Cardiomyopathic Etiology and SERCA2a Reverse Remodeling During Mechanical Support of the Failing Human Heart

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Many hearts in end-stage, chronic failure (CHF) retain the capacity to reverse abnormal expression of genes regulating myocyte calcium cycling when supported with a left ventricular assist device (LVAD). In the present study, we determined whether LVAD-induced upregulation of the gene encoding for the key calcium cycling protein sarcoplasmic endoreticular calcium adenosine triphosphatase subtype 2a (SERCA2a) is influenced by the nature of underlying disease broadly characterized as ischemic (ICM) or idiopathic dilated (DCM) cardiomyopathy. Data from Northern blot analysis of SERCA2a messenger (m)RNA within 84 heart samples (50 CHF [23 DCM and 27 ICM] and 34 CHF+LVAD [18 DCM and 16 ICM]) were used for characterizing gene expression. In addition, measurements of the force-frequency relationship (FFR), a reflection of *in vivo* SERCA2a function, were obtained in myocardial trabeculae isolated from 75 hearts (51 CHF [29 DCM and 22 ICM] and 24 CHF+LVAD [10 DCM and 14 ICM]). SERCA2a mRNA demonstrated upregulation after LVAD that was not influenced by ICM or DCM. However, only in DCM hearts was the proportion of trabeculae exhibiting a normal FFR increased after LVAD. Thus, although upregulated SERCA2a gene expression after LVAD support is independent of myopathic origin, normalization of myocardial FFR, an index of SERCA2a function, is not. These data provide new insight into the process of cardiac "reverse molecular remodeling," and underscore potential differences in the impact of disease processes on posttranscriptional events.

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With increasing use of mechanical support for end-stage failing hearts, perioperative clinicians are now presented with the challenge of not only managing patients during implant or revision/removal of a left ventricular assist device (LVAD), but also caring for those with indwelling LVADs undergoing noncardiac surgery (1,2). Although the massively dilated hearts of patients with end-stage, chronic heart failure (CHF) are often regarded as irrevocably damaged, considerable data now indicate that mechanical circulatory support can lead to reverse structural remodeling of the heart, manifest as reduced left ventricular (LV) mass and chamber size, improved global pump function, and normalized *ex vivo* pressure-volume relations (3–5). Additional investigation of isolated myocytes (6) and isometric LV trabeculae (7) has demonstrated increased contractile function and an enhanced inotropic response to β-adrenergic stimulation after LVAD support that occurs in conjunction with improved cytosolic Ca^{2+} transients (6). Data from our laboratory and others have also demonstrated a variety of subcellular changes within myocytes that contribute to, or result from, normalized structure or function and impact upon the basic biochemical processes of excitation-contraction coupling (5,7,8). This process has been termed "reverse molecular remodeling."

Whereas end-stage heart failure can vary widely in cause, patients are often broadly categorized as having either an ischemic (ICM) or idiopathic (nonischemic) dilated cardiomyopathy (DCM). A common feature of both classifications is a fundamental abnormality in myocyte calcium cycling that becomes functionally manifest as impairment of both contraction and relaxation, particularly in the setting of cardiac stress. Normally, myocyte contraction is initiated by entry of a small amount of activator calcium via the L-type voltage gated calcium channel that then binds to the

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ryanodine-sensitive calcium release channel to cause release of a large amount of calcium from the sarcoplasmic reticulum. After binding to contractile proteins to produce physical shortening of the myocyte, calcium is rapidly moved back into the sarcoplasmic reticulum by the sarcoplasmic endoreticular calcium adenosine triphosphatase subtype 2a (SERCA2a) where it is sequestered on binding proteins until released again for the next cardiac cycle. By mediating this process, SERCA2a plays a prominent role not only in myocyte relaxation (the faster calcium is moved into the sarcoplasmic reticulum the faster relaxation occurs), but also in contraction because the amount of calcium taken up during diastole determines the amount that can be released to produce myocyte shortening during the next systole. Because of this central role in mechanical function of the myocyte, the expression and function of SERCA2a have become a focus of research into the molecular mechanisms of heart failure.

