Abstract—Heart failure is associated with remodeling that consists of adverse cellular, structural, and functional changes in the myocardium. Until recently, this was thought to be unidirectional, progressive, and irreversible. However, irreversibility has been shown to be incorrect because complete or partial reversal can occur that can be marked after myocardial unloading with a left ventricular assist device (LVAD). Patients with chronic advanced heart failure can show near-normalization of nearly all structural abnormalities of the myocardium or reverse remodeling after LVAD support. However, reverse remodeling does not always equate with clinical recovery. The molecular changes occurring after LVAD support are reviewed, both those demonstrated with LVAD unloading alone in patients bridged to transplantation and those occurring in the myocardium of patients who have recovered enough myocardial function to have the device removed. Reverse remodeling may be attributable to a reversal of the pathological mechanisms that occur in remodeling or the generation of new pathways. A reduction in cell size occurs after LVAD unloading, which does not necessarily correlate with improved cardiac function. However, some of the changes in both the cardiac myocyte and the matrix after LVAD support are specific to myocardial recovery. In the myocyte, increases in the cytoskeletal proteins and improvements in the Ca\(^{2+}\) handling pathway seem to be specifically associated with myocardial recovery. Changes in the matrix are complex, but excessive scarring appears to limit the ability for recovery, and the degree of fibrosis in the myocardium at the time of implantation may predict the ability to recover. (Circ Res. 2013;113:777-791.)

Key Words: heart failure ■ left ventricular assist device ■ remodeling ■ reverse remodeling
Heart failure (HF) is associated with a process known as remodeling that consists of adverse cellular, structural, and functional changes in the myocardium. Clinically, this results in progressive enlargement of the ventricle, reduction in contractility, and increase in intracardiac pressures. As a consequence of the reduced contractility, decreased cardiac output occurs, resulting in the syndrome of HF. Remodeling is the central feature that occurs in the progression of HF; it often results after an initial insult and is also associated with an increase in the left ventricular (LV) muscle mass, an increase in ventricular volume, and a change in shape of the ventricle. At the cellular level, the histological changes include myocyte hypertrophy, myocyte slippage, interstitial growth, myocyte lengthening, and apoptosis. The initiators of the remodeling process, however, remain incompletely understood.

Until recently, this remodeling process was thought to be unidirectional and progressive. However, the concept of reversibility has been shown to be incorrect because complete or partial reversal of remodeling has been documented. This can occur at least partially in response to pharmacological agents alone but can also be marked in response to unloading of the heart with LV assist devices (LVADs). LVADs provide near-total unloading of the ventricle, and patients with chronic advanced HF who are supported with an LVAD can show near-normalization of nearly all structural abnormalities of the myocardium or reverse remodeling. However, despite this reversal of structural changes in most patients after a period of support, only a small percentage of patients have shown significant associated improvement in their myocardial function. This improvement in myocardial function can be significant enough to allow the device to be removed (termed myocardial recovery).

Thus, molecular reverse remodeling does not always equate with clinical recovery. We review the molecular changes that occur after LVAD support. The studies comprise 2 categories: (1) those that have examined changes in patients between the time of LVAD implantation and transplantation, which are studies of the effect of LVAD unloading alone and are not necessarily associated with any improvement in cardiac function; and (2) those that have studied molecular changes in patients between LVAD implantation and explantation, which are therefore associated with significant improvement in myocardial function. Of the latter group, patients have experienced sufficient improvement in their myocardial function to have the device removed (remission of HF), and many return to normal myocardial function with sustained recovery of their cardiac function for many years (myocardial recovery) and can return to a normal life and not require transplantation. The exact mechanisms underlying myocardial recovery with LVAD support remain unclear. Reverse remodeling may be attributable to a reversal or U-turn of the pathological mechanisms that occur in remodeling or the generation of new pathways.

We review the molecular changes that have been demonstrated with LVAD unloading in patients bridged to transplantation (BTT) and also those that have occurred in the myocardium of patients who have recovered their myocardial function sufficiently to allow removal of the device. Many investigators have shown improvements in molecular markers in the myocardium of patients BTT, reflecting unloading with a LVAD. Although very few of these patients were tested for recovery (and some might have recovered enough to have the device explanted if tested), we must assume most of these patients did not recover; hence, the molecular changes reflect the changes that occur with ventricular assist device unloading that do not always mean functional and clinical recovery. Changes in both the cardiac myocyte and the surrounding matrix structure are reviewed.

**Effects of LVAD Support at the Tissue, Cellular, and Subcellular Levels**

**Cardiac Myocyte**

The cardiac myocyte represents ≈35% of the cell number in the heart but 70% to 90% of the heart by volume.

**Myocyte Size**

The cardiomyocytes undergo major remodeling during the progression of HF and develop cell hypertrophy. Hematoxylin and eosin staining of myocardial samples taken from patients with end-stage HF demonstrate an increase in the size of cardiomyocytes with loading followed by reduction in myocyte size after long-term LVAD support, and studies have shown a reduction in myocyte hypertrophy as well as improvement in overall histology after LVAD support. Interestingly, Bruckner et al showed a significant correlation between the length of LVAD support and the percentage change in myocyte size. Enlargement of isolated myocytes in HF might be more pronounced in the length as compared with the width or depth.

