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Left Ventricular Assist Device Support Normalizes Left and Right Ventricular Beta-Adrenergic Pathway Properties

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OBJECTIVES	We hypothesized that some aspects of left ventricular assist device (LVAD) reverse remodeling could be independent of hemodynamic factors and would primarily depend upon normalization of neurohormonal milieu.
BACKGROUND	The relative contributions of LVAD-induced hemodynamic unloading (provided to the left ventricle [LV]) and normalized neurohormonal milieu (provided to LV and right ventricle [RV]) to reverse remodeling are not understood.
METHODS	Structural and functional characteristics were measured from hearts of 65 medically managed transplant patients (MED), 30 patients supported with an LVAD, and 5 nonfailing donor hearts not suitable for transplantation.
RESULTS	Compared with MED patients, diastolic pulmonary pressures trended lower ($p < 0.01$) and cardiac output higher ($p < 0.001$) in LVAD patients; V_{30} (ex vivo ventricular volume yielding 30 mm Hg, an index of ventricular size) in LVAD patients was decreased in the LV ($p < 0.05$) but did not change significantly in RV. The LVAD support improved force generation in response to beta-adrenergic stimulation in isolated LV (increase in developed force from 6.3 ± 0.6 to 18.5 ± 4.4 mN/m ² , $p < 0.01$) and RV (increase in developed force, from 10.9 ± 2.0 to 20.5 ± 3.1 mN/m ² , $p < 0.05$) trabeculae. The LVAD patients had higher myocardial beta-adrenergic receptor density in LV ($p < 0.01$) and RV ($p < 0.01$). Protein kinase A (PKA) hyperphosphorylation of the ryanodine receptor 2 (RyR2)/calcium release channel was significantly reduced by LVAD in both RV and LV ($p < 0.01$).
CONCLUSIONS	Improved beta-adrenergic responsiveness, normalization of the RyR2 PKA phosphorylation, and increased beta-adrenergic receptor density in LV and RV after LVAD support suggest a primary role of neurohormonal environment in determining reverse remodeling of the beta-adrenergic pathway. (J Am Coll Cardiol 2005;45:668–76) © 2005 by the American College of Cardiology Foundation

Left ventricular mechanical assist devices (LVADs) reverse many of the molecular, cellular, extracellular, ventricular structural, neurohormonal, and peripheral abnormalities characteristic of end-stage heart failure (1–16). These phenomena are collectively referred to as *reverse remodeling* (1). We recently showed that several aspects of reverse remodeling including left ventricle (LV) myocyte diameter, sarcoplasmic reticulum Ca²⁺-ATPase, isoform 2a (SERCA2a) levels, and force-frequency relations of isolated superfused trabeculae were directly related to LVAD-mediated mechanical unloading of the LV by showing that these changes do not occur in the right ventricle (RV) (17). We postulated, however, that some aspects of reverse remodeling could be independent of hemodynamic factors and would

primarily depend on normalization of neurohormonal milieu (17).

It has recently been shown that LVAD support restores beta-adrenergic responsiveness and reverses receptor down-regulation in failing human hearts (13). It was postulated that this effect might be due to the mechanical unloading of the ventricle. However, beta-blockers also reverse down-regulation of beta-adrenergic receptors, improve myocardial beta-adrenergic responsiveness, and induce some degree of reverse remodeling without significant hemodynamic unloading of either ventricle and without substantial improvement in systemic hemodynamics (18–25).

The RV and LV mainly share a common biochemical milieu, but the hemodynamic effects of LVADs are different in the two chambers (17). Accordingly, we took advantage of this difference to test the hypothesis that reverse remodeling of the beta-adrenergic pathway is independent of hemodynamic factors and primarily depends on systemic factors shared by the two ventricles.

METHODS

Heart harvest and pressure-volume relationships. Data presented in this study were derived from 95 human hearts

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Abbreviations and Acronyms

LV	= left ventricle/ventricular
LVAD	= left ventricular assist device
PKA	= protein kinase A
RV	= right ventricle/ventricular
RyR	= ryanodine receptor
SERCA2a	= sarcoplasmic reticulum Ca^{2+} -ATPase, isoform 2a

of patients with end-stage heart failure after orthotopic heart transplant between October 1999 and July 2003 under a protocol approved by the Institutional Review Board of the New York Presbyterian Hospital. A total of 65 of the hearts were from medically managed patients undergoing transplantation without the need for LVAD support (medical support group), and the other 30 hearts were from patients requiring LVAD support (HeartMate, Thoratec Co., Pleasanton, California) for more than 30 days before transplant. Furthermore, five hearts from nonfailing donors not suitable for transplantation were available.

At the time of transplantation, hearts were preserved with cold (4°C) hypocalcemic, hyperkalemic cardioplegia solution at explant. The passive ventricular pressure-volume relationships were measured by placing compliant balloons in the RV and LV as detailed previously (1,9). Right and left ventricular chamber sizes were indexed by the volume yielding an intraventricular pressure of 30 mm Hg (LVV₃₀ and RVV₃₀, respectively).

