Clinical, Molecular, and Genomic Changes in Response to a Left Ventricular Assist Device

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The use of left ventricular assist devices in treating patients with end-stage heart failure has increased significantly in recent years, both as a bridge to transplantation and as destination therapy in those who are ineligible for cardiac transplantation. This increase is based largely on the results of several recently completed clinical trials with the new second-generation continuous-flow devices that showed significant improvements in survival, functional capacity, and quality of life. Additional information on the use of the first- and second-generation left ventricular assist devices has come from a recently released report spanning the years 2006 to 2009, from the Interagency Registry for Mechanically Assisted Circulatory Support, a National Heart, Lung, and Blood Institute–sponsored collaboration between the U.S. Food and Drug Administration, the Centers for Medicare and Medicaid Services, and the scientific community. The authors review the latest clinical trials and data from the registry, with tight integration of the landmark molecular, cellular, and genomic research that accompanies the reverse remodeling of the human heart in response to a left ventricular assist device and functional recovery that has been reported in a subset of these patients. (J Am Coll Cardiol 2011;57:641–52) © 2011 by the American College of Cardiology Foundation

Clinical Perspective

Currently, the average mortality from the time of diagnosis of heart failure, regardless of whether there is preserved or reduced systolic function, is >60% at 5 years, and this is much higher when patients are in the most advanced stages (1). Patients typically become progressively less responsive to medical therapy and are left with limited options for meaningful improvement in survival or functional capacity and quality of life (2).

Cardiac transplantation is currently the most established treatment for refractory heart failure (1). However, this option is available to fewer than 2,300 patients per year, with a relatively fixed organ supply (1) and no substantive increase anticipated in the near future. The prevalence of heart failure increases with age and affects an estimated 10% of people over 70 years of age, which is the age that many transplantation programs use as a cutoff for accepting candidates. Importantly, the population over age 65 is expected to double in the next 20 years. As a result, the use of left ventricular assist devices (LVADs) in treating patients with end-stage heart failure will increase significantly in the coming years, both as a bridge to transplantation and as destination therapy in those who are ineligible for cardiac transplantation (3).
In 2009, the Centers for Medicare and Medicaid Services set a mandate that patients receiving ventricular assist devices be entered into a national database, the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) (3). INTERMACS is a National Heart, Lung, and Blood Institute funded collaboration between the National Heart, Lung, and Blood Institute, the U.S. Food and Drug Administration, the Centers for Medicare and Medicaid Services, and the mechanical circulatory support scientific community (3). This registry has shown that patients with LVADs alone have overall actuarial survival rates of 74% at 1 year and 55% at 2 years (3). The primary cause of death in the 1,092 primary LVAD patients in INTERMACS from June 2006 through March 2009 was cardiac failure, including right ventricular failure and ventricular tachycardia or ventricular fibrillation (3). The secondary cause of death was infection (3).

At the inception of INTERMACS, the HeartMate XVE pulsatile-flow pump (Thoratec Corporation, Pleasanton, California) was the only approved device for destination therapy in the U.S. In April 2008, the HeartMate II (Thoratec Corporation), a continuous-flow LVAD, received Food and Drug Administration approval as a bridge to transplantation based on the results of the clinical trial (4). Thus, the registry began tracking the evolution of continuous-flow technology in addition to the original pulsatile flow pumps (3). A recent randomized trial showed that patients receiving treatment with continuous-flow LVADs (HeartMate II) as destination therapy exhibited significant improvement in the probability of stroke-free survival and device failure at 2 years compared with those receiving pulsatile LVADs (5). Both continuous-flow and pulsatile devices significantly improved the quality of life and functional capacity (Thoratec HeartMate II Left Ventricular Assist System [LVAS] for Destination Therapy; NCT00121485) (5). However, the survival outcomes with the pulsatile device were nearly identical to those in patients enrolled nearly 10 years earlier in the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure for destination therapy patients, indicating little progress with that pump (6).