However, regression of cellular hypertrophy occurs with unloading alone and is not necessarily associated with clinical and functional recovery. A study of paired myocardial samples of patients at the time of LVAD implantation and explantation in those who showed significant myocardial recovery and in those who did not show significant improvement in myocardial function revealed regression of cellular hypertrophy and cell size in both groups regardless of whether the patients recovered or not.
Apoptosis
A progressive decline in LV function has been linked to loss of nearly one-third of all cardiomyocytes as a result of apoptosis in cardiomyopathy, both in human and in animal HF, with both proapoptotic and antiapoptotic pathways being activated in advanced HF.12 The studies to date show contradictory findings on the effects of LVAD unloading on apoptosis. An increase in mRNA levels for the apoptosis-inhibiting proteins FasEx06del and B-cell lymphoma extra large (Bcl-XL) (the latter a member of the B-cell lymphoma 2 family), along with reduced DNA fragmentation, has been reported after LVAD unloading in patients.13,14 Upregulation of genes associated with cell growth, DNA repair, and apoptosis has been shown in failing hearts after LVAD support.15 Cytoplasmic levels of cytochrome c, a key mediator of the intrinsic mitochondrial apoptotic pathways, have also been shown to be substantially reduced after LVAD support.20 By contrast, other studies have shown a very low level of apoptosis in failing hearts, with normalization of the overexpressed proapoptotic B-cell lymphoma 2 (Bcl-2) and repair and proliferation marker mitochondrial nuclear antigen (PCNA) after LVAD support/unloading.17 De Jonge et al18 have shown a low level of apoptosis in LVAD-unloaded failing hearts, despite the abundant presence of mediators and receptors of apoptosis. The abundant expression of Fas-associated protein with death domain interleukin-1 β-converting enzyme inhibitor protein after LVAD unloading, reported in this study, may have an important role in the inhibition of cardiomyocyte death.20 The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) method used to quantitate apoptosis, however, may underestimate early stages of apoptosis with cytoplasmic damage, which is associated with contractile dysfunction and intact nuclei (termed apoptosis interruptus). Mitogen-activated protein kinases respond to extracellular stimuli (mitogens) and regulate various cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis.19 In mammalian cells, 4 parallel kinase cascades that lead to the activation of members of the mitogen-activated protein kinase family, such as the extracellular signal–related kinase–Akt (an antiapoptotic kinase) and increased glycogen synthase kinase-3β (downstream target of Akt), along with reduced apoptosis, in failing human hearts after LVAD support. Studies in vitro confirm a mechanosensitive regulation of these kinases.20 Nuclear factor κB is a crucial transcription factor regulating genes associated with antiapoptosis21 and can be activated in failing hearts. It also regulates factors involved in cell survival/apoptosis such as interleukin-1, tumor necrosis factor (TNF)-α, B-cell lymphoma extra large, and heme oxygenase-1. Normalization of elevated nuclear factor κB activity with reduced apoptosis has been reported after LVAD support.22,23

In summary, LVAD unloading seems, overall, to have a beneficial effect by acting on parts of the apoptotic pathway, but the pathways involved are complex. Interrupted apoptosis, which may be a more common stage of apoptosis in the failing heart, may be a reversible disease state and offers an attractive target. Further studies are required to recognize the complex, temporal, and spatial relationships to identify the overall effect on cell survival with LVAD unloading.

Calcium Handling
Ca2+ is required for muscle contraction, and alterations in Ca2+ handling by the cardiac myocyte are likely to contribute to the decreased contractility and negative force–frequency relationship seen in HF. There is depletion of internal stores of Ca2+ in HF and impairment of relaxation. LVAD support seems to replete these stores. Reuptake of diastolic Ca2+ into the internal stores mediated by sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a), which is impaired in HF, is improved after LVAD support.24 Chaudhary et al25 showed that myocytes from hearts supported with an LVAD exhibited a faster decay in both early and late stages of the Ca2+ transient compared with myocytes from failing hearts not supported with an LVAD. Upregulation of SERCA2a mRNA has been demonstrated after LVAD support, which was related to the papillary force–frequency relationship.26 Although SERCA2a mRNA upregulation was not influenced by disease type (ischemic cardiomyography or dilated cardiomyopathy [DCM]), only in DCM hearts was the proportion of trabeculae exhibiting a normal force–frequency relationship increased after LVAD. Some of the key changes in excitation–contraction coupling and Ca2+ cycling mechanisms are summarized in Figure 1. The studies described above regarding Ca2+ handling have been of LVAD unloading alone. Hence, many changes in excitation–contraction coupling occur with LVAD support alone and are not specific to myocardial recovery.28 However, some of the changes in excitation–contraction coupling are specifically associated with clinical myocardial recovery after LVAD support as demonstrated by the molecular studies on patients who achieved significant recovery of myocardial function, sufficient to have the device removed.27,29 These studies have shown that a comparison of myocytes taken at implantation in both recovered and nonrecovered patients showed a similar reduction in cell capacitance, an index of cell size. However, action potentials recorded in myocytes from patients who recovered shortened significantly compared with those of patients at the time of implantation, yet no such effect was observed in the nonrecovered group. L-type Ca2+ current fast inactivation was significantly more rapid in recovery vs nonrecovery, and the sarcoplasmic reticulum (SR) Ca2+ content was increased in recovered patients compared with both implantation and the nonrecovery groups. These data suggest that increased SR Ca2+ content is linked with clinical recovery by improving cardiac function. An increase in RNA expression of the sodium–calcium exchanger was also identified in the recovered patients.27 In myocytes from patients with clinical recovery after mechanical and pharmacological treatment, Na+/Ca2+-mediated Ca2+ extrusion was also faster compared with patients who did not recover.27 It should be noted that the patients who did and did not recover in these studies also received medical therapy with angiotensin-converting enzyme (ACE) inhibitors, β-blockers, and aldosterone antagonists, which could affect Ca2+ handling. A significant decrease in expression of the Ca2+-regulating gene exchange protein activated by cAMP has also been identified after LVAD support in recovered patients but not...
Exchange protein activated by cAMP has been shown to tether cAMP to mitogen-activated protein kinase, to regulate calcium-mediated signaling through nuclear factor of activated T cells, and to play an instrumental role in metabolic signaling pathways involving insulin.

Cytoskeletal Proteins

Vatta et al.32 identified a disruption in the N-terminus of dystrophin in 18 out of 20 patients with end-stage cardiomyopathy (dilated and ischemic), and this disruption reversed after LVAD unloading in 4 out of 6 patients (as BTT). In a subsequent study, the same group33 demonstrated disruption of the N-terminal dystrophin in both left and right ventricles of failing hearts, which was reversed in 12 out of 14 patients using either pulsatile or continuous devices. Mutations in dystrophin (which links the actin cytoskeletal network and the sarcolemmal dystrophin–associated complex, which in turn is associated with the extracellular matrix [ECM] matrix) can result in skeletal myopathy and cardiomyopathy. This suggests that dystrophin might play an important role in reverse remodeling.