Myocardial force generation in response to beta-adrenergic stimulation. Baseline force generation and force generation in response to beta-adrenergic stimulation were measured from trabeculae (<0.8 mm² cross-sectional area) isolated from RVs and LVs in a subset of patients as described previously (9,17). Trabeculae were mounted in a bath with one end connected to a force transducer and the other to a micrometer. Two identical baths (one for the LV strip and one for the RV strip) were simultaneously perfused from the same warming flask containing oxygenated Krebs-Ringer solution (37°C; flow rate 1 cc/min; bath volume 1 cc). Trabeculae were equilibrated at slack length for 1 h at a stimulation rate of 1 Hz. Trabeculae were then stretched to L_{max}, the length of maximal force generation, and reequilibrated for 30 min. Adequate muscle oxygenation was confirmed as detailed previously (17). After recording baseline force, contractile response to beta-adrenergic stimulation was measured by switching to a perfusate of identical composition, oxygenation, and temperature containing isoproterenol (1 µg/ml).

Beta-adrenergic receptor density. Beta-antagonist binding studies were performed to determine beta-receptor density in RV and LV myocardium. All experiments were done in duplicate in the presence of 100 mM Tris, 5 mM Mg Cl₂, 1 mM EDTA, pH 7.2; 25 µl [³H]dihydroalprenolol ([³H]DHA) 1 to 40 nM, 25 µl propranolol (10 µM), or buffer, and 200 µg of membrane protein were used for each assay. Samples were incubated at 37°C for 30 min, and

the binding reaction was terminated by rapid filtration on Whatman GF/C filters (Whatman Chemical Separation, Clifton, New Jersey). The filters were dried and counted in 7.0 ml of scintillation fluid (ScintiSafe 30%, Fisher Scientific, Pittsburgh, Pennsylvania) for 5 min.

Immunoprecipitation and back-phosphorylation of ryanodine receptor (RyR). To test RyR2 protein kinase A (PKA) phosphorylation, RV and LV myocardial homogenates were prepared as detailed previously (10) by homogenizing approximately 1.0 g of cardiac tissue in 1.0 ml of a buffer containing 50 mM Tris-HCl (pH 7.4), 200 mM NaCl, 20 mM NaF, 1.0 mM Na₃VO₄, 1.0 mM DTT, and protease inhibitors. Samples were centrifuged (3,000 g for 10 min), and RyR2 was immunoprecipitated by incubating 500 µg of homogenate with anti-RyR antibody in 0.5 ml of a modified RIPA buffer (50 mM Tris-HCl [pH 7.4], 0.9% NaCl, 1.0 mM NaF, 1.0 mM Na₃VO₄, 0.25% Triton X100, and protease inhibitors) overnight at 4°C. Protein A sepharose beads were added, incubated at 4°C for 1 h, washed with 1 × phosphorylation buffer (8 mM MgCl₂, 10 mM EGTA, and 50 mM Tris/piperazine-N,N'-bis[2-ethanesulfonic acid], pH 6.8), and resuspended in 10 µl of a 1.5 × phosphorylation buffer containing either PKA catalytic subunit (Sigma, St. Louis, Missouri) or PKA plus a PKA inhibitor (PKI₅₋₂₄, 500 nM, Calbiochem, San Diego, California). Back phosphorylation of immunoprecipitated RyR2 was initiated with 33 µM Mg-ATP containing 10% [γ -³²P]ATP (NEN Life Sciences, Boston, Massachusetts) and terminated after 5 min at room temperature with 5 µl of stop solution (4% SDS and 0.25 M DTT). Samples were heated to 95°C, size fractionated on 6% SDS-PAGE, and RyR2 radioactivity was quantified using a Molecular Dynamics Phosphorimager and ImageQuant software (Amersham Pharmacia Biotech, Piscataway, New Jersey). Nonspecific phosphorylation (not inhibited by PKA inhibitor) was subtracted, the resulting value was divided by the amount of RyR2 protein (determined by immunoblotting and densitometry), and expressed as the inverse of the PKA-dependent [γ -³²P] ATP signal.

Statistics. Data are expressed as mean ± SD values. One-way analysis of variance with Bonferroni correction was used to select differences between groups. The statistical analysis was conducted with a commercially available statistical software (SPSS 11.5, Chicago, Illinois). A value of p < 0.05 was considered to be statistically significant.

RESULTS

Patient population. Patient demographics are summarized in Table 1. Patients requiring LVAD support tended to be younger and were mostly male. The distribution between ischemic and idiopathic dilated cardiomyopathic patients was similar in both groups. All but four of the LVAD patients were receiving intravenous inotropic support with dobutamine or milrinone before LVAD insertion, while these drugs were used in only 49% of transplant patients

Table 1. Patient Demographics and Hemodynamics (Mean ± SD)

	Medical Support at Transplant	LVAD Support	
		At Insertion	At Transplant
Number of patients	65	30	
Age (yrs)	53.6 ± 11.4	48.4 ± 14.1	
Male/female	42/23*	26/4	
ICM/DCM	29/36	13/17	
CVP (mm Hg)	9.8 ± 6.4	13.4 ± 5.6	10.7 ± 5.4
dPAP (mm Hg)	20.5 ± 6.5†	27.1 ± 6.4¶	13.8 ± 7.4**
mPAP (mm Hg)	27.8 ± 9.2‡	37.4 ± 9.4¶	19.2 ± 7.5††
mAP (mm Hg)	80.1 ± 11.9†	76.5 ± 8.6	86.6 ± 9.8**
CO (l/min)	3.6 ± 1.1§	4.1 ± 1.3	5.5 ± 1.6**
Receiving inotropic support	32 (49%)§	26 (87%)#	1 (3%)††
IABP use	0	10¶	0††
Duration of LVAD support (days)	NA	NA	89.7 ± 72.2

*p < 0.001 vs. LVAD; †p < 0.05; ‡p < 0.01; §p < 0.001 vs. LVAD support at transplant; ||p < 0.05; ¶p < 0.001; #p < 0.01 vs. medical support at transplant; **p < 0.01; ††p < 0.001 vs. LVAD support at insertion.

