membranes were stripped and subsequently incubated in primary total cardiac TnI antibody (Fitzgerald, Inc, 1:2500). Site-specific phosphorylation was calculated by dividing the optical density of the phosphospecific antibody blot by the density of the total protein blot.

Changes in myosin heavy chain isoforms were assessed as previously described20 by separating proteins with modified 6% 1D SDS-PAGE and subsequent staining with BioSafe Coomassie Blue total protein stain. MLC-1 and MLC-2 phosphorylation and isoform changes were assessed using 2D SDS-PAGE,21 and percent phosphorylated MLC-2 and percent atrial isoform of MLC-1 were calculated.

Changes in TnT isoform expression were assessed using modified Western blots as previously described.22 The primary cardiac TnT isoform antibody used was Ab-1 (clone 13 to 11; Thermo Scientific, 1:1000).

Statistical Analysis

Results are expressed as mean±SD. The paired t test was used to compare differences between before- and after-LVAD implantation. The unpaired t test was used to compare differences between before-LVAD implantation failing and nonfailing hearts and be-
The baseline characteristics of patients with end-stage nonischemic cardiomyopathy requiring LVAD as a bridge to cardiac transplantation are provided in Table 1. All patients were inotrope dependent with New York Heart Association functional class IV HF at the time of LVAD implantation, and 7 of 8 patients required mechanical support with an intraaortic balloon pump. The mean duration for the diagnosis of HF at the time of LVAD implantation was 59±22 months. Both first-generation pulsatile displacement LVADs (HeartMate XVE; Thoratec; Pleasanton, CA) and second-generation continuous axial flow LVADs (HeartMate II) were used. The mean duration of LVAD support was 143±41 days. LVAD support resulted in significant reductions in LV echocardiographic dimensions, improvement in EF, and normalization of several hemodynamic measures as summarized in Table 2.

### Results

#### Patient Characteristics

The baseline characteristics of patients with end-stage nonischemic cardiomyopathy requiring LVAD as a bridge to cardiac transplantation are provided in Table 1. All patients were inotrope dependent with New York Heart Association functional class IV HF at the time of LVAD implantation, and 7 of 8 patients required mechanical support with an intraaortic balloon pump. The mean duration for the diagnosis of HF at the time of LVAD implantation was 59±22 months. Both first-generation pulsatile displacement LVADs (HeartMate XVE; Thoratec; Pleasanton, CA) and second-generation continuous axial flow LVADs (HeartMate II) were used. The mean duration of LVAD support was 143±41 days. LVAD support resulted in significant reductions in LV echocardiographic dimensions, improvement in EF, and normalization of several hemodynamic measures as summarized in Table 2.

### Myocyte Size and Contractility Parameters

Significant differences were noted for all myocyte fragment dimensions as summarized in Table 3. Myocytes obtained from patients before-LVAD implantation were significantly larger than myocytes from patients after-LVAD implantation. However, the myocytes from patients after-LVAD implantation were still larger than those obtained from nonfailing hearts. Fmax doubled with LVAD support (3.6±0.9 versus 7.3±1.8 mN/mm², P<0.001) but was still less than half of nonfailing hearts (7.3±1.8 versus 17.6±2.1 mN/mm², P<0.001) (Table 4). The pCa50 was unchanged in all groups, and the Hill coefficient was reduced in both LVAD groups relative to controls.

### Sarcomeric Proteins and Phosphorylation Levels

Total TnI phosphorylation increased with LVAD support (12.8±4.1 versus 21.6±9.4 OD units, P=0.030) (Figure 3 and Table 5). Although no significant differences were noted between after-LVAD implantation failing and nonfailing hearts. Statistical significance was defined as a 2-tailed P<0.05. The R version 2.9.1 (Vienna, Austria) statistical program was used for all analyses.
in the serine 22 and 23 site-specific phosphorylation of TnI, serine 43 site-specific phosphorylation of TnI decreased after LVAD implantation (7.5±2.2 versus 5.2±1.9 OD units, \( P=0.044 \)) (Figure 4). Total phosphorylation levels of MyBPC were higher in both failing groups compared with the nonfailing group (Table 5). Phosphorylation levels of TnT, tropomyosin, and MLC-2 were not significantly different before and after LVAD implantation (Figure 3 and Table 5).

Before and after LVAD implantation, there were no changes noted on 2D SDS-PAGE in the percent phosphorylation of MLC-2 (43±3% versus 41±2%, \( P=0.341 \)) and in the percent atrial isoform of MLC-1 (23±5% versus 21±7%, \( P=0.711 \)). Furthermore, no differences were noted in myosin heavy chain isoforms before and after LVAD implantation, with only the \( \beta \)-isoform being expressed in both groups of patients (Figure 5). Finally, there was no evidence of altered TnT isoform expression in the before-LVAD, after-LVAD, and nonfailing groups. Total TnT content was unchanged among these groups.