The sarcomeric contractile proteins, actin, myosin, tropomyosin, and troponin, and the troponin complex are responsible for cross-bridge cycling of actin thin filaments and myosin thick filaments, ultimately generating the force in striated muscle. Changes in both the sarcomeric and nonsarcomeric cytoskeletal proteins were investigated by microarray in paired myocardial LV samples of patients who recovered ventricular function and underwent explantation of the device compared with patients BTT who did not recover (Figure 2).30,34 In the recovery group, β-actin; α-tropomyosin; α1-actinin; and α-filamin A. Both troponin T3 and α2-actinin decreased at the time of explantation. Some showed differential changes; vinculin and syntrophin (a dystrophin-like protein) decreased in the recovered group but increased in the nonrecovered group.

Some changed only in the nonrecovered group; α2-tubulin, β2-tubulin, and β5-tubulin increased and the dystrophin-like proteins, dystroglycan, syntrophin-α, and syntrophin-β, increased. LIM domain-binding-3 and actin-binding LIM protein-1 increased between implantation and transplantation. LIM domains 1 and 7 increased and decreased, respectively. β1-Laminin, α3-laminin, troponin I, myosin 5C, myosin IXB, and integrin-α10 increased.

Hence, there was an increase in the gene expression of lamin A/C in the recovered, but not in the nonrecovered, patients. Mutations in the lamin A/C gene have been previously shown in families with DCM.35 Lamins A and C are components of the nuclear envelope and are located in the lamina, a structure associated with the nucleoplasmic surface of the inner nuclear membrane. In our study, the increased expression of lamin A/C suggests that it might be restored in recovery.

Vinculin (which is elevated in HF) decreased in the recovered group but increased in the nonrecovered patients. Vinculin is situated at the costameres of the lateral sarcolemma and at the intercalated disc and is one of the major components of the linkage system that connects, via the integrins, the intracellular milieu with the ECM.

Another study36 analyzed the pattern of sarcomeric gene expression in patients BTT with LVAD. Although the patients were BTT and not recovery, a global increase in mRNA levels of sarcomeric proteins was also demonstrated with an increase in 17 sarcomeric genes with LVAD unloading.

Changes in some of the sarcomeric and nonsarcomeric proteins were also investigated at the protein level in patients who...
recovered; at explantation, statistically significant increases in myosin heavy chain, sarcomeric actin, α II spectrin, troponin C, troponin T, cytoskeletal actinin, and smooth muscle α-actin were found.37 Hence, significant changes were shown in sarcomeric, focal adhesion, and cytoskeletal proteins in the myocardium of patients who recovered with subsequently maintained function. De Jonge et al38 also found improvement, but not normalization, in the architecture of actin, tropomyosin, troponins C and T, and titin by immunocytochemistry in the myocardium of patients after LVAD unloading as BTT. Hence, these studies show a specific pattern of changes in both sarcomeric and nonsarcomeric cytoskeletal proteins in the myocardium of patients who recovered compared with those who did not, suggesting that the cytoskeleton might play an important role in functional myocardial recovery.

One of the most striking differences between the recovered and nonrecovered cohorts in the gene array study was the change in β-integrin signaling networks.30,34 The transmembrane integrins β1, β6, and α7 decreased, but integrins α5 and β5 increased at explantation in the recovered patients. The integrin family of proteins spans the cell membrane and links the ECM with the cytoskeleton; hence, they play an important role in mechanosensation and cell signaling. They mediate mechanical stretch signals from the ECM though protein kinase cascades that provoke changes in gene expression, including those in the hypertrophic response. Integrins bind directly to components of the cytoskeleton, such as α-actinin, through their cytoplasmic tails and can orchestrate changes in cellular cytoarchitecture. The changes seen at multiple levels in the integrin pathway suggest that it may play a key role in the process of reverse remodeling and subsequent functional recovery in these patients. This could imply a reversal of impaired mechanosensation whereby altered loading conditions signal reorganization of the cell cytoskeleton.

The Matrix

The ECM (which constitutes 2%–4% of the normal myocardium) is composed of a number of proteins, including fibronectins, elastins, and laminins; however, the most abundant proteins are collagens, of which types I and III represent >90% of the total content in the heart. Collagen maintains the structural composition, transmits forces, and, with elastin, contributes to the elastic properties of the myocardium. In addition, it acts as a ligand-binding protein that affects the function of other components of the myocardium. HF leads to increased collagen type I, type I:III ratio, and cross-linking associated with fibrosis.

Changes in the ECM and collagen metabolism after mechanical unloading have been studied by various groups. Conflicting changes in collagen content after LVAD support have been reported. Some groups report a decrease59–61 in the collagen content, whereas others report an increase.62–64 The reasons for these differing responses, although still unclear, may be related to differences in the causes of HF, duration of unloading,53,65 use of pharmacological therapy, and whether there is myocardial recovery.
An increase in collagen cross-linking after LVAD treatment alone was demonstrated by Klotz et al. These authors showed that total collagen and cross-linked collagen increased during LVAD support, as did the ratio of type I to type III collagen, and concomitantly chamber and myocardial stiffness increased with LVAD support. Hence, their data suggested that LVAD support increases LV collagen cross-linking with a consequent increase in myocardial stiffness, which could impact on ventricular filling. Bruggink et al. demonstrated a biphasic response with an initial increase in fibrosis, followed by a subsequent regression with prolonged LVAD therapy (after 200 days of support). Drakos et al. with advanced image analysis found higher interstitial and total fibrosis in failing hearts and that the collagen content increased further after LVAD unloading.

A specific therapy aimed at promoting myocardial recovery in patients receiving LVADs combines LVAD unloading with drugs meant specifically for reversing remodeling and reducing fibrosis. In particular, this combination therapy adds aggressive medical therapy with ACE inhibitors, angiotensin II receptor blockers, and aldosterone antagonists to maximal LVAD unloading, to reduce fibrosis. Klotz et al. specifically investigated the effect of ACE inhibitors and studied heart samples obtained before and after LVAD implantation in patients who did and did not receive ACE inhibitors. Pre-LVAD angiotensin I and angiotensin II and total and cross-linked collagen were similar between the 2 groups; however, after LVAD support, angiotensin II was reduced in the ACE-I group but increased in the non–ACE-I group. Similarly, cross-linked collagen decreased during LVAD support in the ACE-I group. Myocardial stiffness and LV mass were lower in the ACE-I group. ACE-I normalized the LV and right ventricular matrix metalloproteinase (MMP)-1/tissue inhibitor of MMP (TIMP)-1 ratio, suggesting a mechanism for improved collagen turnover. Collagen content and characteristics of the right ventricle were not affected by ACE-I, suggesting an LVAD unloading effect. Hence, ACE-I therapy was associated with decreased angiotensin II, myocardial collagen content, and myocardial stiffness during LVAD support. Milting et al. also showed that patients receiving ACE-I during ventricular assist device support had lower COL1A1 mRNA content at transplantation than those not receiving ACE inhibitors. These findings support the use of adjunctive pharmacological reverse remodeling and antiﬁbrotic therapies with LVAD support.