An important characterization of a systolic dysfunction evident in heart failure is the myocardial force-frequency relationship (FFR). Normal myocardium exhibits an increase in force when stimulated with greater frequency (positive FFR). With CHF, however, the FFR tends to be negative (decreased force with increasing stimulation frequency), although force is often similar to that measured in trabeculae (isolated muscle strips) from nonfailing hearts at a low stimulation frequency (e.g., 60 bpm). Previous studies have indicated an association between abnormal FFR and altered expression of genes regulating proteins involved with myocyte calcium cycling, particularly SERCA2a, which is down regulated (5,7,9). In this regard, the FFR can be considered in many ways as an *in vivo* index of SERCA2a function (10). However, unlike the study of genetic syndromes in which a gene associated with a specific phenotype is either absent or only partially present from birth, the study of progressive, disease-associated changes in gene expression is often complicated by wide variability in messenger (m)RNA. Furthermore, because of processes such as translational regulation, posttranslational modification, and protein-protein interaction, changes in mRNA may not be directly reflected in the physiological process for which they encode. Whether LVAD support has similar effects on both SERCA2a gene expression and function in ICM and DCM hearts remains unclear. Previous work, however, has demonstrated aspects of LVAD-induced subcellular changes that are, at least in part, dictated by cardiomyopathic etiology (11,12) leading us to hypothesize that SERCA2a reverse remodeling may be influenced differently by ICM and DCM. To test this hypothesis, we examined two relatively large databases for ICM versus DCM differences in SERCA2a mRNA expression in the context of the proportion of isolated trabeculae exhibiting a positive FFR after LVAD support.

Methods

Under protocols and informed consent waivers approved by the IRB of the New York Presbyterian Hospital, LV myocardium was obtained immediately after explant of the native heart in patients undergoing transplantation. Eighty-four samples (50 CHF [23 DCM and 27 ICM] and 34 CHF+LVAD [18 DCM and 16 ICM]) were flash frozen in liquid nitrogen and used for gene expression analysis. Viable myocardial trabeculae harvested from 75 hearts (51 CHF [29 DCM and 22 ICM] and 24 CHF+LVAD [10 DCM and 14 ICM]) were used for FFR analysis. The gene expression and FFR databases were largely independent, with tissue from only 10 hearts included in both. All LVAD patients were supported with the same type of device (TCI; Thermo Cardio Systems Inc., Woburn, MA), as previously described (5).

For all samples, tissue without gross evidence of infarction or scarring was processed, as previously described (5). Briefly, total RNA was extracted from myocardium with guanidinium thiocyanate followed by centrifugation in cesium chloride solutions. Aliquots of total RNA were then separated by electrophoresis, transferred to nitrocellulose membranes, and hybridized with complimentary (c)DNA for rat SERCA2a (1.181 kb EcoRI fragment), and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.3 kb PstI fragment). All cDNA probes were labeled with a ^{32}P-dCTP (3000 Ci/mmol, Amersham, Buckinghamshire, United Kingdom) to a specific activity of 1×10^6 cpm/μg using a multiprimer DNA labeling system (Amersham). The blots were exposed to X-OMAT AR film and autoradiograms then analyzed by laser densitometry in the linear response range of the radiograph films using GAPDH as an internal standard. The relative expression of SERCA2a was quantified as the ratio of target band intensity to GAPDH band intensity standardized to the average of 3 nonfailing heart samples on the blot. The same nonfailing hearts were used for standardization of all blots with a value of 1 arbitrarily designated as normal (5).