**Table 4. Mean Contractile Parameters Among Control Patients and Patients With Nonischemic Cardiomyopathy Before and After LVAD Implantation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>( P^* )</th>
<th>Nonfailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmax, mN/mm(^2)</td>
<td>3.6±0.9</td>
<td>7.3±1.8</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>pCa50</td>
<td>5.89±0.08</td>
<td>6.03±0.28</td>
<td>0.257</td>
<td>5.85±0.14</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td>1.64±0.28</td>
<td>1.55±0.45</td>
<td>0.579</td>
<td>2.30±0.81‡</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Fmax indicates the maximum calcium-saturated developed force normalized to the cross-sectional area of the myocytes; Hill coefficient, the slope of the calcium-force relation and is an index of myofilament cooperative activation; pCa50, the calcium concentration at which the force is half maximal and represents a measure of myofilament calcium sensitivity. Other abbreviation as in Table 1. 

\( ^* \) \( P<0.001 \) for comparison between before-LVAD and after-LVAD implantation.

\( ^† \) \( P<0.05 \) for comparisons of Hill coefficient between before-LVAD and nonfailing heart samples and after-LVAD and nonfailing heart samples.

**Table 5. Phosphorylation Levels of Sarcomeric Proteins in Patients With Nonischemic Cardiomyopathy Before and After LVAD Implantation**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>( P^* )</th>
<th>Nonfailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin binding protein C</td>
<td>6.1±1.2</td>
<td>6.7±1.0</td>
<td>0.328</td>
<td>4.6±0.6‡</td>
</tr>
<tr>
<td>Troponin T</td>
<td>19.2±3.0</td>
<td>18.0±3.8</td>
<td>0.562</td>
<td>20.9±7.0</td>
</tr>
<tr>
<td>Troponin I</td>
<td>12.8±4.1</td>
<td>21.6±9.4</td>
<td>0.030</td>
<td>13.2±1.9</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>7.9±2.6</td>
<td>8.5±2.9</td>
<td>0.431</td>
<td>8.0±1.9</td>
</tr>
<tr>
<td>Regulatory myosin light chain</td>
<td>0.5±0.2</td>
<td>0.7±0.3</td>
<td>0.109</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Serine 22 and 23 phosphorylated troponin I</td>
<td>3.7±2.3</td>
<td>3.4±1.5</td>
<td>0.747</td>
<td>2.8±0.6</td>
</tr>
<tr>
<td>Serine 43 phosphorylated troponin I</td>
<td>7.5±2.2</td>
<td>5.2±1.9</td>
<td>0.044</td>
<td>12.0±1.0‡</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Phosphorylation levels expressed in arbitrary optical density units. Abbreviation as in Table 1.

\( ^* \) \( P<0.001 \) for comparison between before-LVAD and after-LVAD implantation.

\( ^† \) \( P<0.05 \) for comparison between before-LVAD and after-LVAD implantation.

\( ^‡ \) \( P<0.001 \) for comparisons of serine 43 phosphorylated troponin I between before-LVAD and nonfailing samples and after-LVAD and nonfailing samples.

**Discussion**

The main finding of the present study is that in isolated skinned myocytes, there is a marked reduction in maximum developed force in patients with end-stage nonischemic cardiomyopathy compared to nonfailing heart donors. This marked reduction in force is only partially improved with LVAD support. Furthermore, there is strong evidence that structural changes accompany these changes in force at the organ level (reduction in LV size) as well as at the cellular level (reduction of all myocyte dimensions), and these correlate with functional changes at both the organ level (improvement in EF) and the cellular level (improvement in maximum force). Despite these significant changes in structural and mechanical parameters, unloading of the LV with

**Figure 3. Twelve-percent 1D SDS-PAGE images stained with a total phosphoprotein stain (A) (Pro-Q Diamond phosphoprotein gel stain, magnification ×1) and a total protein stain (B) (BioSafe Coomassie Blue, magnification ×1) show the increase in total TnI phosphorylation with ventricular assist device support and higher MyBPC phosphorylation in both failing heart samples and nonfailing heart samples. Sarcomeric protein phosphorylation levels were calculated by dividing the optical density of each sarcomeric protein on the Pro-Q Diamond gel by the optical density of the same protein on the Coomassie gel. MyBPC was normalized to MLC-1 as noted in the text. MLC-1 indicates myosin light chain 1; MLC-2, myosin light chain 2; MyBPC, myosin binding protein C; NF, nonfailing; TnI, tropinin I; Tm, tropomyosin; TnT, troponin T; VAD, ventricular assist device.**
LVAD support did not change the biochemical properties of the sarcomere other than a change in TnI phosphorylation.

