

Altered Myocardial Ca^{2+} Cycling After Left Ventricular Assist Device Support in the Failing Human Heart

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OBJECTIVES	The objective of the present study was to determine whether improved contractility after left ventricular assist device (LVAD) support reflects altered myocyte calcium cycling and changes in calcium-handling proteins.
BACKGROUND	Previous reports demonstrate that LVAD support induces sustained unloading of the heart with regression of pathologic hypertrophy and improvements in contractile performance.
METHODS	In the human myocardium of subjects with heart failure (HF), with non-failing hearts (NF), and with LVAD-supported failing hearts (HF-LVAD), intracellular calcium ($[\text{Ca}^{2+}]_i$) transients were measured in isolated myocytes at 0.5 Hz, and frequency-dependent force generation was measured in multicellular preparations (trabeculae). Abundance of sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase (SERCA), $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), and phospholamban was assessed by Western analysis.
RESULTS	Compared with NF myocytes, HF myocytes exhibited a slowed terminal decay of the Ca^{2+} transient ($\text{DT}_{\text{terminal}}$, 376 ± 18 ms vs. 270 ± 21 ms, HF vs. NF, $p < 0.0008$), and HF-LVAD myocytes exhibited a $\text{DT}_{\text{terminal}}$ that was much shorter than that observed in HF myocytes (278 ± 10 ms, HF vs. HF-LVAD, $p < 0.0001$). Trabeculae from HF showed a negative force-frequency relationship, compared with a positive relationship in NF, whereas a neutral relationship was observed in HF-LVAD. Although decreased SERCA abundance in HF was not altered by LVAD support, improvements in $[\text{Ca}^{2+}]_i$ transients and frequency-dependent contractile function were associated with a significant decrease in NCX abundance and activity from HF to HF-LVAD.
CONCLUSIONS	Improvement in rate-dependent contractility in LVAD-supported failing human hearts is associated with a faster decay of the myocyte calcium transient. These improvements reflect decreases in NCX abundance and transport capacity without significant changes in SERCA after LVAD support. Our results suggest that reverse remodeling may involve selective, rather than global, normalization of the pathologic patterns associated with the failing heart. (J Am Coll Cardiol 2004;44:837-45) © 2004 by the American College of Cardiology Foundation

In laboratory studies of failing human hearts, the most prominent functional abnormalities observed have been slowed rates of relaxation and impaired contractile reserve exemplified by a negative force-frequency response. These functional abnormalities, in turn, have been linked to alterations in calcium cycling and the abundance of key calcium regulatory proteins such as sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA), the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), and phospholamban (PLB) (1-5). Such studies support the hypothesis that defects in calcium homeostasis, particularly reduced calcium

reuptake by the sarcoplasmic reticulum, represent primary contributors to the impaired cardiac contractility.

Recent studies demonstrate improved in vitro myocardial function in failing human hearts that required mechanical circulatory support with a left ventricular assist device (LVAD) (6,7). For example, Dipla et al. reported improved basal relaxation rates and improved frequency-dependent responses (6), whereas Heerdt et al. observed an improved force-frequency relationship in association with increases in SERCA protein abundance (7).

To further examine the physiologic mechanisms responsible for improvements in myocardial contractile function after LVAD support, we studied whole-cell calcium transients in isolated cardiac myocytes and performed further experiments to relate changes in calcium cycling to changes in frequency-dependent contractile reserve and the abundance of calcium handling proteins. Our results indicate that the prolonged terminal decay of the calcium transient observed in myocytes from failing hearts is normalized after LVAD support and that this improvement is associated with an improved force-frequency response. Faster intracel-

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Abbreviations and Acronyms

[Ca ²⁺] _i	= intracellular calcium
HF	= heart failure
HF-LVAD	= failing hearts supported by a left ventricular assist device
I _{NCX}	= Ni ⁺ -sensitive inward current
KHB	= Krebs-Henseleit buffer
LV	= left ventricle/ventricular
LVAD	= left ventricular assist device
NCX	= sodium-calcium exchanger
NF	= non-failing
PLB	= phospholamban
RV	= right ventricle/ventricular
SERCA	= sarcoplasmic reticulum calcium adenosine triphosphatase

lular calcium ([Ca²⁺]_i) transient decay was also associated with functionally significant reductions in NCX protein abundance although SERCA abundance did not change significantly with LVAD support. These studies indicate that improved myocyte relaxation and contractile reserve after LVAD support reflect a change in the functional balance between SERCA and the NCX for [Ca²⁺]_i reuptake.

METHODS

Subjects and tissue preparation. Human left ventricular (LV) myocardial tissue was obtained at the time of cardiac transplantation from 27 patients with severe heart failure (HF) and from 15 patients who required LVAD support before cardiac transplantation (HF-LVAD). Left ventricular tissue was also obtained from 18 non-failing hearts (NF) of brain-dead organ donors that could not be used for transplantation. Temple University Institutional Review Board approved the studies on explanted heart tissue.