Clinical heart failure may present with changes referred to as remodeling that include eccentric dilation of the ventricular chamber, a reduction in contractile function, and increases in cardiac filling pressures and wall stress. These clinical phenotypic changes of heart failure are paralleled by significant histologic, cellular, and molecular changes in most structural and functional components of the myocyte, including alterations in myocyte geometry and size, progressive interstitial fibrosis, up-regulation of cytokines and inflammation, and changes in myosin isoforms, as well as reductions in myocardial energetics, beta-receptor density, and calcium-handling proteins; this is otherwise described as recapitulation of the fetal gene program. These cellular and molecular changes may arise from a response to changes in volume, stretch, pressure, and inflammation that occur as a result of diet, additional environmental modifiers, and/or genetic mutations. A progressive body of evidence has shown a trend toward normalization of these perturbations, or reverse remodeling, with LVAD support. However, response to an LVAD is unique to each patient, suggesting that underlying factors are critical in the response to therapy.

### Functional Improvements in Myocytes in Response to LVAD Unloading, Linked to Gene Expression Changes

Translational research from human heart tissue before and after support with an LVAD has shown that myocytes in end-stage failing hearts have the capacity to remodel. These changes are accompanied by alterations in gene expression. We summarize findings in the field that have led to important steps forward in our understanding of the molecular changes and contractile properties of cardiac myocytes before and after unloading with an LVAD.

There is evidence of early mechanical improvements in failing hearts treated with LVADs (7–15). These early findings were important in showing that the contractile performance of myocytes was partially recovered in response to an LVAD. Myocyte contractile performance was significantly greater in isolated myocytes from failing human hearts after LVAD support compared with failing human hearts without LVAD support in patients bridged to transplantation (Table 1) (8). Improvements in the magnitude of shortening were seen in isolated myocytes after LVAD support in response to beta-adrenergic agonists (Table 2) (8). Similar to work in isolated myocytes, developed tension in response to beta-adrenergic stimulation in isolated trabecular muscle preparations was also improved (13) (Fig. 1). Basal relaxation was also improved in myo-

### Table 1: Contractile Characteristics Are Improved in Myocytes After Support With an LVAD

<table>
<thead>
<tr>
<th>Contractile Characteristic</th>
<th>HF Monocytes (n = 57)</th>
<th>HF LVAD Monocytes (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnitude of shortening (% RCL)</td>
<td>6.9 ± 0.5*</td>
<td>9.6 ± 0.7*</td>
</tr>
<tr>
<td>Time to peak contraction (s)</td>
<td>0.75 ± 0.04</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Time to 50% relaxation (s)</td>
<td>1.45 ± 0.11</td>
<td>0.55 ± 0.02</td>
</tr>
</tbody>
</table>

*Significant difference between the HF and the HF LVAD monocytes at p < 0.05. Adapted from Díaz et al. (8).

HF = heart failure; LVAD = left ventricular assist device; RCL = resting cell length.
cytes after LVAD support (Table 2) (8). However, relaxation in response to isoproterenol was not improved (Table 2) (8). This was confirmed in isolated trabecular preparations in a separate study (Fig. 1) (13). Figure 1 also shows the significant magnitude in maximal rates of developed rise and fall in tension in response to the beta agonist isoproterenol. Significant improvements are seen in both rise and fall in tension with LVADs compared with failing hearts. In sum, these findings suggest that LVAD support improves developed tension and relaxation rates at rest and also improves developed tension in response to beta agonists. However, LVAD support did not improve relaxation rates after stimulation with beta agonists.

**Beta-adrenergic signaling.** Improvements in developed tension with LVADs have been shown to be accompanied by increased beta-adrenergic receptor density (13,16). Early studies showed that beta-adrenergic density was improved with LVAD therapy in 17 patients compared with 8 nonfailing patients (13). A recent study confirmed these findings (15) (Fig. 2). Whether this improvement in beta-adrenergic density translates to intermediate biochemical improvements in the beta-adrenergic signaling pathway that are associated with improved developed tension are not known. It is possible that other molecules in the signaling pathway may still be “desensitized” that control the “brake” on this important pathway that regulates myocyte contractility and remodeling.