The MMPs and their inhibitors, TIMPs, play a key role in maintenance of the ECM and are responsible for collagen denaturation and degradation. They are altered in chronic HF. An imbalance between MMPs and TIMPs is thought to lead to adverse ECM and LV remodeling. Increased MMP-9 and reduced TIMP expression (TIMP-1, TIMP-3, and TIMP-4) have been shown in HF. We have previously reported high myocardial MMP-1 and MMP-8 expression in patients with deteriorating conditions requiring LVAD support along with increased TIMP-4 expression. Others have reported that after LVAD support, MMP-1 and MMP-9 tended to decrease with a normalization of the MMP-1/TIMP-1 ratio and an increase in TIMP expression (TIMP-1 and TIMP-3). In patients receiving concomitant ACE inhibitor therapy during LVAD support, reversal of TIMP-1 levels with normalization of the MMP-1/TIMP-1 ratio has been reported. Myocardial expression of collagens (COL1A1 and COL3A1), fibronectin, MMP-1 to MMP-14, TIMP-1 to TIMP-4, connective tissue growth factor, transforming growth factor-β1, and Thy-1 cell surface antigen were measured at LVAD implantation and again at explantation or transplantation in patients who did and did not develop sustained myocardial recovery. The nonrecovery group had higher levels of profibrotic markers (COL1A1, transforming growth factor-β1, and Thy-1 cell surface antigen) at implantation compared with the recovery group. Of all the genes analyzed, only the gene TIMP-4 showed a significant change in the recovery patients, with levels consistently decreased at explantation, and did not change in nonrecovered patients.

Moreover, in patients who recovered, higher expression levels of the profibrotic genes (COL1A1, COL3A1, fibronectin, and Thy-1 cell surface antigen) at explantation correlated with poorer subsequent function after explantation at 1, 2, and 5 years in the recovery group. Hence, patients who did not recover had higher myocardial expression of profibrotic genes at LVAD implantation. In the recovered patients, higher levels at explantation were negatively associated with subsequent function, suggesting that low ﬁbrotic markers are associated with long-term recovery.

Segura et al. compared the histological features at the time of implantation in patients who were subsequently weaned-off LVAD support because of improved myocardial function to those of patients who remained on support without evidence of clinical remission. The group who subsequently had the LVAD removed tended toward less overall ﬁbrosis at implantation. The same investigators conﬁrmed their ﬁndings in a group of patients from Hareﬁeld Hospital. Again at implantation, the group who subsequently recovered had less ﬁbrosis (both overall ﬁbrosis and perivascular/interstitial ﬁbrosis) than those who did not recover. Less ﬁbrosis may therefore predict patients with a higher probability of LVAD removal and myocardial recovery after LVAD support. Another study has shown that patients BTT who gain the maximum improvement in LV ejection fraction (LVEF) during LVAD support had less ﬁbrosis at the time of device implantation. Furthermore, Yamada et al. found a correlation between the amount of ﬁbrosis and brain natriuretic peptide change after LVAD implantation, such that patients with less ﬁbrosis had signiﬁcantly improved brain natriuretic peptide levels. These studies suggest that a preimplant assessment of the degree of ﬁbrosis may predict myocardial improvement during LVAD support.

**Metabolic Enzymes**

A wide range of metabolic derangements occur in HF. Most of these result in depletion of myocardial ATP, phosphocreatine, and creatine kinase, with decreased efficiency of mechanical work. Generally, improvement in cardiomyocyte metabolic dysfunction occurs after LVAD unloading, although the mechanisms involved are still unclear.

Cardiolipin is a specific lipid component of the mitochondrial inner membrane whose presence is essential for maintaining mitochondrial ultrastructure, oxidative ATP formation, and substrate transport. Reversal of myocyte cardiolipin composition with LVAD unloading in ischemic but not in dilated HF has been reported. LVAD support of failing hearts...
can also potentiate endogenous nitric oxide–mediated regulation of mitochondrial respiration. Furthermore, reversal of decreased uncoupling protein 3 expression after LVAD treatment may be an important mechanism for reducing the formation of O₂-derived free radicals. These findings suggest that LVAD unloading can reverse decreased metabolic gene expression in the failing human heart.

In the failing heart, despite increases in some ATP synthesizing pathways (such as glycolysis), other pathways such as the creatine kinase–phosphocreatine system decrease. However, ATP levels have been shown to be ≈30% lower in the failing compared with the normal human myocardium. The reduction of the creatine pool shown in HF is related to HF severity. Elevated myocardial arginine:glycine amidinotransferase levels have been reported in failing hearts requiring LVAD support, and these normalized after myocardial recovery sufficient to allow device explantation. This change was specific to patients who recovered and was not seen in patients who had LVAD unloading but no myocardial recovery. Arginine:glycine amidinotransferase is a rate-limiting enzyme that catalyzes the formation of guanidinoacetate, the immediate precursor of creatinine, and hence is a rate-limiting enzyme in the creatine synthesis pathway. It is well-known that changes in circulating levels of creatine have a reciprocal effect on the levels of arginine:glycine amidinotransferase enzyme activity, and it is possible that the increased level of arginine:glycine amidinotransferase expression newly described in this study is a direct response to the depleted myocardial creatine pool in HF; hence, these changes suggest a response to HF that involves elevated local creatine synthesis. Interestingly, creatine is central to energy metabolism in tissues with high energy demand such as the myocardium, so this finding may be important.

The Immune System

The innate immune system plays a major role in the progression and regression of HF. Expression of Toll-like receptors (which modulate the immune and inflammatory responses) leads to the activation of signaling pathways that induce the expression of cytokines, chemokines, and costimulatory molecules. Increased expression of Toll-like receptors and proinflammatory cytokines (interleukin-1, interleukin-1β, interleukin-6, and TNF-α) have been demonstrated in severe HF and in the myocardium of those decompensating and requiring LVAD support. The causes of immune system activation by the cardiomyocyte are thought to be in response to different forms of injury.