All hearts were arrested in situ with cold, blood-containing, cardioplegia solution and promptly transported to the laboratory in Krebs-Henseleit buffer (KHB) solution as previously described (6). The right ventricle (RV) of the heart was removed for preparation of isolated trabeculae. An epicardial vessel supplying the LV free wall was cannulated for perfusion digestion, and transmural samples were obtained from viable LV free wall myocardium adjacent to the region of perfusion. Tissue slices were immediately snap frozen in liquid nitrogen and stored at -80°C.

Myocyte isolation and volume measurement. After cardiectomy, a perfusion-based myocyte dissociation procedure was initiated as previously described (6,8). Myocytes were resuspended in 1% w/v bovine serum albumin, 10 mM taurine, and 0.25 mM CaCl₂. All solutions were equilibrated with 95% O₂ and 5% CO₂. Initial yields of rod-shaped myocytes ranged from 10% to 60% in calcium-containing buffer. An aliquot of freshly isolated myocytes was fixed in an iso-osmotic solution of 1.5% glutaraldehyde and enriched by Ficoll gradient centrifugation as described by Gerdes *et al.* (9). Median

myocyte volume for each cell suspension was determined using a Coulter Channelyzer technique as previously described (9). **Myocyte intracellular Ca²⁺ measurements.** A stock solution of Fluo-3AM was prepared using Pluronic F-127 (20% w/v in dimethyl sulfoxide) (Molecular Probes, Eugene, Oregon). A 1-ml aliquot of myocytes in resuspension buffer was incubated with Fluo-3AM at a final concentration of 4.4 μM. Myocytes were studied in a chamber on the stage of an inverted microscope (Nikon Eclipse TE300); superfused at 1 to 2 ml/min with Tyrode solution (150 mM NaCl, 5.4 mM KCl, 1 mM CaCl₂, 1.2 mM MgCl₂, 10 mM glucose, 2 mM pyruvate, 5 mM HEPES, pH 7.4, 37°C). Rod-shaped myocytes were chosen on the basis of the absence of spontaneous contractions in 1 mM Ca²⁺. Fluo-3AM was excited at 480 nm with a xenon lamp, and the emitted light at 530 nm was recorded to represent the cytosolic Ca²⁺ transient ([Ca²⁺]_i). Intracellular calcium measurement was represented as a pseudo-ratio as previously described (10). Myocytes were field stimulated at a rate of 0.5 Hz, and [Ca²⁺]_i was stored on computer for analysis using pClamp software (Axon Instruments, Union City, California). Indicated parameters were measured on three consecutive transients from each cell (see "Results").

Protein extraction. Frozen heart tissue (150 mg) was pulverized by mortar and pestle cooled in liquid nitrogen and then homogenized using a Brinkman Polytron for 10 s in 1.5 ml of homogenization buffer containing 30 mM Tris-HCl pH 7.6, 2 mM ethylenediaminetetraacetic acid, 0.6 M NaCl; leupeptin, aprotinin, antipain at 10 μg/ml; 1 mM phenylmethylsulfonyl fluoride, 2.5 mM benzamidine and phosphatase inhibitor cocktail (Sigma, St. Louis, Missouri). Triton X-100 was added to 0.5% v/v, and the homogenate was rocked in the cold room for 30 min. An aliquot of homogenate was diluted six-fold with the homogenization buffer to a final salt concentration of 0.1 M NaCl and centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was collected, and the protein concentration was determined by Lowry assay.

Analysis of calcium-handling proteins. Tissues homogenization, protein extraction, and electrophoresis were performed as previously described (11). The proteins (5 or 30 μg) were resolved by electrophoresis on either 15% or 10% sodium dodecyl sulfate-polyacrylamide gels for immunoblot analysis of PLB and SERCA2 or NCX, respectively. Western blot analysis was performed and analyzed as previously described (11). Antibody dilutions and sources were as follows: 1:1,000 for SERCA antibody (Affinity BioReagents Inc., Golden, Colorado), and 1:1,000 for mouse monoclonal anti-Na⁺/Ca²⁺ exchanger (Research Diagnostics Inc., Flanders, New Jersey). The NCX antibody recognizes the 120-kDa mature protein and a 70-kDa proteolytic fragment as well as a 160-kDa form of NCX that is believed to be either an unreduced product of or an unprocessed form of the protein. Values for total NCX protein abundance are expressed as the summation of the 120-kDa and 70-kDa NCX protein bands quantified from