**Beta-adrenergic signaling in recovery patients.** More recent work assessed beta-adrenergic signaling pathways in an unbiased analysis using a gene chip platform in 6 paired heart samples from patients who achieved sufficient myocardial recovery to allow explantation of the devices (17,18).

### Table 2 Isoproterenol Response on Contractile Parameters

<table>
<thead>
<tr>
<th>Contractile Parameter</th>
<th>Disease Group</th>
<th>Baseline</th>
<th>Isoproterenol</th>
<th>Delta Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnitude of shortening (% RCL)</td>
<td>HF</td>
<td>6.43 ± 0.73</td>
<td>8.92 ± 0.78*</td>
<td>2.49 ± 0.58†</td>
</tr>
<tr>
<td></td>
<td>HF-VAD</td>
<td>7.63 ± 0.88</td>
<td>12.56 ± 1.01‡</td>
<td>4.93 ± 1.42</td>
</tr>
<tr>
<td>Time to 50% relaxation (s)</td>
<td>HF</td>
<td>1.28 ± 0.19</td>
<td>0.95 ± 0.16</td>
<td>0.32 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>HF-VAD</td>
<td>0.70 ± 0.07‡</td>
<td>0.53 ± 0.03</td>
<td>0.17 ± 0.08</td>
</tr>
</tbody>
</table>

*Significant difference (p < 0.0125) between baseline and isoproterenol (paired t test). †Significant difference (p < 0.05) between HF and HF-VAD myocytes on the delta scores (t test for independent samples). ‡Significant difference (p < 0.0125) between HF and HF-VAD myocytes (t test for independent samples). Reproduced, with permission, from Dipla et al. (8). HF = heart failure; RCL = resting cell length; VAD = ventricular assist device.

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**Figure 1 Response of Muscles to 1 μmol/l Isoproterenol**

Muscles from nonfailing hearts (HF), failing hearts without left ventricular assist device (LVAD) support, and failing hearts with LVAD support. (A) Time to peak tension (TPT); (B) time to half relaxation (THR); (C) maximal rate of tension rise (+dT/dt); (D) maximal rate of tension fall (−dT/dt). Data are shown as change from baseline contraction (mean ± SEM). This study shows no change in TPT (A) or THR (B) between NF, failing hearts, and failing hearts with LVAD support. Significant decreases in +dT/dt (C) and −dT/dt (D) were seen in the failing hearts that were improved with LVAD support. n = number of muscles/number of hearts. *p < 0.05 versus NF. Adapted from Ogletree-Hughes et al. (13).
A novel combination therapy consisting of an LVAD combined with pharmacologic therapy including the selective beta-2 agonist clenbuterol has shown promise in restoring ventricular function in patients with heart failure (17). The aim of this study was to identify common genes and signaling pathways whose expression was associated with reversal of heart failure and restoration of ventricular function. Microarray analysis was performed on 6 paired human heart samples harvested at the time of LVAD implantation and at the time of LVAD explantation for recovery of ventricular function. Follow-up data showed that the improvements in ventricular function were maintained for more than 5 years after explantation. This dataset represents the first description of signaling pathways associated with the functional recovery of end-stage human heart failure and the identification of new targets in the human heart that are modified by this combination therapy. Analyzing the gene expression profiles from these patients at the time of LVAD explantation compared with LVAD implantation (heart failure) resulted in the identification of pathways significantly enriched with genes whose expression was statistically different between heart failure and recovery. Instead of a gene-by-gene approach, this study used a network analysis, with the goal of identifying pathways that are altered in the process of recovery from heart failure (18). Put simply, gene lists are imported into the Ingenuity database (Ingenuity Systems, Inc., Redwood City, California), and the program subsequently overlays these genes into several described networks to identify the best overall fit. As seen in Figure 3, significant changes in genes in the beta-adrenergic signaling pathway in recovered hearts included Rap guanine nucleotide exchange factor 4 (exchange protein directly activated by cyclic adenosine monophosphate 2 [Epac2], down-regulated at explantation 2.2-fold), protein kinase, regulatory subunit, type I alpha (down-regulated at explantation 1.5-fold), phosphodiesterase 1A (down-regulated at explantation 1.5-fold), phosphodiesterase 3B (down-regulated 1.5-fold), and calcineurin A (up-regulated 1.4-fold) (18). The down-regulation in Epac2 was unique to the recovered group and confirmed in a replication cohort, suggesting that this target may be important for recovery. (This target is discussed again in the following section on calcium handling.) It is important to keep in mind that expression changes in messenger ribonucleic acid (mRNA) need to translate to functional changes in biochemical signaling to be causally related to functional recovery. Studies of this nature will be critical for moving the field forward.