Our findings confirm that contractile dysfunction in human HF at least in part resides at the level of the cardiac myofilament. This is independent of cell loss or changes in the cardiac interstitium and argues that therapies to treat HF need to acknowledge sarcomeric function. To place these changes in context, a prior study using a similar experimental protocol in human samples reported ∼30% reductions in myocyte Fmax in patients with diabetes with preserved EF compared with control patients (14.6±1.7 versus 20.6±3.7 mN/mm², P<0.05). The Fmax of the failing groups before and after LVAD implantation in the current study are more dramatic (∼60%) than that seen in this prior report, which is consistent with the severe reductions seen in contractile function at the organ level as assessed by EF.

There are a number of cellular and molecular changes in the heart that occur with LVAD support, and these encompass the most basic aspects of genetic regulation and involve a complex, interconnected cascade of changes. Although there have been 2 prior studies that assessed changes in myocyte contractility with LVAD, the present study is the first to directly measure isometric force of skinned myocytes from paired samples before and after LVAD support. The 2 prior studies (1 of which used paired pre- and post-LVAD samples) involved patients with both ischemic and nonischemic/idiopathic cardiomyopathies. Using in vitro motility and unloaded cell shortening assays, the authors reported improvements in contractility in myocytes obtained from patients with HF versus patients with HF supported by LVAD. The present assessment of myocyte contractility also differs from the 2 prior reports in that we limited our analysis to only those patients with nonischemic cardiomyopathy (a global pathological process that involves the entire LV) as opposed to ischemic cardiomyopathy (a regional pathological process based on areas of infarct/ischemia from coronary artery disease). The prior reports included data from patients with ischemic cardiomyopathy; thus, depending on the regions of LV infarction, the before-LVAD tissue (from the LV apex) would be inherently different than the after-LVAD tissue, making comparisons difficult. The results of the present study confirm that among patients with nonischemic cardiomyopathy, improvement in contractility at the sarcomeric level is possible with LVAD support; however, normalization of forces to levels seen in nonfailing hearts does not occur with standard LVAD protocols. This finding is concordant with organ-level assessment of contractility where EF improved from 10.0% to 25.6% with LVAD support, which is still half that seen in nonfailing control hearts and may partly explain clinically why the majority of LVADs cannot be successfully explanted.

The cellular mechanisms by which contractility is depressed in these HF samples and why there is only partial reversal with mechanical unloading is not unequivocally answered in the current study, although evidence suggests both structural and biochemical mechanisms. The fact that the Hill coefficient, a marker of protein cooperativity, was reduced in both HF groups coupled with the changes in MyBPC phosphorylation suggests that functional interactions between actin and myosin might have been compromised in both HF groups. This hypothesis is supported by the cell morphology data suggesting that sarcomere cross-bridge (and perhaps lattice) spacing is tightly coupled with contractile performance both in the 2 HF groups and in the donor heart specimens, a finding that reflects previous studies demonstrating that contractile parameters in isolated muscle were influenced in a step-wise fashion by osmotic determinants that influenced lattice architecture.

The mechanism for the partial recovery of contractile function after LVAD support might partly reflect contractile protein phosphorylation changes. Total TnI phosphorylation was lower before LVAD support compared with after LVAD and nonfailing samples, which may be because of downregulation of β-adrenergic receptors in inotrope-dependent patients with end-stage HF. The increase in TnI phosphorylation after LVAD might reflect normalization of β-adrenergic receptor function, but there was an increase in TnI phosphorylation at serine 43 (a putative PKC site) in the before-LVAD samples that decreased after LVAD support. This finding is consistent with a substantial amount of literature that suggests that HF in both humans and experimental animals is associated with an increase in PKC activation and that PKC-dependent phosphorylation of TnI is associated with depressed myofilament force development. The finding also provides a plausible explanation about why phosphatase treatment of myofilament preparations from patients with HF...
improves contractile performance despite low levels of overall contractile protein phosphorylation. The fact that there was no change seen in TnI phosphorylation at the putative protein kinase A site serine 22 and 23 despite postulated changes in adrenergic receptor activity before and after LVAD implantation may reflect the somewhat promiscuous nature of this site that can be targeted by both protein kinase A and PKC, the former likely increasing after LVAD implantation and the later declining.

Not seen in the present study were changes in phosphorylation of other contractile proteins or isoform shifts before and after LVAD support. In particular, and in contrast to studies in experimental animals, we did not see a shift at the protein level in the α/β myosin heavy chain isoform ratio or in TnT isoform distribution. This finding is particularly important to note because mechanical unloading of the normal LV in experimental animals results in atrophy associated with a recapitulation of the fetal gene program, whereas in the current study, unloading was associated with a normalization of ventricular size without isoform changes.