From individual tissue samples, trabeculae <1 mm in diameter were excised and immersed in ice cold Krebs-Ringer solution with 30 mM of 2,3-butanedione monoxime before mounting in a bath with one end connected to a force transducer and the other to a micrometer. During superfusion with 37°C oxygenated Krebs-Ringer solution (rate = 1 mL/min; bath volume = 1 cm^3), trabeculae were stimulated at 1 Hz and allowed to equilibrate for at least 1 h at slack length. The trabeculae were then progressively

Table 1. Patient Demographics Within and Between Databases

	CHF				CHF+LVAD			
Database	mRNA		FFR		mRNA		FFR	
Disease	DCM	ICM	DCM	ICM	DCM	ICM	DCM	ICM
Number	23	27	29	22	18	16	10	14
men/women	17/6	23/4	20/9	19/3	14/4	14/2	7/3	12/2
Age (yr)	47 ± 2	59 ± 1*	48 ± 3	54 ± 2	49 ± 4	57 ± 2*	36 ± 3†	58 ± 4*
LVAD duration (days)	0	0	0	0	97 ± 12	98 ± 17	92 ± 26	103 ± 18

FFR = force frequency relationship; DCM = nonischemic dilated cardiomyopathy; ICM = ischemic cardiomyopathy; CHF = chronic heart failure; LVAD = left ventricular assist device. * Designates DCM versus ICM difference within database; † Designates DCM versus DCM difference between databases.

stretched to L_{max}, the length of maximal force generation. After re-equilibration, stimulation frequency was increased every 5 min at 0.5-Hz increments to a maximum of 2.5 Hz (150 bpm). Data were continuously recorded in digital form, and for each preparation force, development at increasing stimulation frequency was referenced to the baseline level at 1 Hz and designated as 1. A positive FFR was defined as an increase in the normalized force of ≥5% more than baseline at any point during the incremental increase in stimulation frequency.

Patient age along with duration of LVAD support between ICM and DCM patients were compared within and between the mRNA and FFR databases by one-way analysis of variance with Tukey *post hoc* test. Sex differences (proportion of populations that were women) were assessed by z-test with Yates correction. To determine the influence of ICM or DCM, mRNA data were categorized in relation to myopathy (ICM or DCM) and LVAD use (yes or no) and tested for normality and equal variance. Data were then analyzed by two-way analysis of variance and Tukey *post hoc* test. For both ICM and DCM hearts, the proportions of isolated trabeculae demonstrating a positive FFR after LVAD support were compared with those with a positive FFR but no LVAD support by z-test with Yates correction. For all statistical analyses, a P value ≤0.05 was considered significant.

Results

Table 1 depicts demographic data for the individual groups within both the mRNA and FFR databases. For all patients, those with DCM were younger, particularly in regard to the LVAD-supported hearts from the FFR database. The average duration of mechanical support for patients with LVADs was not different between ICM and DCM groups within either database. For all groups, there was an equal preponderance of men.

As shown in Figure 1, there was a wide distribution of SERCA2a mRNA values among samples obtained from individual patients in the CHF and CHF+LVAD groups. The range of data is similar for both ICM and DCM, and when the means of all data (ICM + DCM) are compared, there is a difference between CHF and CHF+LVAD. Figure 2 depicts these data subdivided according to condition (CHF or CHF+LVAD) and