CO = cardiac output; CVP = central venous pressure; DCM = idiopathic dilated cardiomyopathy; dPAP = diastolic pulmonary artery pressure; IABP = intraaortic balloon pump; ICM = ischemic cardiomyopathy; LVAD = left ventricular assist device; mAP = mean arterial pressure; mPAP = mean pulmonary artery pressure; NA = not applicable.

(p < 0.01). Ten patients received intraaortic balloon pumping before LVAD insertion. The mean duration of the LVAD support was 89.7 ± 72.2 days (range 30 to 360 days). Inotropic support was discontinued in all LVAD patients, except one, within one week of LVAD insertion.

Hemodynamic data were collected under anesthesia at the time of LVAD insertion and at the time of heart transplantation for both groups (Table 1). Hemodynamic parameters at LVAD insertion showed significant elevated central venous pressure (p < 0.05) and pulmonary artery diastolic and mean pressures (p < 0.001) versus the medically supported group. Data for the LVAD patients at time of transplantation were obtained with the device active and in “auto” mode; LVAD support decreased pulmonary diastolic and mean pressures and improved arterial blood pressure and cardiac output (p < 0.01) significantly versus the medically supported group.

LV and RV size. Ventricular size, indexed by V₃₀, is summarized in Figure 1. The mean LVV₃₀ of hearts from medically supported patients was significantly larger than that of hearts after LVAD support (p < 0.05) and trended

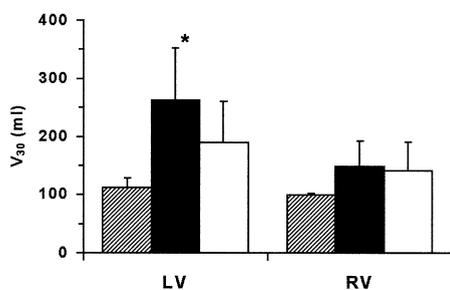


Figure 1. V₃₀, the ex vivo volume providing a ventricular pressure of 30 mm Hg, is an index of ventricular size. Left ventricular (LV) V₃₀ was decreased after left ventricular assist device (LVAD) support, but right ventricular (RV) V₃₀ did not change significantly. Ruled bars = nonfailing hearts; black bars = medical support; open bars = LVAD support. All data are mean ± SD. *p < 0.05 vs. LVAD support and nonfailing hearts.

toward levels from nonfailing hearts. In contrast, there was no change between the LVAD and MED group with respect to RVV₃₀.

Myocardial force generation in response to beta-adrenergic stimulation. The cross-sectional area of LV and RV endocardial trabeculae were similar between nonfailing, medical-supported, and LVAD-supported hearts (LV, 0.49 ± 0.13, 0.46 ± 0.14, and 0.45 ± 0.19 mm², respectively; RV, 0.42 ± 0.19, 0.41 ± 0.23, and 0.43 ± 0.07 mm², respectively). The myocardial responses to beta-adrenergic stimulation of individual LVAD-supported, medically supported, and nonfailing hearts are shown in Figure 2. These tracings show that, while the response to isoproterenol is significantly blunted in myocardium of the medically supported hearts, it is robust and similar to that observed in the nonfailing myocardium in both right and left ventricular myocardium of LVAD-supported hearts. Baseline and isoproterenol-stimulated force generation, summarized in Figures 3A and 3B for all muscles studied confirm that, as in the examples of Figure 2, LVAD support was associated with improved beta-adrenergic responsiveness in both LV and RV myocardium. Baseline developed force production was similar in LV (MED, 10.25 ± 0.85 mN/mm²; LVAD, 9.79 ± 1.41 mN/mm²; nonfailing, 9.53 ± 1.31 mN/mm²) and RV (MED, 13.92 ± 1.56 mN/mm²; LVAD, 23.44 ± 4.1 mN/mm²; nonfailing, 22.48 ± 4.3 mN/mm²). However, the increase in force generation in response to isoproterenol was substantially larger in LVAD-supported patients than in medically supported patients for muscles from both the LV and RV (Fig. 3C, Table 2). In addition, while the maximum rate of force increase (+dF/dt) and the maximum rate of force decline (-dF/dt) were similar at baseline in all groups in LV (+dF/dt: nonfailing, 59.4 ± 13.9; MED, 72.5 ± 6.2; LVAD, 69.0 ± 7.2 mN/mm²·s and -dF/dt: nonfailing, -40.9 ± 6.9; MED, -50.7 ± 4.4; LVAD, -51.9 ± 8.9 mN/mm²·s) and RV (+dF/dt: nonfailing, 117.5 ± 6.6; MED, 99.4 ± 10.2;