TNF-α is a proinflammatory cytokine associated with DCM in animals and humans. Increased levels of TNF-α have been shown in the myocardium of patients needing LVAD support. Torre-Amione et al demonstrated a reduction of TNF-α expression after LVAD unloading, and their data suggested that the magnitude of the change was a useful marker for recovery of cardiac function. However, Razeghi et al have shown that there was no correlation between the clinical indices of cardiac improvement and the decreased levels of myocardial TNF-α expression after LVAD support.

Follistatins

Another group of mediators that have recently been implicated in remodeling and reverse remodeling are the follistatins (FST) and their related proteins, FSTL1 and FSTL3. They act by neutralizing activins, members of the transforming growth factor-β1 superfamily, which are implicated in diverse biological processes, including cell proliferation and differentiation, wound healing, inflammation, and fibrosis. Follistatins have also been implicated in skeletal muscle regeneration. Elevated myocardial expression of FSTL1 and FSTL3 has recently been shown in patients with HF. Furthermore, elevated levels of FSTL1 and FSTL3 in patients at the time of LVAD implantation were shown to return to normal after myocardial recovery and LVAD explantation in patients treated with an LVAD and drug combination therapy, but not in patients who did not recover. FSTL3 expression correlated with molecular markers of disease severity. Microarray analysis showed that FST and FSTL1 expressions correlate with ECM-related and calcium-binding proteins, whereas FSTL3 is associated with cell signaling and transcription. FSTL1 also showed positive correlation with the endothelial cell marker CD31, suggesting a potential link with improved vascularization. FSTL1 levels before treatment correlated with cardiac function after explantation, suggesting initial levels influence long-term outcome. FST-like genes may therefore be linked to both disease severity and mechanisms underlying recovery and offer novel therapeutic targets.

The Sympathetic Nervous System

Patients with HF exhibit increased sympathetic efferent neuronal activity, leading to excessive exposure of the myocardium to norepinephrine. Reduced norepinephrine uptake occurs because of a reduction in the norepinephrine transporter activity. Excessive exposure of the heart results in worsening HF with the development of arrhythmias, downregulation of α and β receptors, apoptosis, increased oxygen consumption and myocardial wastage, loss of contractile reserve, and other symptoms. Reduced norepinephrine uptake results in low metaiodobenzylguanidine concentrations in the neurons and leads to an enhanced metaiodobenzylguanidine washout rate. A lower heart to mediastinal (H/M) rate (or higher washout rate) is associated with a worse prognosis and has been proposed to be a superior predictor of outcome to ejection fraction (EF). In DCM, cardiac 123I-metaiodobenzylguanidine uptake is reduced in proportion to the severity of HF. Two studies have shown that LV unloading by an LVAD improves the sympathetic innervations of the failing heart (as assessed by 123I-metaiodobenzylguanidine myocardial scintigraphy). The first showed a significant reduction in the washout rate and an increase in the H/M ratio after a short period of LVAD unloading. The second very detailed study prospectively followed-up patients who did and did not recover and also assessed their true underlying myocardial function with the pump speed reduced. There was not only a reduction in the washout and an increase in the H/M ratio after LVAD unloading but also the percentage change in the delayed H/M ratio and washout rates were greater in the patients who recovered, and these changes were sustained in follow-up scans of the patients who underwent explantation. There were parallel improvements in echocardiographic parameters of LV function and reductions in plasma catecholamine levels. These studies suggest that LVAD
unloading restores norepinephrine reuptake mechanisms and improves the sympathetic innervation of the failing heart and this is associated with myocardial recovery.

**Regeneration of the Ventricle Could Occur in Myocardial Recovery**

A recent study\(^76\) examined cardiomyocyte DNA content, nuclear morphology, and the number of nuclei per cell before and after LVAD support. The number of polyplody cardiomyocytes and cardiomyocyte DNA content declined, whereas an increase in multinucleated cardiomyocytes was observed. This suggested that the polyplody of cardiomyocytes in the failing human heart was a result of hypertrophic growth associated with repeated rounds of DNA synthesis that did not result in cell division and may mean that hypertrophic stimuli throughout LVAD unloading may lead to beneficial cardiomyocyte duplication and regeneration. This suggests that at least a proportion of cardiomyocytes are not terminally differentiated and could re-enter the cell cycle during a regenerative process. Additional stem cell treatment could potentiate this ability in the future.

**Clinical Factors and Adjuvant Therapies Influencing Reverse Remodeling and Recovery**

**Duration of Unloading**

Animal models of prolonged unloading of nonfailing myocardium by means of heterotopic transplantation\(^77\) or LVAD\(^78\) suggest that prolonged mechanical unloading can lead to cardiomyocyte atrophy. An animal study of failing hypertrophied hearts indicated that unloading resulted in a decrease of cardiac myocytes in size beyond normal values.\(^79\) In human studies, cardiomyocyte size decreased with LVAD unloading although not beyond the size of normal cardiac myocytes\(^77\) and, in one of these studies, electron microscopy suggested no evidence of cardiomyocyte atrophy or degeneration even with >6 months of LVAD support. Development of atrophy could mean that the heart would not be strong enough to be reloaded.

Furthermore, although improvements in Ca\(^{2+}\) handling occur during myocardial recovery with LVAD support, animal studies suggest that prolonged LV unloading (by heterotopic abdominal transplantation) can result in cardiac myocytes with smaller sarcomere shortening amplitude and delayed sarcomere relaxation, despite a normal Ca\(^{2+}\) transient amplitude, with reduced myofilament sensitivity to Ca\(^{2+}\) and reduced SR Ca\(^{2+}\) uptake.\(^80\) This suggests that prolonged unloading can cause impaired contractility and relaxation with preserved Ca\(^{2+}\) release mechanisms but impaired cytoplasmic Ca\(^{2+}\) removal mechanisms. Studies with shorter periods of unloading were not associated with contractile dysfunction.\(^79\)\(^81\) Another study showed normalization of SERCA and SERCA/phospholamban ratios early after LVAD implantation, with reversion back to failing levels with prolonged LVAD support.\(^82\) There is also growing evidence that alterations in T-tubule structures (which are colocalized to areas of impaired Ca\(^{2+}\) release) are an important cause of deterioration in cardiac myocyte function in a range of cardiac diseases.\(^82\) One study showed irregular distribution of the T-tubules and profound disruption of the openings of the T-tubules and the cell surface in cardiac myocytes after prolonged unloading\(^82\) with a smaller proportion of T-tubule openings in their ordinary position. Interestingly, T-tubules might be regulated by stretch-sensitive molecules.