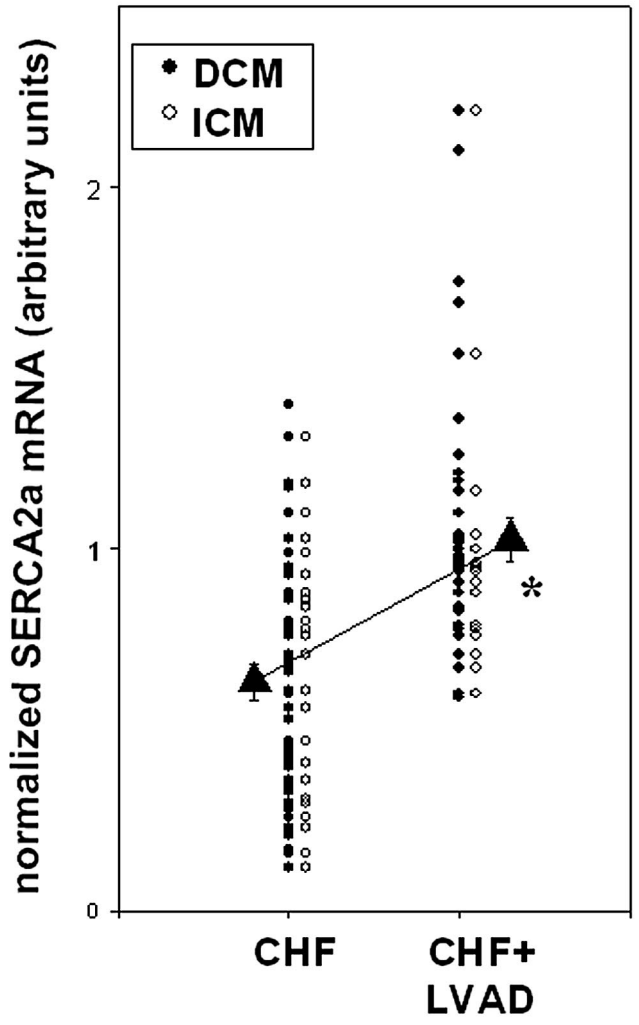

Figure 1. Individual data points for myocardial sarcoplasmic endoreticular calcium adenosine triphosphatase subtype 2a (SERCA2a) messenger (m)RNA values (n = 84) in chronic heart failure (CHF) patients; LVAD = left ventricular assist device; closed circles represent patients with globally dilated cardiomyopathy (DCM); open circles represent patients with ischemic cardiomyopathy (ICM). Triangles depict the mean (with SE) of all CHF or CHF+LVAD data. *designates difference from CHF; $P \leq 0.05$.

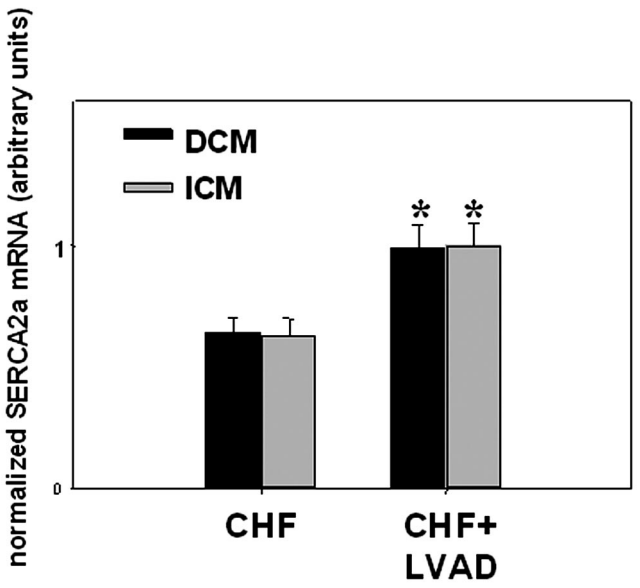

Figure 2. Comparison of myocardial sarcoplasmic endoreticular calcium adenosine triphosphatase subtype 2a (SERCA2a) messenger (m)RNA data when categorized by myopathy (DCM = globally dilated cardiomyopathy; ICM = ischemic cardiomyopathy). *designates difference from CHF; $P \leq 0.05$. Data are presented as mean ± SE.

disease (ICM or DCM) and demonstrates that the CHF versus CHF+LVAD differences are present regardless of myopathic origin.

Representative tracings depicting positive and negative FFR responses are shown in Figure 3. Pooled data are shown in Figure 4 and demonstrate that in contrast to the mRNA data (no ICM versus DCM difference), the proportion of trabeculae exhibiting a positive FFR after LVAD was increased more than that in non–LVAD-supported hearts only in DCM patients.