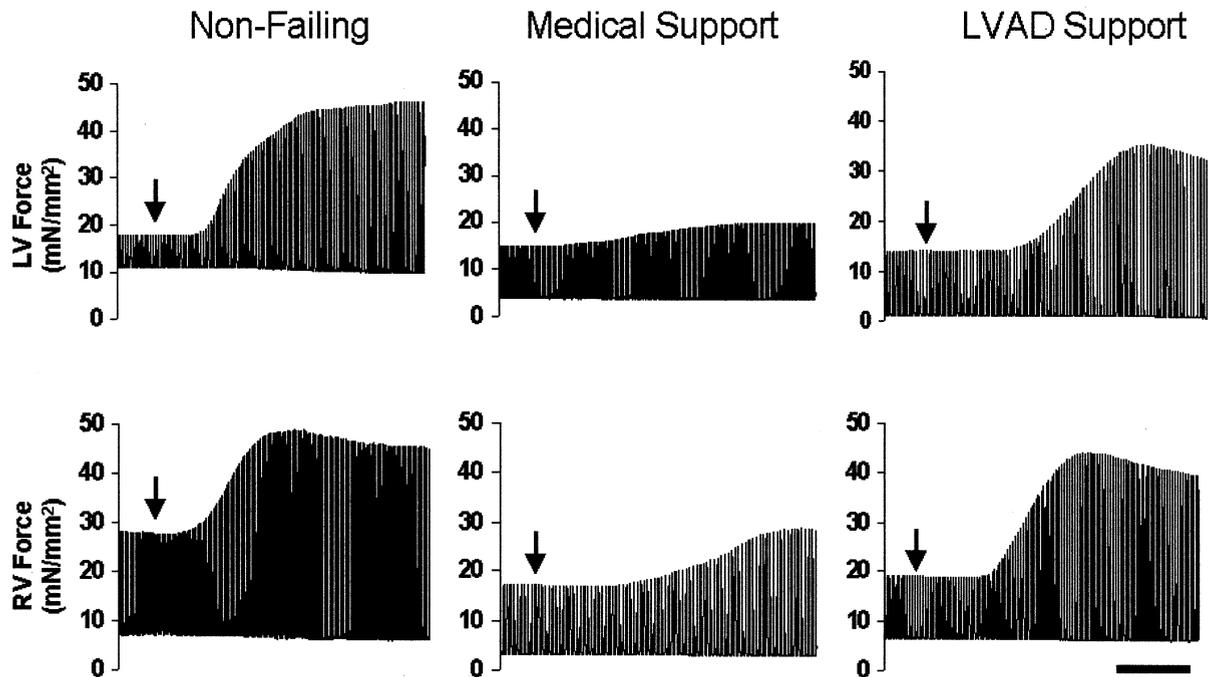


Figure 2. Representative force tracing from right ventricular (RV) and left ventricular (LV) endocardial trabeculae showing the responses to isoproterenol ($1 \mu\text{g/ml}$). Whereas responses were blunted in RV and LV muscle of medically managed transplanted hearts, left ventricular assist device (LVAD) support markedly improved responses in both ventricles similar to nonfailing hearts. **Arrows** = time when perfusion was switched to solution containing isoproterenol. Scale bar = 30 s.

LVAD, $136.3 \pm 24.4 \text{ mN/mm}^2\cdot\text{s}$ and $-\text{dF}/\text{dt}$: nonfailing, -110.1 ± 1.4 ; MED, -73.7 ± 8.2 ; LVAD, $-107.3 \pm 21.7 \text{ mN/mm}^2\cdot\text{s}$) trabeculae, LVAD support lead to a significant improvement during isoproterenol stimulation (Table 2).

Inotropic therapy, commonly used before transplant and LVAD insertion, is known to modify beta-adrenergic responsiveness. We, therefore, compared responses in patients receiving and not receiving such treatment. For medically treated patients, the increase in myocardial contractile force in trabeculae from patients receiving intravenous inotropic agents before transplant (mean increase in isometric developed force generation of $6.40 \pm 1.37 \text{ mN/mm}^2$ for LV and $9.16 \pm 2.38 \text{ mN/mm}^2$ for RV) was no different than that in myocardium from patients treated without inotropic agents (mean increase in isometric developed force generation of $7.82 \pm 1.18 \text{ mN/mm}^2$ for LV and $10.86 \pm 1.5 \text{ mN/mm}^2$ for RV). Only one of the LVAD patients was receiving inotropic support before transplant.

It is also known that chronic beta-blocker treatment can improve inotropic response to beta-adrenergic stimulation. Ten of the medically treated patients for whom muscle data are available were receiving beta-blockers up to the time of transplant. In these patients the mean increase of developed force in response to isoproterenol was $9.18 \pm 0.72 \text{ mN/mm}^2$ ($n = 10$), which compared with $6.6 \pm 1.22 \text{ mN/mm}^2$ for patients who never received a beta-blocker ($n = 19$, $p < 0.05$). Thus, beta-blocker treatment can improve beta-adrenergic responsiveness but to a much lesser degree than do LVADs. For RV trabeculae the mean increase in

developed force generation in response to isoproterenol was $11.35 \pm 2.86 \text{ mN/mm}^2$ in patients receiving beta-blocker therapy ($n = 5$) and $9.59 \pm 2.25 \text{ mN/mm}^2$ in patients not receiving beta-blocker treatment ($n = 11$), but this difference did not reach significance. There was not sufficient data to make this assessment for the LV or RV trabecular data of LVAD patients, because only two were taking beta-blockers at the time of transplant.