**Calcium handling.** Impaired myocardial relaxation is a physiologic characteristic of heart failure. The reported improvements in basal relaxation rate with LVADs have not been definitively linked to changes in calcium handling. Elegant functional experiments have been carried out to assess calcium transients and expression of calcium handling proteins (7,19). In a study by Chaudhary et al. (7), LVAD-supported failing myocytes exhibited faster decay in both early and late stages of the \([\text{Ca}^{2+}]\) transient compared with myocytes from failing hearts not supported with LVADs. These same findings were seen in patients who recovered. An increase in ribonucleic acid (RNA) expression of the \(\text{Na}^{+}\)–\(\text{Ca}^{2+}\) exchanger was identified in this study from recovered patients (19). In the study with nonrecovered patients, changes were not measured at the RNA level, and no changes were detected at the protein level of the \(\text{Na}^{+}\)–\(\text{Ca}^{2+}\) exchanger (7), thus not offering an explanation for the faster decay times in the \([\text{Ca}^{2+}]\) transients (20,21). A summary of calcium handling changes in response to an LVAD is shown in Figure 4.

**Calcium handling in recovery patients.** Expanding on these findings, recent work by Terracciano et al. (26) showed the largest improvements in action potential duration and sarcoplasmic reticulum calcium content in patients who achieved clinical recovery in response to LVADs and pharmacological therapy (Fig. 5) (Harefield Recovery Protocol Study). These findings help distinguish contractile and molecular differences in myocytes from hearts that achieve clinical recovery compared with those that achieve partial recovery (26). Another recent study by Ogletree et al. (27) demonstrated that the benefits of unloading on calcium handling and contractility are time dependent. Patients with shorter durations of support (<115 days) achieved significant increases in sarco/endoplasmic reticulum \(\text{Ca}^{2+}\)–adenosine triphosphatase expression levels and corresponding increases in developed tension (27). These improvements, however, reverted back to failing levels in the patients with longer durations of support (Fig. 6) (27). The reason these changes are not persistent is unknown, but it...
could be an indication of adverse remodeling signals with prolonged unloading.

A recent phase II clinical trial, the CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) study, is already testing sarco/ endoplasmic reticulum Ca2+/adenosine triphosphatase 2a gene therapy in patients with end-stage heart failure, with some initial indications of efficacy (28). A second potential target for drug development may be Epac2. Significant decreases in the calcium-regulating gene Epac2 were identified after LVAD support in 11 recovered hearts (Fig. 7) (18). This decrease in Epac2 was unique to recovered hearts, as the pattern was not found in nonrecovered hearts (Fig. 7) (18). Epac2 tethers cyclic adenosine monophosphate to mitogen-activated protein kinase, calcium-mediated signaling through nuclear factor of activated T cells, and metabolic signaling pathways. The roles of both Epac2 and exchange protein directly activated by cyclic adenosine monophosphate 1 in multiple cell types and tissues, including the heart, nerves, and pancreas, are just beginning to be understood (20,21).