Discussion

Although a variety of LVAD technologies have become increasingly applied for both acute and long-term support of patients with severe heart failure, only recently has it been suggested that some patients awaiting transplant may actually demonstrate sufficient recovery to allow for LVAD removal without subsequent transplantation (13). This has led to the concept of using LVADs not only as a "bridge to transplant," but also as part of a "bridge to recovery" strategy, in which mechanical support of various degrees may be combined with secondary interventions to sustain reverse remodeling and maintain normalized mechanical function (14). The prospect of such a therapeutic course becomes of particular interest to cardiothoracic anesthesiologists and intensivists who will ultimately become involved with the perioperative care of these complex patients. Hopefully, better understanding of

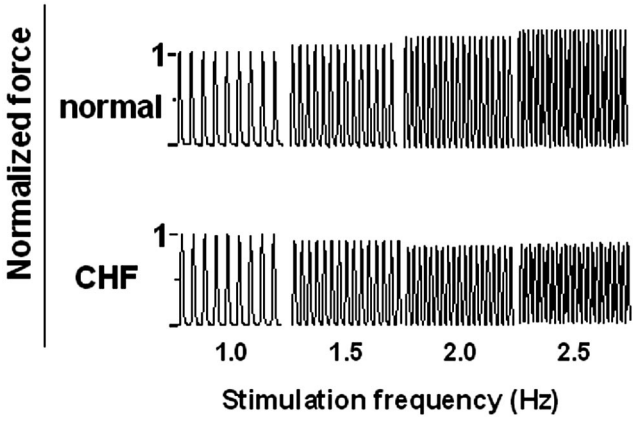

Figure 3. Representative trabecular force tracings depicting a normal positive response force (increased force with increasing frequency of stimulation) and an abnormal negative response (decreasing force with increasing stimulation frequency) as seen in failing myocardium.

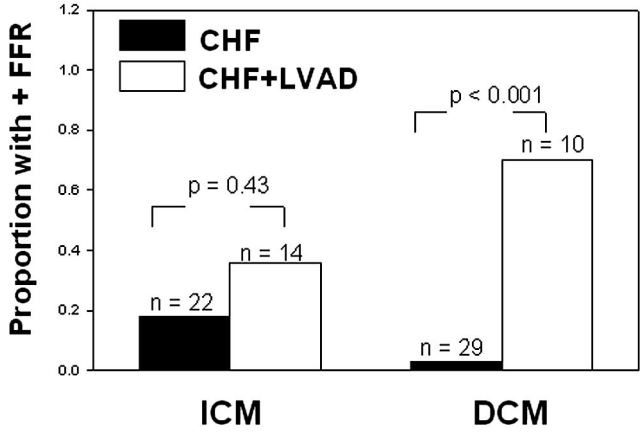

Figure 4. The proportion of trabeculae from ischemic cardiomyopathy (ICM) and globally dilated cardiomyopathy (DCM) hearts with a positive force frequency relationship (FFR). For ICM, the 95% confidence interval for the chronic heart failure (CHF) versus CHF+left ventricular assist device (LVAD) difference is −0.465–0.115; for DCM the 95% confidence interval is −0.956 to −0.376.

the process of reverse remodeling and functional recovery will aid the development of adjunctive therapies and promote design of optimum use protocols to improve patient outcome. The present study demonstrates that although LVAD-induced normalization of SERCA2a gene expression is not influenced by myopathic origin, normalization of the FFR within isolated muscle trabeculae, an index of SERCA2a protein function, occurs predominantly in DCM hearts. These data underscore the fact that the path between an alteration in gene expression and an eventual change in physiological function has multiple components and suggest that the factors modulating this process vary between ICM and DCM.