Table 1. Clinical Characteristics of Patients

	Age	Gender	LVEF (%)	Etiology	Medications
NF (n = 10)	57 ± 2	10 M 8 F	57 ± 3	N/A	Dopa 7/18, CCB 1/18, ARB 1/18, BB 2/18, Nit 1/18
HF (n = 32)	56 ± 2	27 M 6 F	13 ± 1	13 Idio 15 Isch 4 Other	Dob 22/32, Mil 20/32, Dig 26/32, Diu 25/32, Nit 14/32, ACE 26/32, ARB 15/32, Amio 10/32, CCB 4/32, BB 6/32
HF-LVAD (n = 15)	52 ± 3	11 M 4 F	N/A	9 Idio 6 Isch	Dob 2/15, Mil 3/15, Dig 3/15, Diu 4/15, Nit 2/15, ACE 3/15, ARB 2/15, Amio 4/15, CCB 2/15

ACE = angiotensin-converting enzyme inhibitor; Amio = amiodarone; ARB = angiotensin receptor blocker; BB = beta-blocker; CCB = calcium channel blocker; Dig = digoxin; Diu = diuretic; Dob = dobutamine; Dopa = dopamine; HF = heart failure group; HF-LVAD = left ventricular assist device supported group; Idio = idiopathic; Isch = ischemic; LVEF = left ventricular ejection fraction; Mil = milrinone; N/A = not applicable; NF = non-failing group; Nit = nitrate.

Western blots. Monoclonal anti-PLB antibody was used at 0.05 µg/ml and has been shown to recognize both phosphorylated and dephosphorylated forms of the protein.

Assessment of NCX current. In freshly isolated myocytes loaded with Fluo-3, whole cell patch clamp recordings were performed at 37°C using 1 to 4 mega ohm pipettes as described previously. In the presence of Cesium (in/out) to block potassium currents and thapsigargin to block calcium reuptake by the sarcoplasmic reticulum, a voltage-ramp (67 mV/s) from -100 mV to +130 mV was employed to increase cytosolic free calcium. After this loading procedure, the Ni⁺-sensitive inward current was measured following a rapid return to the resting membrane potential as a bioassay for NCX transport capacity. Simultaneously, [Ca²⁺]_i was measured with Fluo-3 as described above.

Muscle strip preparation and force-frequency experiments. The excised cardiac apex was placed in KHB solution containing 20 mM 2,3-butanedione monoxime and 0.25 mM CaCl₂ gassed with 95% O₂-5% CO₂. Thin (<500 µm), non-branching, free-running trabeculae from the RV free wall were carefully removed with a small cube of tissue on either side and mounted in a tissue bath as previously described (12,13). The KHB solution was replaced by 2,3-butanedione monoxime-free KHB solution, and the bath CaCl₂ was increased stepwise from 0.5 to 1.75 mM over 15 min. Trabeculae were equilibrated, and muscle length was set at 80% L_{max} [0.8 × (L_{max} - L_o)], as previously described (14). After an additional 30-min equilibration period, steady-state twitches were established (0.5 Hz, 37°C, 1.75 mM Ca²⁺), and the frequency of stimulation was increased sequentially from 0.5 Hz to 1.0 Hz, 1.5 Hz, 2.0 Hz, and 2.5 Hz.

Statistical analysis. All data are expressed as mean ± SEM. Comparisons between the three experimental groups were made using one-way analysis of variance for indepen-

dent groups. For intergroup comparisons, Tukey post hoc analysis was used to locate the significantly different means. A p value of <0.05 was considered statistically significant for all hypothesis testing.

RESULTS

Clinical characteristics. The clinical characteristics of the HF patients are presented in Table 1. Ages of the patients in the three experimental groups did not differ significantly. Fifteen of the 32 failing hearts without LVAD support and 6 of the 15 patients with LVAD support had coronary artery disease and ischemic cardiomyopathy, whereas the balance had a nonischemic etiology for failure. The duration of clinical HF before transplantation varied widely among failing hearts, but the average duration was similar in patients with and without LVAD support. In contrast, the number of cardiovascular medications being taken at the time of transplantation was far greater in the non-LVAD failing hearts.

Myocyte volume. Myocyte volume measurements revealed marked cellular hypertrophy in the failing hearts. Myocytes from HF hearts (n = 11) were an average of 35% larger than myocytes from NF hearts (n = 7) (NF 32,210 ± 2,469 µm³ vs. HF 50,025 ± 3,438 µm³; p < 0.01). The LVAD support was associated with a significant decrease in myocyte volume, with myocytes an average of 26% larger in HF hearts than in cells from HF-LVAD hearts (n = 7) (HF-LVAD 36,933 ± 4,511 µm³; HF-LVAD vs. HF p < 0.01). The LVAD-supported and NF myocytes were not significantly different.