**Cytoskeletal proteins and integrins in recovery patients.**

Myocardial recovery has also been associated with a specific pattern of changes in sarcomeric, nonsarcomeric, and membrane-associated proteins (29). These changes are high-

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**Figure 3 Identification of Genes in the cAMP-Mediated Signaling Pathway Whose Expression Was Altered in Association With Recovery**

**Shaded symbols** represent genes whose expression was significantly altered in explanted versus implanted samples (18). cAMP = cyclic adenosine monophosphate.
Affymetrix (Santa Clara, California) microarray analysis was performed on paired samples (before and after LVAD implantation) and analyzed with reference to sarcomeric and nonsarcomeric cytoskeletal proteins (29). In the recovery group, of the nonsarcomeric proteins, lamin A/C and spectrin increased from before to after LVAD implantation (29). The sarcomeric proteins beta-actin, alpha-tropomyosin, alpha-1-actinin, and alpha-filamin A increased in the recovered group, while troponin T3 and alpha-2-actinin decreased at explantation (29). Vinculin decreased after LVAD support in the recovered group but increased in the nonrecovered group (29). Further work is needed in this area to define if these changes that occur in expression of cytoskeletal proteins and are uniquely associated with recovery are integral to promoting improvements in contractility that are necessary for recovery.

Integrins are bidirectional signaling molecules and play a role in mechanotransduction by mediating mechanical (stretch) signals from the extracellular matrix, via protein kinase cascades that provoke changes in gene expression, including those involved in the hypertrophic response. Data suggest that the integrin pathway may play a key role at the molecular and cellular levels in processes of reverse remodeling and subsequent functional recovery occurring in these patients (29). Work in a pre-clinical rat model has shown similar findings (31). Online Figure 1 summarizes the changes in the integrin signaling pathway along with the cytoskeletal proteins that occurred in the recovery cohort (29).
integrin signaling, several new targets were identified in an unbiased approach, including those that regulate metabolism. Arginine:glycine amidinotransferase (AGAT), a rate-limiting enzyme in the creatine synthesis pathway, was significantly down-regulated after unloading in the recovered hearts, returning to normal levels in direct contrast to the up-regulation of AGAT in patients with heart failure compared with donor hearts (18,32). These changes in AGAT mRNA levels suggest a response to heart failure that involves elevated local creatine synthesis (18,32). The mechanisms leading to induced AGAT expression are unknown but may be a response to the depletion of the local creatine pool, which we and others have shown to be a feature of heart failure.

Insulin growth factor (IGF)-I mRNA was elevated at the time of LVAD explantation in the patients who received pharmacologic therapy, which included clenbuterol (33). There were 2 groups distinguishable, those with low IGF-I mRNA at implantation who showed significant increase during recovery and those with high IGF-I mRNA at implantation who remained high (33). IGF-I levels correlated with stromal cell–derived factor mRNA measured both in LVAD patients and in a wider cohort of patients with heart failure (33). Microarray analysis of implantation and explantation samples of recovery patients further revealed elevated IGF-II and IGF binding protein-6. IGF-I may act to limit atrophy and apoptosis during reverse remodeling and to promote repair and regeneration in concert with stromal cell–derived factor. It may have important effects on fibroblasts (33). A previous analysis in 19 paired patient samples before and after LVAD support with the HeartMate XVE device in a cohort of patients who were transplanted (did not achieve full recovery) revealed expression changes in the Forkhead transcription factor family and genes governing the angiotensin/insulin signaling axis (34). This analysis also identified a significant correlation in expression between Forkhead box 03A and angiotensin II type 1 receptor (34). More
work will need to be done to determine the role and integration of IGF-I/Forkhead and angiotensin II in remodeling and recovery and localize these signals to myocytes and/or fibroblasts. In a later section, we discuss maladaptive fibrotic remodeling in response to LVADs and the potential role of angiotensin-converting enzyme (ACE) inhibition in alleviating ventricular stiffness and fibrosis. A recent study by Bhavsar et al. (35) showed that IGF-I is released preferentially from fibroblasts over cardiac myocytes. Thus, the interaction between angiotensin II and IGF-I both mechanistically and clinically is still not well understood.