Finally, the duration of LVAD support did not correlate with the magnitude of force response to isoproterenol in this group. Note, however, that LVAD patients supported for <30 days were excluded from study.

Beta-adrenergic receptor density. Scatchard analysis of [^3H]DHA binding demonstrated a significant difference in beta-adrenergic receptor density in the medically supported group compared with the LVAD-supported and nonfailing group in both the LV and RV. Representative Scatchard plots of LV and RV myocardial samples for all groups are shown in Figures 4A and 4B. Averaged values for the receptor density (indexed by the plateau of the binding curve, B_{max}) are presented in Figure 4C and Table 3. In hearts receiving LVAD treatment before transplant, B_{max} was significantly increased in both LV and RV in comparison with medically supported hearts ($p < 0.01$) and reached almost normal levels. No significant differences existed in the receptor affinity (K_d) in any of the tissue studied (Table 3).

Although beta-agonist therapy can, in general, influence beta-receptor density, this was not the case in these end-stage heart failure patients, a finding that is concordant with

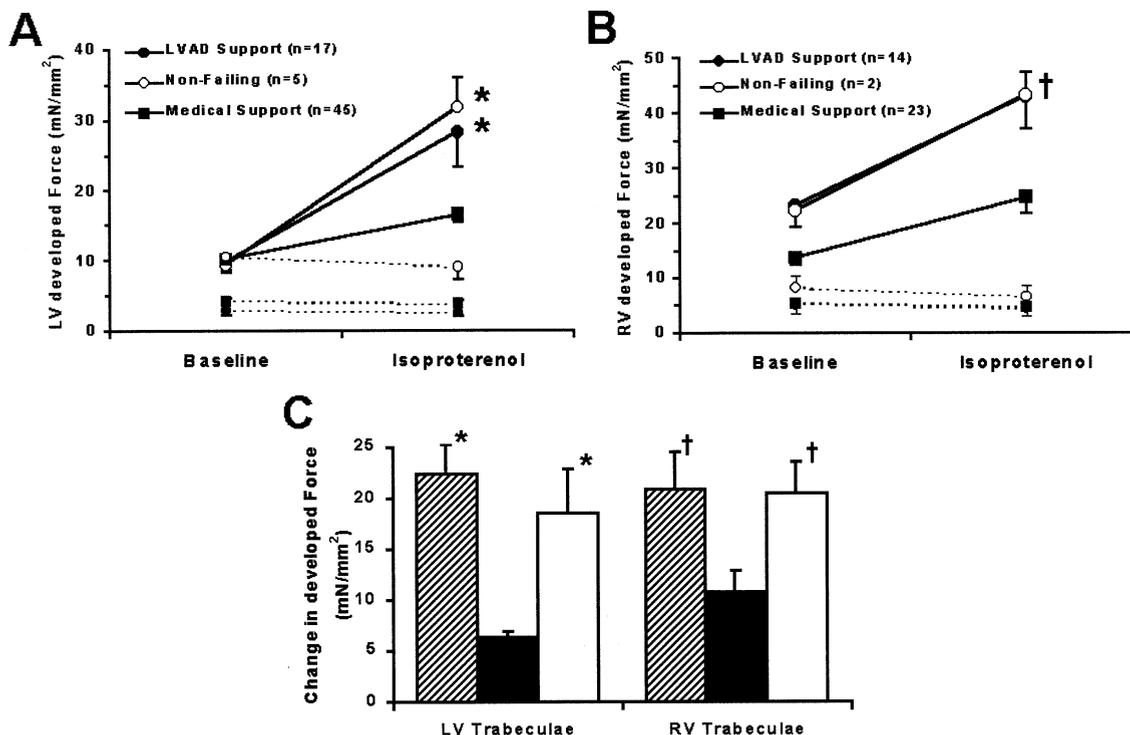


Figure 3. Change of developed (filled lines) and diastolic (dashed lines) isometric force of left ventricle (LV) (A) and right ventricle (RV) (B) endocardial trabeculae from baseline to peak response to isoproterenol perfusion (1 $\mu\text{g}/\text{ml}$). Left ventricular assist device (LVAD) support resulted in significantly increased developed isometric force generation in both the LV and RV muscle. (C) Change in isometric developed force generation after isoproterenol perfusion. Ruled bars = nonfailing hearts; black bars = medical support; open bars = LVAD support. All data are mean \pm SEM. * $p < 0.01$, † $p < 0.05$ vs. medical support.

the lack of difference in beta-adrenergic-stimulated contractile force noted above. Among patients not receiving beta-blockers, beta-receptor density averaged 24.9 ± 4.3 fmol/mg and 30.5 ± 3.5 fmol/mg for the LV and RV, respectively, in patients not receiving beta-agonist therapy before transplant, compared with 23.2 ± 1.1 fmol/mg and 25.6 ± 0.6 fmol/mg, respectively, in those receiving beta-agonist therapy.