Myocyte calcium transients. Calcium transients (0.5 Hz, 1 mM Ca²⁺, 37°C) were recorded in 20 HF myocytes, 5 NF myocytes, and 15 HF-LVAD myocytes. The time constant (τ) for the initial portion of the [Ca²⁺]_i decay (DT_{initial}) was

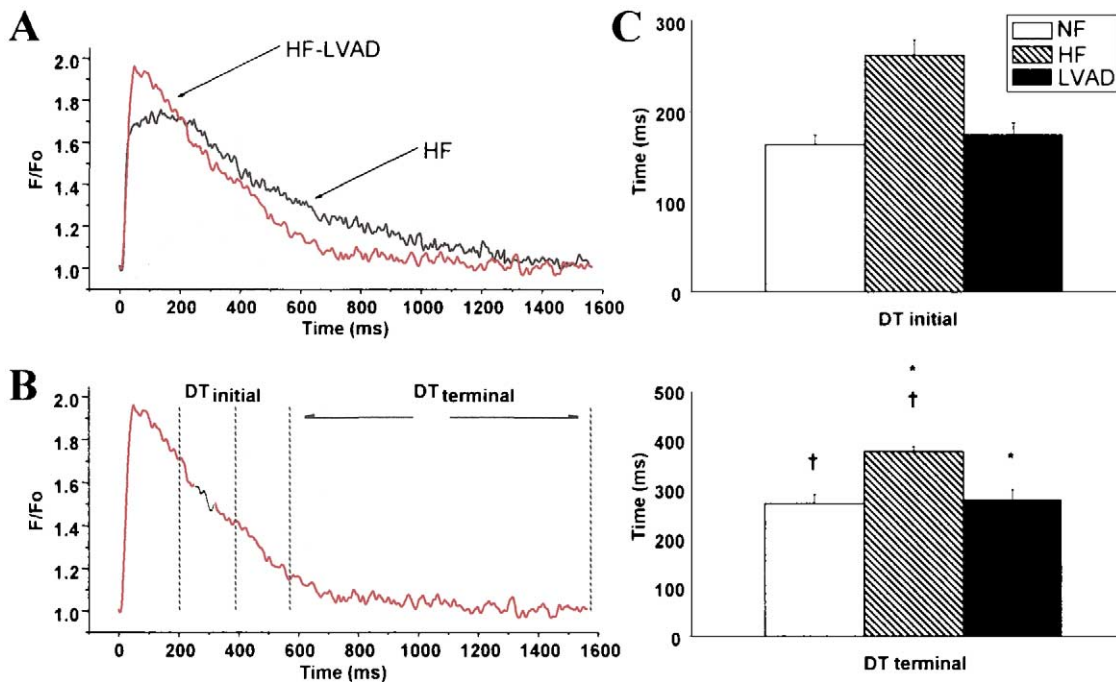


Figure 1. Effects of left ventricular assist device (LVAD) support on [Ca²⁺]_i transients in cardiac myocytes from failing hearts with and without previous LVAD support. Data were collected during field stimulation at 0.5 Hz, and [Ca²⁺]_i was measured by Fluo-3 fluorescence. **(A)** Raw fluorescence intensity traces from representative myocytes. **(B)** Schematic illustrating curve-fitting technique for initial and delayed portions of the [Ca²⁺]_i transient decay. **(C)** Average initial and delayed time constants for the [Ca²⁺]_i transients in each of the three experimental groups (NF = non-failing; HF = failing; HF-LVAD = LVAD support). Data are expressed as mean ± SEM. *p < 0.0001 HF-only versus HF-LVAD; †p < 0.001.

measured by fitting a mono-exponential curve to each transient beginning at 150 ms after the peak and continuing for 200 ms. The terminal portion of the [Ca²⁺]_i decay (DT_{terminal}) was described by fitting a mono-exponential curve beginning at the point where Ca²⁺ had fallen to 75% of its peak and ending at the start of the next stimulus marker (Fig. 1B). Data from the three experimental groups are presented in Figure 1C. Although the peak [Ca²⁺]_i level did not differ between the three experimental groups (data not shown), there were inter-group differences in the [Ca²⁺]_i decay. The DT_{initial} tended to be longer in HF myocytes than in NF and HF-LVAD myocytes (HF 263 ± 30 ms; NF 163 ± 13 ms; HF-LVAD 175 ± 17 ms; HF vs. NF p = 0.11; HF vs. HF-LVAD p = 0.06). For the DT_{terminal}, there were striking differences, with the HF

myocytes significantly prolonged compared with both the NF and the HF-LVAD myocytes (HF 376 ± 18 ms, NF 270 ± 21 ms, HF-LVAD 278 ± 10 ms; HF vs. NF, p < 0.0008; HF vs. HF-LVAD, p < 0.0001). Thus, sustained LVAD support is associated with increases in the rate of [Ca²⁺]_i transient decay in failing human myocytes.