These studies raise the question of optimal timing and suggest that adverse remodeling could sometimes occur with very prolonged unloading. Hence, an initial improvement in ventricular function may be followed by subsequent deterioration in some cases, which might partly explain why LVAD unloading is not always accompanied by myocardial recovery.

**Adjuvant Therapy: Use of Clenbuterol**

Clenbuterol is a novel, selective, \(\beta\)-2 adrenergic receptor agonist that is a potent synthetic pharmacological analog to epi-nephrine. It was initially used in the meat industry to increase muscle bulk.\(^83\)

Two types of hypertrophy can be induced by different stimuli and mechanisms: pathological hypertrophy and the physiological type.\(^84\)\(^85\) Pathological myocardial hypertrophy, as seen in pressure overload, involves re-expression of proteins as fetal isoforms. Cardiac \(\alpha\)-actin is the usual predominant type of sarcomeric \(\alpha\)-actin; however, in pathological hypertrophy, skeletal \(\alpha\)-actin is induced instead, and downregulation of SERCA2 and phospholamban occurs with increased interstitial collagen.\(^84\)

Physiological myocardial hypertrophy is defined as increased LV mass with normal systolic and diastolic LV functions, normal relaxation times, normal morphology, extracellular structure (LV collagen concentration and morphology) and gene expression (SERCA2 and phospholamban mRNA), and LV re-expression of atrial natriuretic factor mRNA (a nonspecific molecular marker of LV hypertrophy), but without contractile protein isoform switching from cardiac to skeletal \(\alpha\)-actin.\(^84\) Clenbuterol induces this type of myocardial hypertrophy that can be summarized morphologically as the induction of physiological hypertrophy associated with improved function, functionally as enhanced systolic and diastolic function, histaologically associated with prevention of increased fibrosis, and molecularly by physiological gene expression.\(^86\)\(^87\)

Unloading a rat heart leads to myocardial atrophy (Figure 3). Infusion of clenbuterol into a rat via a minipump leads to an increase in cell surface area along with prolongation of the action potential, increased Ca\(^{2+}\) transient amplitude, increased SR content, and increased SERCA expression (Figure 4).\(^79\)

Animal models\(^79\) show that when unloading is combined with clenbuterol administration in a HF model, an improvement in myocardial function occurs. This does not happen if clenbuterol is administered without unloading. Some data suggest that acute administration of clenbuterol might even impair myocardial function, and it is its chronic administration that is important to be beneficial to cardiac function.

Concerns about cardiac muscle atrophy after chronic LVAD myocardial unloading form the possible explanation of the low incidence of myocardial recovery and incidence of subsequent failure in some patients. It was hypothesized in the Harefield combination protocol that to achieve consistent successful device explantation, regression of myocardial pathological hypertrophy should be followed by the induction of physiological hypertrophy by clenbuterol, which may have a role in
preventing disuse atrophy in completely unloaded hearts. In addition, the ability of clenbuterol to induce skeletal hypertrophy may be beneficial because many patients with severe HF have atrophy of their skeletal muscles. Investigators showed chronic β2-agonist administration also induces slow-to-fast fiber type transition, resulting in an increase in greater, faster contractions with increased stroke power and reduced contraction and relaxation times. It induces insulin growth factor 1, through which skeletal muscle hypertrophy is mediated.

Terracciano et al also identified that chronic administration of clenbuterol induced beneficial changes in Ca2+ regulation, energy metabolism, and organ and cellular hypertrophy in rat hearts. Clenbuterol induced cardiac hypertrophy, increased Ca2+ transients and SR Ca2+ content without changes in the rate of Ca2+ decline in isolated ventricular myocytes, increased expression of SERCA2a, phospholamban, and Na+/Ca2+ exchanger, and increased oxidative carbohydrate use in the heart. The increased Ca2+ transients generate larger contractions. The increased contractility at the cellular level was not observed at the organ level, although optimal cardiac performance was already present in normal animals that were studied. Clenbuterol also increases the carbohydrate (rather than fatty acid) contribution to cardiac oxidative metabolism after chronic treatment, and glucose and pyruvate are better substrates for cardiac cells under stress.

**Role of Reloading the Ventricle**

It is possible that intermittently lowering the pump speed to open the aortic valve, increase the afterload, and, hence, increase myocardial work might be an alternative way to work and therefore retrain the LV. Intermittently lowering the pump speed might act as an alternate method to clenbuterol to prevent atrophy and cause physiological hypertrophy, making explantation more durable. This concept is already in place to some degree with the devices that reduce their speed for a few seconds every minute, such as the Jarvik pump.

**Continuous vs Pulsatile Flow Unloading**

There has been an almost total conversion away from use of volume displacement and pulsatile design LVADs to the new second generation and third generation of continuous flow (CF) designs. The question has been raised regarding whether, despite far superior clinical outcomes with the CF design, there is similar, greater, or reduced LV unloading. LV wall stress has been thought to be the most important stimulus for activation of all the downstream adverse compensatory mechanisms triggered by perturbations in LV function leading to the HF phenotype. Thus, maximal reduction of wall stress seems important. The data available suggest that CF pumps might achieve less reduction in LV end-diastolic pressure than the pulsatile flow pumps. However, the ability to adjust pump speed and LV decompression suggests that this, too, is variable. The secondary induction of significant ventricular arrhythmias with excessive speeds and reduction in LV size attributable to physical contact of the LV wall with the inlet cannula of the device may limit CF pumps as well. In theory, CF pumps might unload less; however, they unload continuously, whereas the pulsatile pumps are asynchronous with the cardiac cycle and might only intermittently load the ventricle.