LVAD treatment reverses RV and LV PKA hyperphosphorylation of cardiac RyR2. Ryanodine receptor was immunoprecipitated (IP) from RV and LV samples of

normal (n = 3), medically managed (n = 2), and LVAD-supported (n = 4) hearts and were back-phosphorylated with [γ - ^{32}P]ATP in the presence of PKA (Fig. 5A); the increased signal (darker band) corresponds to higher amounts of ^{32}P transferred to RyR2 by PKA, which is indicative of reduced RyR2 PKA phosphorylation in vivo. A similar reaction carried out in the presence of PKA inhibitor (PKI) was performed to quantify background levels of nonspecific ^{32}P incorporation. As shown in representative examples (Fig. 5A), the RyR2 ^{32}P signals after treatment with PKA were significantly higher in samples from normal and LVAD samples than from medically managed patients, indicating that the in vivo PKA phosphorylation of RyR2 was significantly reduced by LVAD treatment as previously reported (11). Moreover, the LVAD-associated reduction in RyR2 PKA hyperphosphorylation was similar in LV and RV (Fig. 5B).

Table 2. Mechanical Parameters of Isolated Trabeculae at Isoproterenol Stimulation

	+dF/dt, mN/mm ² ·s	-dF/dt, mN/mm ² ·s
LV		
NF	260.3 \pm 40.0	-220.8 \pm 34.5
MED	150.8 \pm 12.2*	-114.8 \pm 9.2*
LVAD	317.4 \pm 71.3	-281.4 \pm 62.5
RV		
NF	603.5 \pm 75.1	-407.0 \pm 53.5
MED	291.1 \pm 45.0†	-232.3 \pm 35.4
LVAD	474.9 \pm 63.4	-355.7 \pm 49.8‡

* $p < 0.01$ and † $p < 0.05$ vs. NF and LVAD; ‡ $p < 0.05$ vs. MED.
LV = left ventricular; LVAD = left ventricular assist device support; MED = medical support; NF = nonfailing; RV = right ventricular; +dF/dt = maximum rate of force increase; -dF/dt = maximum rate of force decline.

DISCUSSION

Consistent with our prior study (17), LVAD support normalized systemic hemodynamics, markedly reduced hemodynamic load on the LV, but did not significantly change the preload on the RV. Thus, hemodynamic changes were associated with reverse structural remodeling of the LV, but not the RV. Despite these differential hemodynamic and structural remodeling effects of LVAD support, we observed

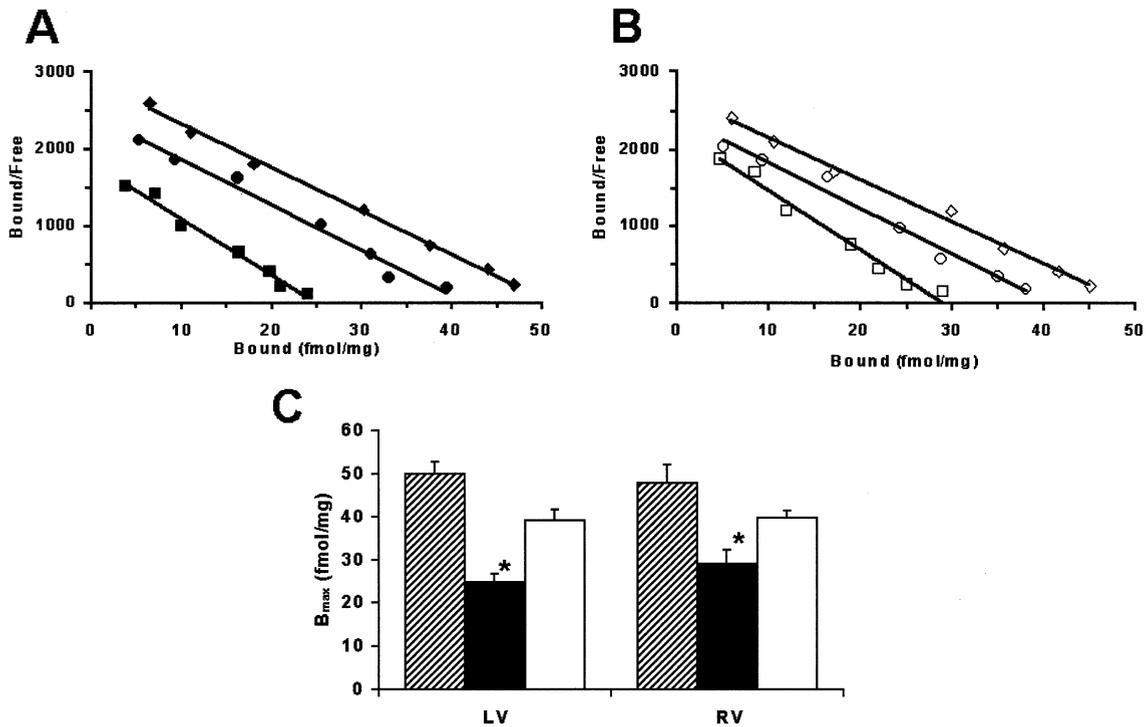


Figure 4. Representative Scatchard blots of [³H]DHA binding in left ventricular (LV) (A) and right ventricular (RV) (B) samples are shown of nonfailing (triangles), medical (squares), and left ventricular assist device (LVAD)-supported hearts (circles). (C) Density of beta-adrenergic receptors in LV and RV myocardial samples of nonfailing (ruled bars), medical (black bars), and LVAD-supported hearts (open bars). Data reported are the means ± SD of maximal binding (B_{max}) values determined from Scatchard transformation of saturated binding data. *p < 0.01 vs. nonfailing and LVAD-supported hearts.

equal recovery of beta-adrenergic myocardial responsiveness, beta-adrenergic receptor density, and reversal of RyR2 hyperphosphorylation in the RV and LV. This suggests that reverse remodeling of the beta-adrenergic pathway during LVAD support is primarily mediated by systemic factors (such as biochemical milieu) and is not directly mediated by hemodynamic factors.