Calcium regulatory protein abundance. The abundance of key calcium regulatory proteins, SERCA, PLB, and NCX, in each of the three experimental groups is presented in Table 2 and Figure 2. We observed highly significant decreases in SERCA protein abundance in both the HF and HF-LVAD patient groups compared with the NF group. Although no significant differences were observed in PLB protein abundance among the groups, the SERCA/PLB ratio in the HF and LVAD patients was reduced by 2.9-fold (p < 0.05) compared with the NF patient group. Moreover,

Table 2. Summary of Western Blot Protein Abundance and Myocyte Morphometry

	NF (n = 12)	HF (n = 12)	HF-LVAD (n = 8)
PLB	5.27 ± 1.01	5.40 ± 0.93	6.20 ± 1.42
SERCA2a	6.27 ± 0.56	3.03 ± 0.6‡	3.54 ± 0.66†
SERCA2a/PLB ratio	1.94 ± 0.55	0.68 ± 0.11*	0.66 ± 0.13*
NCX	5.14 ± 0.68	12.92 ± 1.78‡	8.56 ± 1.11*§
SERCA/NCX ratio	1.48 ± 0.22	0.30 ± 0.07‡	0.49 ± 0.16†

*p < 0.05, †p < 0.01, ‡p < 0.001 versus NF; §p < 0.08 versus HF. Values are mean ± SEM.

NCX = Na⁺/Ca²⁺ exchanger; PLB = phospholamban; SERCA = sarcoplasmic reticulum calcium adenosine triphosphatase; SERCA2a = SERCA type 2a. Other abbreviations as in Table 1.

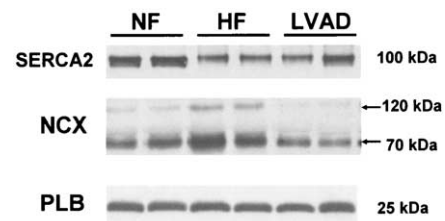


Figure 2. Western blot demonstrating protein abundance for sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA2), sodium-calcium exchanger (NCX), and phospholamban (PLB). NF = non-failing; HF = failing; LVAD = left ventricular assist device support.

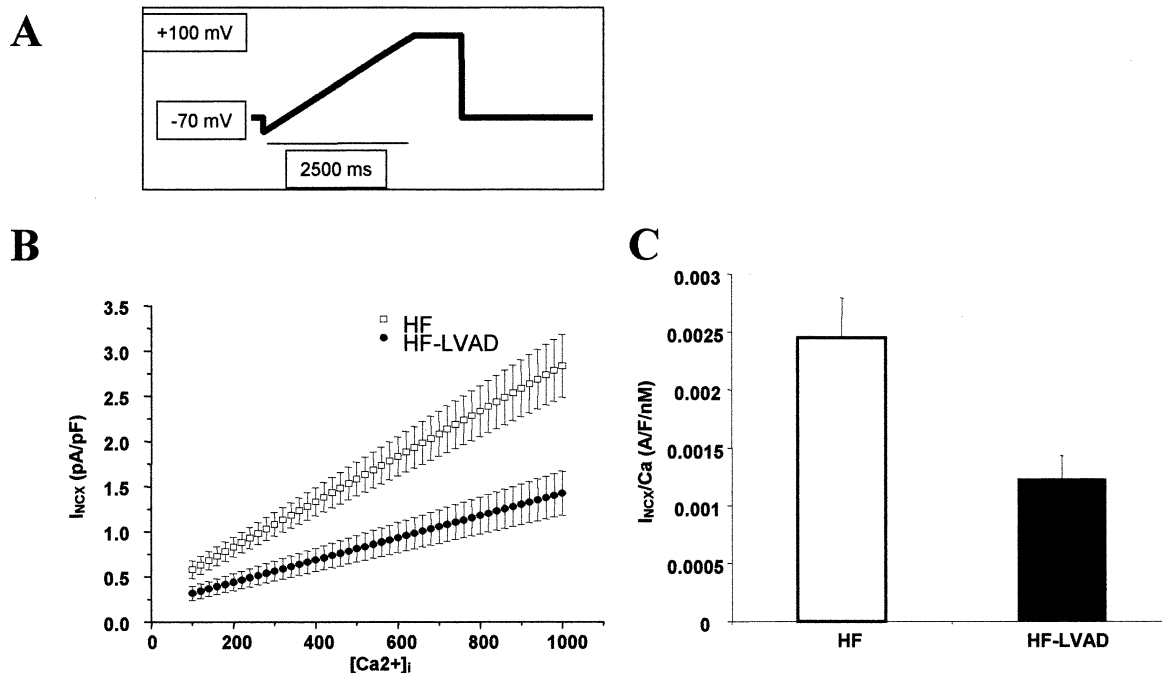


Figure 3. Assessment of forward-mode sodium-calcium exchanger transport capacity in isolated cardiac myocytes from failing hearts with and without previous left ventricular assist device (LVAD) support. **(A)** Schematic illustration of the voltage-ramp (67 mV/s) protocol employed to increase cytosolic-free calcium (see “Methods”). **(B)** The Ni⁺-sensitive inward current (I_{NCX}) is plotted as a function of $[Ca^{2+}]_i$ after membrane repolarization. **(C)** Average data are depicted from failing myocytes with and without previous LVAD support (n = 31, n = 10). HF= failing; HF-LVAD = LVAD support. Data are expressed as mean \pm SEM.