The majority of studies to date of the molecular changes of reverse remodeling have been performed in patients who have been supported with a pulsatile rather than a CF pump, so it is not yet known whether all of the changes that have been reported to date will be observed with CF devices.
Clinical Relevance

As noted, there is an abundance of data that demonstrate near-normalization of multiple key components of the cardiac myocyte structure (the cytoskeleton, Ca\(^{2+}\) handling proteins, and others), metabolic substrates, as well as changes in the matrix and increased myocardial contractility, which collectively suggest that myocardial recovery should be possible in patients who are supported with an LV AD for a period of time. However, this does not always equate with significant clinical myocardial recovery (although it is commonly not promoted or tested for). A summary of the clinical outcomes in patients is presented.

In 1994, Frazier et al\(^8\) at the Texas Heart Institute, Houston, were the first to describe functional improvement in patients supported with the Heartmate pulsatile device. They found that in all cases (12 DCM, 6 ischemic), LVAD support resulted in a significant reduction in the cardiothoracic ratio, a decrease in LV dimensions, and a 43% improvement in LVEF from baseline.\(^8\) These patients also showed improved hemodynamics, including a reduction in pulmonary capillary wedge pressure and improvement in the cardiac index. This was the first report suggesting that significant improvement of cardiac function was possible with LVAD unloading.

Levin et al\(^8\) then measured the end-diastolic pressure–volume relation at the time of cardiac transplantation in patients treated with medical therapy and those unloaded with LVAD support. The end-diastolic pressure–volume relations of hearts from medically treated patients were shifted toward markedly larger volumes, but end-diastolic pressure–volume relations of hearts from LVAD-supported patients were similar to those of normal hearts. This suggested that chronic hemodynamic unloading of sufficient magnitude and duration can result in reversal of chamber enlargement and normalization of cardiac structure (reflected by end-diastolic pressure–volume relation), even in advanced stages of HF.

The first report of actual recovery and device explantation with a period of LVAD support was in a series of 5 patients with advanced nonischemic HF. One patient died of a noncardiac cause 10 days after LVAD removal, but the other 4 remained alive and well at 35, 33, 14, and 2 months after LVAD removal.\(^9\) However, Mancini et al\(^1\) reported that in a retrospective review of 111 patients (46% of whom had
DCM), only 5 (4.5%) patients had sufficient myocardial recovery for the device to be explanted. Furthermore, only 1 patient remained alive and well with maintained LV function at 15-month follow-up—1 died of HF 3 months after explantation, 1 died suddenly, and 2 patients required LVAD reimplantation. These authors argued that significant recovery only occurred in a small percentage of patients.

Hence, although recovery of LV function sufficient to explant the device had now been shown, there was skepticism, and the rate was felt to be small by many, and durability of recovery had not been proven. However, larger programs began reporting their experience with LVAD explantation for recovery. Farrar et al reported results from the Thoratec registry and showed that out of 271 nonischemic patients, 22 (8.1%) patients underwent explantation. Of these 22, 17 patients were still alive and well at latest follow-up. Of the remaining 5 patients, 2 patients died and 3 patients had undergone transplantation. The overall transplant-free survival was 86% and 77% at 1 and 5 years, respectively, after explantation. Of the 22 patients, 2 had idiopathic DCM, 3 had combined cardiomyopathy and myocarditis, 12 had myocarditis, 4 had postpartum cardiomyopathy, and 1 patient had viral cardiomyopathy. Simon et al retrospectively reviewed the Pittsburgh program and showed that out of 154 patients, 10 (6.4%) patients underwent explantation. The 8 nonischemic patients were from a total of 74 nonischemic patients (11%). Of the 10 patients who underwent explantation, 8 (80%) were alive and free from transplantation after 1.6±1.1 years. Hence, these series suggested recovery from chronic HF was possible. However, recovery of chronic DCM still needed to occur in a wider number and needed to be sustained to be fully proven.

The Berlin group has been interested in testing their patients for myocardial recovery for some time. They reported their first 5 patients with DCM weaned from the device in 1997 and have updated their series in a number of reports during a 10-year period. Their initial report showed that all of the first 5 patients who underwent explantation remained device-free for 51 to 592 days. By 2008, they reported a total of 81 patients weaned from LVADs, biventricular ventricular assist devices, and right ventricular assist devices. Of adults who had received an LVAD for nonischemic cardiomyopathy (excluding myocarditis) as a BTT, a total of 35 out of 188 (18.5%) patients underwent explantation (12). Of these, 30 out of 35 patients had the device explanted electively and in 5 it was precipitated by pump-related complications. There were no established criteria to define recovery and suggest safety in removing the device. In 8 of the electively weaned patients, the LVAD was removed with a subnormal EF (30%–44%) and large LV end-diastolic diameters (LVEDD, 56–60 mm). Post explantation 5- and 10-year survival rates with their own heart were 76% and 71%, respectively, and the overall 5- and 10-year survival rates after LVAD removal (including the post-transplant survival for patients with HF recurrence) were 79% and 75%, respectively. At 5 years, there was a 61% probability of freedom from HF recurrence. If the patient remained stable 1 year after explantation, then the freedom from HF recurrence rates at 5 and 10 years after LVAD explantation were 84% and 62%, respectively. However, there was no standardized use of an oral HF drug regimen, and this group of patients had quite a high proportion with incomplete recovery before explantation, which is likely to contribute to a higher HF relapse rate.

Seven United States institutions formed the LVAD Working Group to study the incidence of myocardial recovery in their LVAD-supported patients. They studied 67 patients (37 nonischemic and 30 ischemic cardiomyopathy) receiving the pulsatile Heartmate XVE device. After 30 days, significant improvement occurred in LVEF (17±7% to 34±12%), LVEDD (7.1±1.2 to 5.11±1.1 cm) and LV mass (320±113 to 194±79 g) compared with before LVAD implantation. Thirty-four patients (32%) had EF >40% with partial device support. However, the LVEF decreased again over time to the pre-LVAD measurements by 120 days. Peak myocardial oxygen consumption improved significantly on LVAD support (13.7±4.2 to 18.9±5.5 mL/kg per minute at 30 vs 120 days). Only 6 (9%) subjects underwent explantation for myocardial recovery. However, because not all centers were experienced with pump-off studies, the patients in this study were not tested by echo off the pump, but at full and reduced LVAD support (LVAD flow at 4 L/min), and cardiopulmonary exercise tests were performed on the pump. Furthermore, ACE inhibitors and β-blockers were encouraged, but there was no protocol-prescribed medical regime during LVAD support, and explantation depended on individual-centered experience and decision. However, this was a prospective serial observation of the natural history of LVAD support on ventricular size and function. Hence, no group had still performed any active intervention to promote recovery or had performed any systematic accurate testing to demonstrate possible recovery.