**Micro-ribonucleic acid (miRNA).** miRNAs constitute one of the more abundant classes of molecules that

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**Figure 7** Epac2 mRNA Levels

(A) Exchange protein directly activated by cyclic adenosine monophosphate 2 (Epac2) messenger ribonucleic acid (mRNA) levels were significantly reduced in recovered hearts after ventricular assist device support \( (n = 11, \ p < 0.01) \). **(B)** No significant differences were seen in nonrecovered hearts. \( (n = 5, \ p = NS) \) (18).

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**Figure 8** Changes in the Cytoskeletal Pathway That Occurred in Recovered Patients Only

Reproduced, with permission, from Birks et al. (29), adapted from Towbin and Bowles (30).
regulate genes in animals. MiRNAs are small, endogenous, noncoding RNAs (36). Many of these miRNAs have been shown to inhibit post-transcriptional processing (36,37). Recent work by Matkovich et al. (38) suggests that adding miRNA profiling to mRNA profiling enhances the ability of mRNA profiles to categorize the clinical status of heart failure before and after biomechanical unloading. The results of this study by Matkovich et al. (38) confirmed 3 earlier identified miRNAs associated with heart failure (miR-24, miR-125b, and miR-195) (38,39). In addition, Matkovich et al. (38) extended earlier work in mouse models (39,40) showing that miR-21, miR-23a, and miR-199a-3p were also regulated in human heart failure. One of the most exciting aspects of the study by Matkovich et al. (38) was the reversibility of the miRNAs with LVAD support. Table 3 lists miRNAs that normalize after LVAD support. It is not yet known which miRNA expression changes are associated with clinical end points that correlate with recovery.

**Natriuretic peptides and chromogranin A are normalized by LVAD support.** Despite divergent etiologies, heart failure is characterized by the activation of neurohormonal systems: catecholamines, natriuretic peptides, and components of the renin-angiotensin axis are increased and have been found to have pathophysiological and prognostic implications. Some of these molecules exert local paracrine activation, but their plasma levels have been demonstrated to be markers of clinical outcomes. Increased cardiac atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) expression in patients with heart failure is associated with increased expression of the natriuretic peptides metabolizing natriuretic peptide receptor C receptors and blunted responsiveness of guanylate cyclase A to ANP by reduced cyclic guanosine monophosphate synthesis.

Unloading the failing heart with an LVAD is associated with a decrease in natriuretic peptides and re-establishment of the local responsiveness of guanylate cyclase A to ANP (41). Cardiac expression of ANP, BNP, and natriuretic peptide receptor C mRNA also correlated significantly with cardiomyocyte diameters. In contrast to the latter, the levels of the natriuretic peptides are fully reversed to the level of the controls, indicating that their expression is partly independent of cardiac hypertrophy and regulated by heart failure–associated factors such as cardiomyocyte stretch (41). Evidence that unloading may be sufficient for a decrease in natriuretic peptides (and that recovery is not necessary) comes from pre-clinical data, in which natriuretic peptides are decreased in heterotopic transplants of failing rat hearts (42,43).

Chromogranin A is an acidic calcium-binding protein and is the major soluble constituent in secretory vesicles throughout the neuroendocrine system. Chromogranin A was found to be significantly up-regulated during heart failure and is co-stored with catecholamines and natriuretic peptides. BNP and chromogranin A are co-stored in the myocardium of patients with dilated cardiomyopathy, whereas this colocalization was not found in healthy controls (44). Wohlschlager et al. (45) investigated the expression of natriuretic peptides and chromogranin A by immunohistochemistry and morphometric quantification before and after LVAD implantation. In a different set of patients, chromogranin A was evaluated in the plasma (45). We demonstrated that in line with ANP and BNP, chromogranin A is significantly increased in patients with congestive heart failure compared with healthy controls and decreased by ventricular support (45). Moreover, sarcoplasmic colocalization of BNP and chromogranin A is diminished after unloading. However, because of its low expression, the negative regulation of chromogranin A is not reflected by plasma levels, so chromogranin A does not appear to be an appropriate biomarker for the monitoring of “reverse cardiac remodeling” after unloading (45) (Online Fig. 2).