We have previously shown through a comparison of LV and RV properties of LVAD-supported hearts that hemodynamic unloading, not neurohormonal factors, is primarily responsible for decreasing myocyte diameter, decreasing ventricular chamber size, increasing relative collagen content, increasing SERCA2a expression, and improving the myocardial force–frequency relationship (17). We hypothesized, however, that this may not necessarily be the case for other aspects of reverse remodeling. Given the strong dependence of regulation

of beta-adrenergic signaling on circulating catecholamine levels and recent evidence showing that LVAD support normalizes neurohormonal milieu (11,26) (including circulating norepinephrine levels), restores beta-adrenergic receptor density, and improves the ability of cardiac muscle to respond to beta-adrenergic stimulation (13), we hypothesized that functional restoration of this pathway would be mediated by systemic factors, a hypothesis that is confirmed by the present results.

Importantly, because structural and other key aspects of reverse remodeling identified in the present and our prior study (17) occur only in the LV, the results of the present study indicate that restoration of beta-adrenergic pathway function is not sufficient to reverse all of the abnormalities of end-stage failing hearts requiring transplantation.

We have previously shown that the calcium release channel of the sarcoplasmic reticulum (SR) is PKA hyper-

Table 3. B_{max} and K_d Values for [³H]DHA Binding to Beta-Adrenergic Receptors in LV and RV of Nonfailing, Medical, and LVAD-Supported Hearts

	Nonfailing		Medical Support		LVAD Support	
	LV	RV	LV	RV	LV	RV
B _{max} (fmol/mg)	50.0 ± 2.8	48.0 ± 4.2	24.8 ± 1.9*	29.0 ± 3.4*	39.2 ± 2.5	39.8 ± 1.7
K _d (nM)	18.0 ± 0.4	17.6 ± 0.38	13.1 ± 0.5	15.2 ± 1.52	16.5 ± 0.38	16.9 ± 0.69

Values are means ± SD for maximal binding (B_{max}) and receptor affinity (K_d) as determined from Scatchard transformations of saturation binding data. *p < 0.01 compared with nonfailing and left ventricular assist device (LVAD) support.

LV = left ventricle; RV = right ventricle.

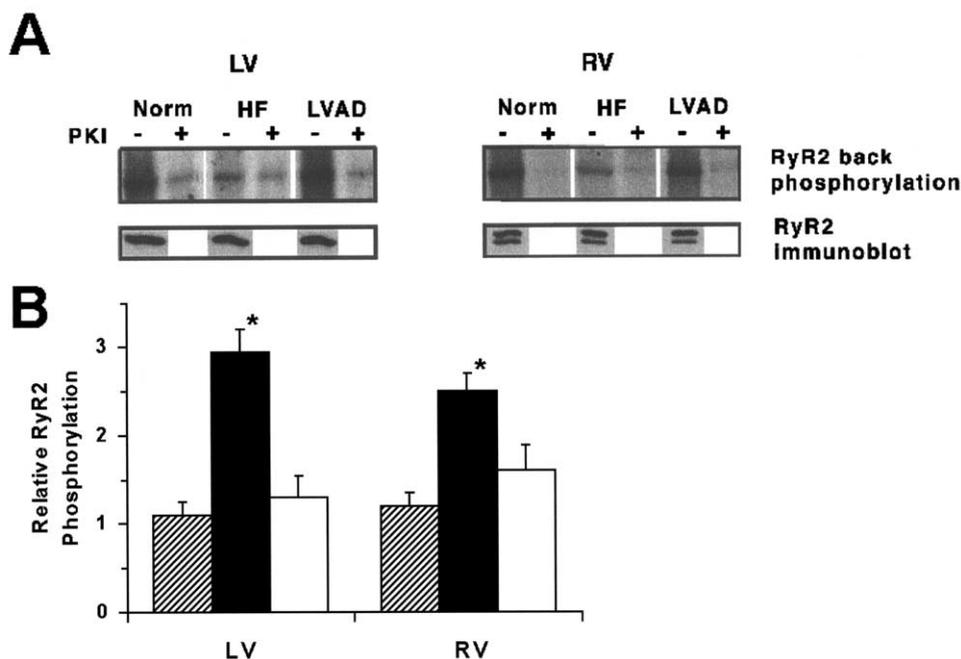


Figure 5. (A) Ryanodine receptor 2 (RyR2) immunoblots (lower panel) and back phosphorylation gels. For the latter, the darker bands correspond to higher amounts of ³²P transferred to RyR2 by protein kinase A (PKA), which is indicative of reduced RyR2 PKA phosphorylation in vivo. Note double band present in right ventricle (RV) RyR2 immunoblot. There is no special significance to the double bands in the RyR2 samples from the RV—moreover, given that the samples from nonfailing, medically, and left ventricular assist device (LVAD)-supported hearts are all equivalent (equal densities of the top and bottom bands) indicates that these samples are comparable and that measurements of the PKA phosphorylation levels are not affected by the presence of the two bands. Moreover, this does not represent nonspecific degradation as that would result in a smear. (B) RyR2 phosphorylation was markedly increased in failing myocardium, but was normalized by LVAD support in both ventricles. *p < 0.01 vs. nonfailing and LVAD-supported hearts. LV = left ventricle; HF = heart failure; PKI = protein kinase A inhibitor.