The mechanisms for disease-dependence of reverse remodeling are unclear. For all myopathies, the mechanical distortion of myocytes that accompanies increased pressure and volume triggers a sequence of events that eventually lead to forward remodeling of individual cells. Whereas the major stimulus is probably physical stretch, autonomic neurotransmitters and intracardiac paracrine/autocrine mediators also contribute to a cascade of immediate, and ultimately prolonged, molecular and cellular events mediated in part by altered expression of genes within both myocytes and noncontractile elements of the myocardium (15). The capacity for LVADs to directly provide pressure and volume unloading of the LV and indirectly normalize the neurohormonal milieu has been described (3,7,16), and multiple lines of investigation have indicated a wide variety of secondary subcellular changes (4–17). Data from our laboratory have documented increased expression of the SERCA2a gene after LVAD support (5), and microarray analysis (that did not report SERCA2a) has indicated a range of other genes that are also altered (12). As with studies of reverse structural remodeling (3,18), the LVAD-induced alterations in myocardial genomic footprint, as defined by microarray, have been reported to be more prominent in DCM hearts (12). DCM tends to be a global process influencing most myocytes that, although impaired, are viable. Accordingly, reduction of LV pressure and volume in conjunction with normalization of the neurohormonal milieu may provide a relatively uniform stimulus that impacts upon a relatively uniform fundamental deficit. In contrast, other investigation has revealed normalization of mitochondrial ultrastructure after LVAD that occurs only in the setting of ICM (11). This response has been attributed to the fact that ICM is a more heterogeneous process than DCM, so that relatively normal tissue interspersed with diseased myocardium may exhibit an enhanced capacity to recover once the metabolic stress of increased pressure and volume are relieved. How multiple disease-dependent variables specifically interact to potentially influence gene expression is uncertain, and, in the context of the current study, seemingly unimportant in terms of SERCA2a gene expression. However, the observation that the proportion of hearts exhibiting a positive FFR after LVAD was increased only in DCM hearts highlights the fact that whereas underlying disease may not have had differential effects on transcription, there may well be upstream effects on translation, posttranslational modification, or modulators of protein function to enhance SERCA2a activity (19,20).

Results of the study need to be interpreted in the context of certain limitations. First, although sample sizes for both mRNA and FFR analysis were quite large in comparison to previous studies, the data do not allow for further investigation into the possible influences of other variables, such as age, sex, and pharmacotherapy. Since aging alone has been shown to alter expression of calcium cycling genes (particularly SERCA2a) and to dampen the process of compensatory remodeling in response to increased pressure and volume (21), there is also a theoretical basis for a possible effect on reverse remodeling. In this regard, the finding that in the FFR database DCM patients supported with an LVAD were younger than those in the mRNA database raises the question of whether functional recovery could be more pronounced in young hearts. Similarly, data demonstrating sex-related differences in sarcoplasmic reticulum calcium cycling in heart failure (22) indicate the possibility that the fundamental molecular abnormality may be influenced by gender, with attendant effects on the nature of any LVAD-induced reversal. In addition, experimental and clinical studies have indicated that drugs such as angiotensin converting enzyme inhibitors and β-adrenergic blockers can prevent or reverse structural, and perhaps molecular forward remodeling, in the setting of heart failure (23,24). Whereas our database analysis did not indicate pharmacotherapy differences among groups, a complete data set was not available for every patient, so we cannot say with absolute certainty that there are not differences. Second, the study does not shed additional light on the functional significance of normalized SERCA2a gene expression. Although short-term augmentation of SERCA2a expression in isolated human myocytes via gene transfer is associated with enhanced contractile function (25), and our own data show normalization of myocardial FFR after LVAD that is coincident with SERCA2a upregulation (5), posttranslational events, and interaction with regulatory proteins probably play a significant role in determining any eventual effects of altered gene expression on mechanical performance. Indeed, results of the current study showing an LVAD effect on FFR only in DCM hearts support the prospect of myopathy-dependent factors having substantial influence on the transition from increased SERCA2a mRNA to altered cellular function. Accordingly, extrapolation of altered mRNA message to specific changes in myocardial function in individual hearts is difficult.

In summary, whereas the data demonstrate upregulated SERCA2a gene expression in the failing heart supported with an LVAD, they do not reveal a differential influence of ICM or DCM on the process. In contrast, normalization of the FFR in isolated trabeculae, an index of *in vivo* SERCA2a function, occurs predominantly in DCM hearts. Taken together, these results underscore the fact that the path between gene expression and physiological function has multiple

components, and suggest a variable influence of ICM and DCM.

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