### Summary

In summary, left ventricular unloading in patients with end-stage heart failure on LVAD support has been shown to improve myocardial structure and function, including improving beta-adrenergic responsiveness and myocyte contractility. The phenotypic changes in the heart associated
with LVAD support may arise from changes in mRNA, miRNA, or post-translational mechanisms such as ubiquitination or phosphorylation events, as discussed by Margulies (11). Several working groups, including our own, have defined changes in gene expression that occur with LVAD support that may be important in improving beta-adrenergic responsiveness and calcium handling, including partial restoration of beta-receptor density and altered regulation of the genes in G-protein-coupled signaling and calcium handling. A gene expression profile analysis using gene chips with 199 myocardial samples from failing, LVAD-supported and nonfailing human hearts demonstrated that the majority of transcriptional changes accompanying morphological and functional alterations in the heart were modest (46). Changes in miRNA expression in a recent study were more robust (38). Protein expression has been mainly restricted to calcium handling, beta-adrenergic, and extracellular matrix pathways, and much work is yet to be done. Pairing pre-clinical work in model organisms in which these targets (genes or miRNA) are overexpressed or deleted and their function is understood in the context of heart failure will be helpful. Overexpressing genes in which phosphorylation sites (or other site of post-translational modification) are mutated will aid in the identification of how post-translational mechanisms regulate myocardial function and remodeling. However, rodent models do not recapitulate human heart failure. Secondly, heterotopic transplantation has limitations in modeling assist devices. The next generation of studies will take advantage of state-of-the-art technology to yield more information from human myocytes from core biopsies and define how changes in RNA and protein may alter morphology and contractility.

A recent histological study by Drakos et al. (47) found that unloading with LVADs in 15 patients receiving the HeartMate I device (pulsatile) resulted in a 33% increase in microvascular density, although the total luminal area was decreased, so the functional patency of these vessels is unclear.

Some remodeling responses to mechanical unloading may be maladaptive. The most prominent advancement of this hypothesis is the concept of myocardial atrophy and reversal, which is being investigated in the Harefield Recovery Protocol Studies described earlier. Mechanisms regulating cardiac atrophy are unknown, although it is thought that the ubiquitin-proteasome degradation pathway that is active in skeletal muscle and the coronary vascular bed plays a role and may represent an overcompensation because of the lack of opposing hypertrophic stimuli after chronic unloading (48,49). Another concept in maladaptive remodeling is that of fibrosis and extracellular matrix turnover. One study by Klotz et al. (50) of collagen content and subtypes after LVAD support found a significant increase in total and cross-linked collagen in the myocardium compared with nonfailing and medically managed patients with heart failure, which correlated with increased left ventricular stiffness. Interestingly, the majority of LVAD patients after implantation were not on ACE inhibitors, which have been demonstrated to improve fibrosis and remodeling (51,52). A subsequent retrospective cohort study by the same group, comparing LVAD patients who did and did not receive ACE inhibitor therapy after implantation, demonstrated a significant decrease in collagen content and myocardial stiffness in the cohort with LVADs plus ACE inhibitors (16). At the molecular level, quantitation of matrix metalloproteinases and their inhibitors demonstrated near normalization of the ratio of matrix metalloproteinase 1 to tissue inhibitor of metalloproteinase 1 in LVAD patients receiving ACE inhibitors, suggesting a mechanism for the improvement collagen turnover. These findings support the hypothesis that maximizing optimal medical management after ventricular unloading with LVADs may promote myocardial recovery. In sum, the modest gene expression changes are sufficient to change vascular and fibrotic properties in the heart. However, a more thorough understanding of the unique therapies and underlying genetic and environmental properties will further aid in our understanding of the biological pathways through which unloading with LVADs leads to remodeling and recovery. A better understanding of the biology may allow us to alter the engineering properties of the devices and/or patient selection criteria (see the following discussion) to improve outcomes.