We developed a specific strategy at Harefield Hospital that combines LVAD mechanical unloading with specific pharmacological interventions in an attempt to specifically maximize the incidence of recovery in patients with DCM and to improve the durability of recovery after explantation. This protocol systematically and regularly tests the underlying cardiac function with the pump turned off (or with no net forward flow if a CF pump) at very regular intervals to assess the response to treatment. This regime consists of an LVAD combined with drugs known to enhance reverse remodeling, followed by the use of the β-2 adrenergic receptor agonist clenbuterol to prevent atrophy/induce physiological hypertrophy.

The strategy is divided into 2 phases. The pharmacological interventions of the first phase of the therapy are designed to act on component parts of the myocardium with the aim of reversing the pathological hypertrophy, remodeling, and normalizing cellular metabolic function. In this first phase, the following oral HF drugs are initiated immediately after weaning of inotropic support and titrated to the following maximum doses: lisinopril 40 mg daily; carvedilol 25 mg 3 times daily; spironolactone 25 mg daily; digoxin 125 mg daily; and losartan 150 mg daily. The rationale of the first phase is to combine mechanical unloading with the drugs known to enhance reverse remodeling. Whereas patients often do not tolerate large doses of these drugs while in severe HF because of their renal failure and hypotension, they tolerate them well once supported with a ventricular assist device that restores their blood pressure and renal function. The ACE and angiotensin II inhibition are also chosen (along with the aldosterone antagonist) to reduce fibrosis (see the molecular data provided).
The second stage of pharmacological therapy is instituted after maximal regression in the LVEDD has been achieved while the LVAD is in place. Once the LVEDD measured with the pump off or essentially off for 15 minutes is <60 mm, the initial nonselective β-blocker carvedilol is switched to a β1-selective blocker (bisoprolol or metoprolol), leaving the β2 receptors unblocked for clenbuterol, which is then added to the phase I drugs.

Using the Harefield strategy of combining LVAD unloading with reverse remodeling therapy, followed by clenbuterol administration, 2 prospective studies were performed at Harefield Hospital77-79 with patients with nonischemic cardiomyopathy receiving an LVAD. The population of the first study consisted of 20 patients with advanced decompensating New York Heart Association class IV DCM who received a pulsatile HeartMate LV AD as a BTT. Patients with myocarditis were excluded to focus on patients with chronic HF (duration of HF symptoms, 4.5±4.5 years).

Of the 15 patients who received a complete course of the combination therapy, 11 (73%) reached sufficient recovery to undergo explantation after a duration of support of 320±186 days (range, 63–603). The LVEF (with the pump off for 15 minutes) was 64±8% before implantation vs 12±6% before explantation as having New York Heart Association class I with mean EF 58.1±13.8%, LVEDD 59.0±9.3 mm, LV end-systolic diameter 4.2±10.7 mm, and myocardial oxygen consumption 22.6±5.3 mL/kg per minute. The cumulative freedom from death and recurrence of HF in the group that underwent explantation was 83.3% at 1 and 3 years.

These prospective studies77-79 showed that reversal of severe HF secondary to nonischemic cardiomyopathy can be achieved in a high proportion of patients using either pulsatile or CF pumps combined with aggressive pharmacological therapy and regular testing. The rate and durability of recovery in this series using this strategy are significantly higher than previously reported. It is not clear what contribution clenbuterol plays in the success of the combination therapy because it is initiated once the heart is mostly reverse-remodeled and there is already significant improvement in myocardial function. Clenbuterol is likely to improve the durability of recovery by causing physiological hypertrophy. The protocol is also remarkable for the phase I uptitration to very high doses of an ACE inhibitor, BB, angiotensin receptor blocker, and angiotensin-2 antagonist, chosen for their reverse remodeling and antifibrotic effects and for regular testing of underlying myocardial function.

Interestingly, the first patient who underwent explantation by using this protocol had a familial cardiomyopathy and still has normal cardiac function and normal exercise capacity >12 years after explantation. Several other patients with familial cardiomyopathy have been recovered using this protocol as they have at the Texas Heart Institute (O.H. Frazier, personal communication), suggesting that even genetic structural chronic HF can sustainably recover after LVAD support.

Summary
Hence, LVAD unloading is often associated with beneficial molecular changes in both the cardiac myocyte and matrix, although these changes are not always associated with clinical myocardial recovery. After LVAD unloading, a reduction in cell size occurs; however, again, this does not necessarily correlate with an improvement in cardiac function.

However, improvements in myocardial function significant enough to remove the device and return the patient to a normal quality of life10 can occur in a proportion of patients. Some of the changes in both the cardiac myocyte and the matrix after...
LVAD support are specific to myocardial recovery. In the cardiac myocyte, increases in the sarcomeric and nonsarcomeric proteins and some of the improvements in the Ca2+ handling pathway (i.e., of excitation–contraction coupling) seem to be specifically associated with myocardial recovery. Changes in the matrix are complicated, but it seems excessive scarring may limit the ability for recovery and the degree of fibrosis in the myocardium at the time of implantation may predict the ability to recover. In general, myocardial recovery seems to be attributable to improvements in the myocyte and is hindered by scarring in the matrix. There is also some evidence that new cell formation may occur, and stem cells may have an important role in myocardial recovery in the future.

These studies show that the myocardial plasticity of remodeling is clearly bidirectional at many levels, and regression of pathological abnormalities is possible, as well as progression; this regression is also possible across the full spectrum of disease severity. One long-term goal of this field is to learn more about the molecular mechanisms underlying remodeling and reverse remodeling to find and develop new therapeutic targets and strategies to induce further significant reverse remodeling and durable myocardial recovery. Reverse remodeling may be attributable to a reversal of the pathological mechanisms that occur in remodeling, or to the generation of new pathways. Some new targets such as specific metabolic enzymes have already come from the molecular study of myocardial recovery and LVAD unloading. These could then be applied to a wider population of patients with HF to treat their myocardial dysfunction and HF.

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