the abundance of the NCX protein was 2.5-fold higher in HF than in NF but was only 50% greater in LVAD-supported hearts than in NF hearts. Nevertheless, in both HF and HF-LVAD hearts the SERCA/NCX protein ratio was significantly reduced compared with NF.

NCX transport capacity. As described, we examined the functional significance of the decrease in NCX protein abundance after LVAD support. Data from these experiments (Fig. 3) demonstrate that HF-LVAD myocytes exhibit decreased forward-mode Ni⁺-sensitive inward current (I_{NCX}) compared with HF myocytes across a wide range of intracellular Ca²⁺ levels. These findings suggest that functionally significant reductions in NCX transport capacity are associated with decreased NCX protein abundance in LVAD-supported hearts.

Force-frequency experiments. Figure 4 and Table 3 show representative raw data tracings and twitch parameters during progressive increases in stimulation frequency. Under basal conditions (0.5 Hz), RV trabeculae from NF hearts exhibited relatively low developed tension, rate of force development (+dF/dt), and rate of relaxation (−dF/dt). With increasing stimulation frequencies, we observed a positive force-frequency relationship in the NF trabeculae, a negative force-frequency relationship in the HF trabeculae, and an intermediate response in the HF-LVAD trabeculae. At the highest stimulation frequency, +dF/dt and −dF/dt were equivalent in NF and HF-LVAD trabeculae and considerably greater than rates observed in HF trabeculae.

DISCUSSION

The principal finding of this investigation is that the decay in myocyte $[Ca^{2+}]_i$ transient is prolonged in failing myocytes compared with non-failing myocytes and that LVAD support of the failing heart is associated with a faster decay of the $[Ca^{2+}]_i$ transient. These changes in the $[Ca^{2+}]_i$ decay are associated with partial restoration of the abnormal force-frequency relationship observed in failing hearts. As in several previous studies, we observed that functional abnormalities in calcium cycling and rate-dependent contractile reserve in HF are associated with decreases in SERCA and increases in NCX abundance. However, the observed functional improvements after LVAD support occurred in the absence of increased SERCA abundance and were associated with only partial, though functionally significant, decreases in NCX abundance. These findings suggest that alterations in cardiac myocyte $[Ca^{2+}]_i$ homeostasis represent a mechanism for previously reported improvements in myocardial relaxation and defects in the force-frequency response after LVAD support, but they do not exclude possible contributions of other factors, including myofilament Ca²⁺ sensitivity, action potential duration, and intracellular sodium.

Prolonged Ca²⁺ transients in failing myocytes. One of the hallmarks of a failing human heart is an impairment of relaxation that is manifested as abnormal diastolic filling patterns, and prolonged relaxation times in isolated myo-