**Study limitations.** The LVAD population presents a unique and valuable opportunity to obtain myocardial tissue of patients with end-stage heart failure at the time of implantation, and often at the time of heart and/or LVAD explantation, after a period of unloading. There are, however, some recognized limitations of carrying out molecular studies on the LVAD patient population. Study sizes tend to be small, which is a particular challenge when performing large-scale transcriptome analysis, although this limitation is being overcome by multicenter collaborations and the overall increase in the use of LVADs. Tissue procurement must also be carefully planned, and the tissue received from the apical transmural tissue core may be fibrotic and not representative of the whole myocardium. In addition, the clinical presentation, duration of support, and medical management are uncontrollable variables, creating a heterogeneous patient population. Animal models have been created in an attempt to study ventricular unloading in a more controlled fashion. These include a thoracic inferior vena cava constriction model described by Lisy et al. (53,54), which demonstrates myocardial atrophy with chronic unloading, with significant up-regulation of the renin-angiotensin-aldosterone system axis and decrease in ANP and BNP expression. Although the myocardial atrophy findings have important implications for research in human LVAD patients, this model creates a biventricular low cardiac output state from a presumably normal baseline and thus is not analogous to the physiologic effect of implanting an LVAD. Another established animal model is that of heterotopic cardiac transplantation, described by
multiple groups, including Tevaearai et al. (55). In this model, heart failure is induced in a “donor” cohort of rabbits, after which the hearts are transplanted heterotopically by anastomosing the aorta and pulmonary trunk to the recipient rabbits’ carotid artery and internal jugular vein, respectively. The heart is perfused only through the coronary circulation, through the coronary sinus, and returned to the recipient via the right heart, creating a completely unloaded left ventricle (55). Although this model is also intriguing, it also involves a transplantation, removing the organ from the systemic physiology of the heart failure “donor,” and also subjecting the heart to the immunological and biochemical milieu associated with transplantation. Given the limitations of animal models, it is critical to continue advancing translational research in the LVAD patient population to apply the understanding of unloading responses in heart failure to the development of clinical predictors and medical therapy for these patients.

**Final clinical perspective and future directions.** Recent clinical trials have shown that both pulsatile and continuous-flow LVADs significantly improve the quality of life and functional capacity of patients with heart failure (4,5). Translational research in the area of LVAD support has provided new information on signaling pathways. Tissue procurement, deoxyribonucleic acid sequencing, RNA sequencing, and epigenetic tools will help in our further understanding of the underlying signals regulating the reverse remodeling and recovery potential of the failing human heart. Investigation in the LVAD patient population has been limited by the fact that there is no consensus on medical management toward, and clinical monitoring of, myocardial recovery. For instance, the findings correlating ACE inhibitor use and favorable decrease in myocardial stiffness and collagen subtypes suggest a greater role for continuing optimal medical management after LVAD placement. The elucidation of myocardial atrophy pathways may also illuminate the concept of optimizing or weaning unloading after LVAD placement to minimize atrophy and promote recovery. Our current understanding of the role of extracellular matrix in heart failure and the ability of the fibroblast to remodel in response to LVADs represents a new focus area. Further elucidating these pathways will allow for the discovery of novel therapeutic strategies for patients in earlier stages of heart failure. Identification of the biomarkers and factors that will aid in risk stratification for patients with LVAD therapy will help move this field forward and enhance patient care.

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**REFERENCES**


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APPENDIX

For supplementary figures and their legends, please see the online version of this article.