phosphorylated in end-stage heart failure (10). Protein kinase A hyperphosphorylation causes defective RyR2 function (including increased channel open probability due to increased calcium-dependent activation). The defective RyR2 function results in “leaky” channels that can deplete SR calcium stores and contribute to reduced calcium transients that likely contribute to impaired contractility in failing hearts. We have recently shown that these abnormalities are reversed in the LV by LVAD support (10). We have also recently demonstrated that RyR2 structure and function can be normalized by beta-blocker administration in dogs with pacing-induced heart failure (24). The hyperadrenergic state of heart failure may underlie the defects in RyR2 structure (depletion of FK506 binding protein 12.6 and reduced phosphatase levels in the RyR2 macromolecular complex) and function (increased open probability and increased calcium-dependent activation) (10). The normalization of RyR2 structure and function by LVADs and beta-blockers may be explained by the fact that both therapies effectively reverse the hyperadrenergic state of heart failure; LVADs, by reducing circulating catecholamine levels, and beta-blockers, by blocking the cellular actions of circulating catecholamines. These observations are complimentary in pointing toward primary biochemical mechanisms as being responsible for improved RyR2 structure and function, independent of hemodynamic factors.

One factor, which has complicated interpretation of data

related to reverse remodeling from studies involving hearts of LVAD and transplant patients, has been differences in medical regimen within and between groups. Most LVAD patients receive inotropic treatment before LVAD insertion, but this is usually withdrawn within about a week of surgery. Additionally, many medically supported patients now receive beta-blocker treatment before transplant. Prior studies have shown that especially metoprolol therapy is able to maintain or upregulate beta-receptor density, while conflicting data regarding carvedilol have been published with some studies suggesting downregulation of beta-receptor density (27,28) and another study showing an upregulation (25). However, myocardial force generation in isolated right atrial trabeculae in response to maximum stimulation with isoproterenol was no different (29). In the present study, we did not see that preoperative beta-agonist treatment significantly influenced beta-receptor density or overall beta-adrenergic myocardial contractile responsiveness in the medically treated end-stage heart failure patients (change in developed force generation in response to isoproterenol with carvedilol was 8.31 ± 0.60 mN/mm² in comparison with metoprolol with 9.96 ± 1.90 mN/mm²; p = NS). In addition, although pretransplant utilization of beta-blockers did upregulate beta-receptor density, the magnitude of this effect was far less than observed in the LVAD group. Thus, systematic changes in medical treatments do not influence the major conclusions of this study.

By design, LVAD patients supported for <30 days were excluded from this study. We have previously shown that 30 days of support is sufficient for molecular remodeling and for a majority of structural characteristics of reverse remodeling to reach near maximal values (12). It was, therefore, anticipated that we would not see any association between duration of LVAD support and recovery of beta-adrenergic myocardial responsiveness.

Study limitations. Left ventricular assist devices have been shown in the past to normalize neurohormone and other cytokine levels (2,7,11,26). We did not measure these factors in our group of patients. However, in prior studies the degree of normalization of these factors has been profound and uniform (i.e., every neurohormone examined thus far that is abnormally elevated in heart failure is normalized by LVAD support). Therefore, even had they been measured, it would not have been possible to mechanistically link recovery of the beta-adrenergic pathway to normalization of a particular neurohormone or cytokine. In addition, we assessed the myocardial force response of isolated trabeculae to beta-adrenergic stimulation with isoproterenol only to the maximum dose and did not perform a dose-response relationship. Only a small amount of myocardial tissue was available for measuring beta-receptor density and RyR2 phosphorylation, so the statistical power is low, and these results should be seen with caution. In addition, we did not find any upregulation in beta-receptor density in patients receiving beta-agonist therapy before transplantation. However, it is known that beta-blocker therapy is able to maintain or upregulate beta-receptor density (27–29). Because of the low samples size, a differentiation of beta-blocker therapy in effectiveness of beta-receptor density was not possible. Finally, availability of normal human tissue is becoming increasingly limited in our institution, and RV functional data from only two nonfailing hearts were available for this study. Over the past 5 years since our studies began, we have obtained only five normal hearts and none in the last 12 months. Therefore, the present study primarily shows improvements in RV myocardial properties during LVAD support compared with medically supported hearts, but does not definitively show that LVADs truly normalize RV myocardial properties in comparison with LV properties.

Conclusions. When used to bridge critically ill patients to transplant, LVAD support has been associated with improved ventricular function to the point where LVADs have been explanted without transplantation (30–32). Although this recovery does not appear to be uniform and may not be permanent in most patients undergoing LVAD explantation (30–34), an understanding of the mechanisms underlying the unprecedented degree and scope of reverse remodeling could lead to development of less invasive means of achieving the same goal. Prior studies have indicated the prime importance of hemodynamic factors in mediating many aspects of reverse remodeling. The results of the present study provide new and important insight into

mechanisms of reverse remodeling by showing that systemic factors (e.g., biochemical milieu) are important for normalizing the myocardial beta-adrenergic axis.

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**Left ventricular assist device support normalizes left and right ventricular
beta-adrenergic pathway properties**

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