Limitations
We acknowledge a number of limitations to this study. The overall number of patients in the analysis is small, and all were from a single-center LVAD program. There were no differences in the results between patients supported with pulsatile-flow HeartMate XVE pumps versus continuous-flow HeartMate II pumps, although our small numbers preclude accurate subgroup analysis to definitively test for differences in the type of mechanical unloading. In addition, the echocardiographic measures of LV size and EF during LVAD support were obtained from retrospective review and, thus, were obtained on the LVAD settings that were clinically indicated for the patient. Although these assessments partially reflect the influence of the LVAD, some component of native LV function also contributes because on-pump measures of LV size and EF often are used in the initial screening assessments to determine whether patients should have further testing to assess for recovery. Myocyte dimensions were measured from the isolated skinned myocyte fragments used for mechanical force measurements rather than a specific histological stain of intact tissue. Despite this different methodology, the cross-sectional areas obtained in the present study were very similar to those reported recently using such traditional histological techniques, and the overall trends are well in line with other published reports.

The nonfailing heart tissue was obtained from organ donors, and the majority of these patients were receiving adrenergic agents before and during harvest, so adrenergic-mediated pathways likely were activated in these patients. Furthermore, concomitant medical therapy during LVAD support, such as β-blockade, varied and may have influenced some of the sarcomeric mechanical properties as has been previously demonstrated. In addition, although we believe that the comparison between LVAD (before and after) and nonfailing donor hearts is illuminating, it is worth noting that the tissue procurement strategies were, by necessity, somewhat different in that the nonfailing and after-LVAD hearts were excised en bloc, whereas the before-LVAD specimens were taken from small tissue cores obtained from beating hearts. Finally, the before- and after-LVAD samples were obtained in the operating room after the patient was induced with general anesthesia and placed on cardiopulmonary bypass. These procedures may have affected the sarcomeric protein phosphorylation background. Despite these limitations, the current study remains, to our knowledge, the most comprehensive examination of the effects of LVAD support on the sarcomere and, thus, merits attention.

Conclusion
There is significant improvement in LV and myocyte size with LVAD support but only partial recovery of EF and myocyte contractility. The architectural changes of LVAD support are associated with a limited spectrum of biochemical changes, which suggest that both lattice spacing and PKC activation might contribute to contractile dysfunction. These data further suggest that additional interventions beyond mechanical unloading may be needed to alter biochemical remodeling of the sarcomere to further enhance myofilament and organ-level recovery.

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Disclosures
None.

References
Sustained recovery of the failing left ventricle (LV) during pressure-volume unloading with an LV assist device (LVAD) is rare and may be related to incomplete recovery of sarcomeric contractility. In this study, we evaluated contractility and biochemistry at the most contractile level of the heart: the sarcomere. Force development in muscle is the result of actin and myosin interactions and cross-bridge cycling, processes regulated by modifications of the sarcomeric contractile proteins. Sarcomeric contractility was assessed by measuring isometric forces on skinned LV myocytes from samples.

**Clinical Perspective**

Sustained recovery of the failing left ventricle (LV) during pressure-volume unloading with an LV assist device (LVAD) is rare and may be related to incomplete recovery of sarcomeric contractility. In this study, we evaluated contractility and biochemistry at the most contractile level of the heart: the sarcomere. Force development in muscle is the result of actin and myosin interactions and cross-bridge cycling, processes regulated by modifications of the sarcomeric contractile proteins. Sarcomeric contractility was assessed by measuring isometric forces on skinned LV myocytes from patients with nonischemic cardiomyopathy before and after LVAD placement. We found that contractile dysfunction at the level of the sarcomere was present in failing hearts and paralleled organ-level contractile dysfunction as assessed by ejection fraction. Furthermore, there were improvements in LV and myocyte size with partial recovery of sarcomeric force after LVAD placement, but LVAD-supported myocyte forces were still half of that seen in nonfailing hearts. The persistence of sarcomeric contractile dysfunction may be one of the reasons most LVADs cannot be explanted in clinical practice. In assessing for biochemical alterations of sarcomeric proteins after LVAD implantation, there were changes in troponin-I phosphorylation that may account for some of the improvement in sarcomeric force, but the other sarcomeric contractile proteins revealed minimal biochemical changes, suggesting that other interventions (in addition to mechanical unloading with an LVAD) may be needed to optimize troponin-I phosphorylation, modify other sarcomeric protein biochemistry, or both to further enhance sarcomeric and organ-level recovery.
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