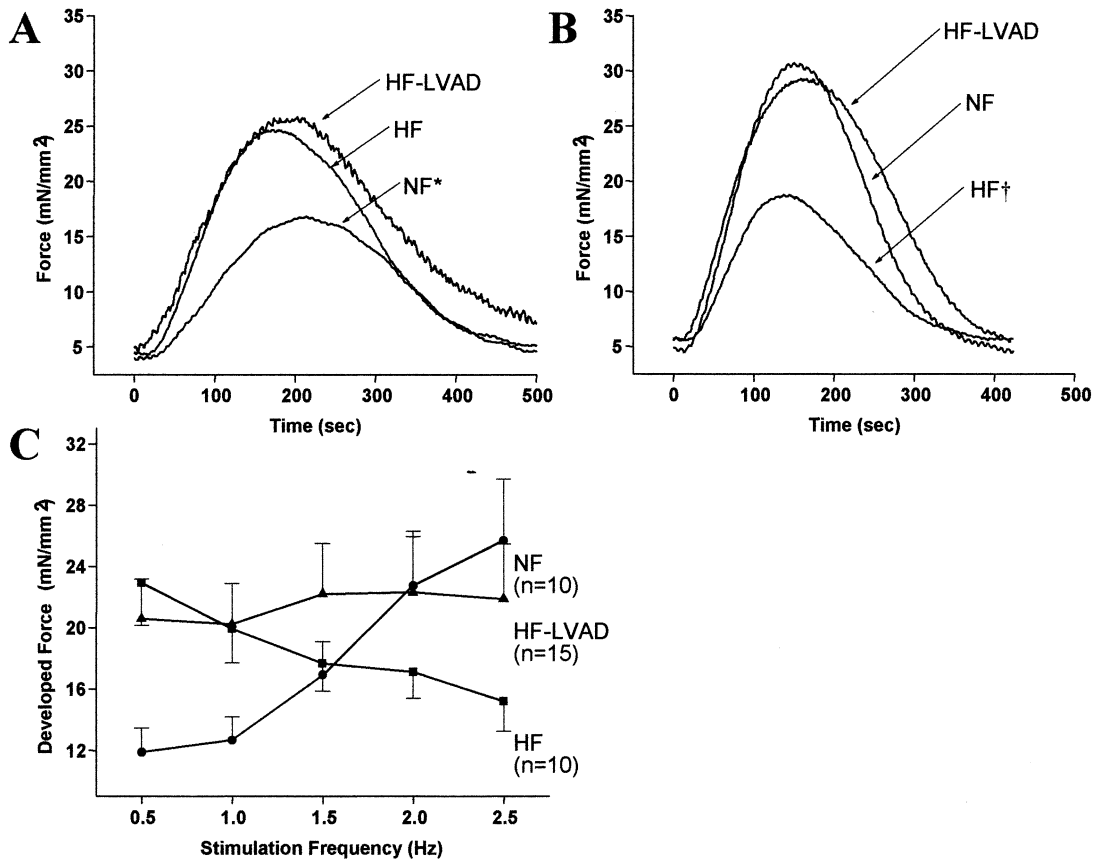


Figure 4. Force generation by isolated right ventricular trabeculae from non-failing, failing, and left ventricular assist device (LVAD)-supported hearts. **(A)** Representative force transients at low stimulation frequency (0.5 Hz) showing higher developed force in failing and LVAD-supported trabeculae than in non-failing muscles. **p* < 0.05 NF versus HF and HF-LVAD. **(B)** Representative force transients at high stimulation frequencies (2.5 Hz) showing higher developed force in non-failing and LVAD-supported trabeculae compared with unsupported failing trabeculae. †*p* < 0.05 NF versus HF. **(C)** Averaged data (± SEM) in which developed force is plotted as a function of stimulation frequency in each of the three groups. A clearly positive force-frequency relationship is seen in non-failing myocardium, a clearly negative force-frequency relationship is seen in unsupported failing myocardium, and a flat force-frequency relationship is seen after LVAD support.

cytes and muscle strips. In the few studies that have measured calcium transients in failing human myocytes, prolongation of the [Ca²⁺]_i transient has been closely associated with prolongation of cellular relaxation (6,15–17). Several of these studies have also observed variability in the shape of the “failing” [Ca²⁺]_i transient, including an initial followed by a delayed decline in cytosolic Ca²⁺ levels (16–18). Using a previously published approach that describes [Ca²⁺]_i transient decay with two separate time constants (initial and terminal) (19–21), we were able to faithfully model the transients observed in our isolated myocytes. We observed that the time constants for the

initial (*p* < 0.11) and terminal (*p* < 0.0008) portions of the decay of the [Ca²⁺]_i transient were prolonged in HF versus NF myocytes. These findings are consistent with previous studies in failing human myocytes as well as several animal models of HF (15,22–25). Our laboratory has previously reported that the initial portion of the contraction and myocyte [Ca²⁺]_i transient of failing human myocytes is sensitive to sarcoplasmic reticulum calcium depletion and is primarily under the control of SERCA transport. On the other hand, the terminal portion of the [Ca²⁺]_i transient decay, which is dependent on the timing of repolarization, is more likely to reflect the combined actions of SERCA-

Table 3. Twitch Parameters From Isolated Trabeculae

	0.5 Hz			2.5 Hz		
	NF (n = 10)	HF (n = 10)	HF-LVAD (n = 15)	NF (n = 10)	HF (n = 10)	HF-LVAD (n = 15)
DevT	11.9 ± 1.6*§	22.9 ± 2.8	20.6 ± 2.6	25.7 ± 4.0	15.2 ± 2.0*	21.9 ± 3.6
+dF/dt	91.1 ± 15.5‡	202.0 ± 16.1	154.8 ± 17.5	282.0 ± 56.2	147.5 ± 16.7*	280.3 ± 55.9
-dF/dt	73.6 ± 12.5†	143.9 ± 13.4	106.0 ± 12.3	200.8 ± 42.7	105.7 ± 15.3*	192.2 ± 36.5

**p* < 0.05, †*p* < 0.01, ‡*p* < 0.001 NF versus HF; §*p* < 0.05 NF versus HF-LVAD; ||*p* < 0.05 HF versus HF-LVAD. Values are mean ± SEM. DevT = developed tension; +dF/dt = maximal force rise; -dF/dt = maximal force decline. Other abbreviations as in Table 1.

mediated reuptake and forward-mode Na/Ca exchange (outward Ca^{2+} transport) (26).

In this context, the prolonged early decay of the $[\text{Ca}^{2+}]_i$ transient in failing myocytes is consistent with both the decrease in SERCA and the increase in NCX abundance that we observed, compared with non-failing myocytes. The prolonged terminal decay of the $[\text{Ca}^{2+}]_i$ transient in failing myocytes is more consistent with the reduced SERCA abundance and activity than the increase in NCX protein abundance because the latter would tend to accelerate the terminal decay of the $[\text{Ca}^{2+}]_i$ transient, all other things being equal. This dominant action of SERCA-mediated $[\text{Ca}^{2+}]_i$ transport during terminal portions of the $[\text{Ca}^{2+}]_i$ transient is consistent with recent studies examining the quantitative contributions of SERCA and NCX to decreases in cytosolic $[\text{Ca}^{2+}]_i$ in failing human myocytes: 69% SERCA versus 31% NCX (27). However, action potential prolongation in failing myocardium may also delay calcium extrusion via the voltage-sensitive NCX transport (17).

Faster Ca^{2+} transient decay after LVAD support. Previous studies have shown increases in the rates of in vitro myocardial relaxation in isolated myocytes (6) and isolated muscle strips (28,29) from LVAD-supported hearts compared with non-supported control hearts. Though we and others (6,28) have hypothesized that LVAD-induced alterations in Ca^{2+} homeostasis might contribute to contractile improvements, the present study is the first to directly measure $[\text{Ca}^{2+}]_i$ transients in adequate numbers of LVAD-supported human myocytes. We observed a faster decay of both the early ($p < 0.08$) and especially the late ($p < 0.0001$) components of the $[\text{Ca}^{2+}]_i$ transient in the LVAD-supported myocytes, with no significant differences observed between myocytes from HF-LVAD and NF hearts. In our studies, the faster initial decay of the $[\text{Ca}^{2+}]_i$ in HF-LVAD myocytes was not associated with significant increases in SERCA protein abundance and SERCA/PLB ratio. However, our analysis does not preclude the possibility that an altered phosphorylation state of PLB and the ryanodine receptor after LVAD support might have contributed to the phasic $[\text{Ca}^{2+}]_i$ decay rates (30). An alternative hypothesis is that reduced reverse-mode Na/Ca exchange, the increased SERCA/NCX ratio, and perhaps greater sarcoplasmic reticulum Ca^{2+} loading are responsible for the faster early decay of $[\text{Ca}^{2+}]_i$. Indeed, the functional significance of the LVAD-associated decrease in NCX protein abundance was affirmed by our I_{NCX} bioassay, indicating that NCX transport capacity was decreased in LVAD-supported myocytes independent of the timing of repolarization or sarcoplasmic reticulum function.

LVAD-associated increases in the rate of $[\text{Ca}^{2+}]_i$ decay were particularly consistent for the terminal portions of the transient. In general, the reductions in NCX transport capacity that we observed with LVAD support would have been expected to slow, rather than accelerate, the rate of terminal $[\text{Ca}^{2+}]_i$ decay. Accordingly, likely contributors to the faster terminal decay actually observed in myocytes after

LVAD support include shortening of the action potential duration, as we have previously observed (30), and possibly a normalization of the elevated $[\text{Na}]_i$ recently reported in failing myocardium (31). Both changes would tend to favor a faster $[\text{Ca}^{2+}]_i$ decay and a decrease in reverse-mode Na/Ca exchange. A shorter action potential duration would additionally promote better synchrony of SERCA-mediated $[\text{Ca}^{2+}]_i$ reuptake and NCX-mediated $[\text{Ca}^{2+}]_i$ extrusion with a faster terminal $[\text{Ca}^{2+}]_i$ decay even in the presence of an absolute decrease in NCX transport capacity. In addition, we cannot exclude a functional contribution of the non-significant increase in SERCA protein abundance that we observed after LVAD support, or an increase in SERCA transport activity.

Improved frequency-dependent responses after LVAD support. An important characteristic of the failing heart is a decrease in contractile reserve upon increased rate of stimulation. To evaluate the impact of changes in intracellular Ca^{2+} handling on contractile reserve, we employed isolated trabeculae from NF, HF, and HF-LVAD-supported hearts. At slow stimulation frequencies, we observed intact contractile performance in failing trabeculae. However, in contrast to the positive force-frequency response in the NF trabeculae, we observed a negative force-frequency response in the failing trabeculae consistent with previous reports (2-5,31-35). Trabeculae from LVAD-supported hearts demonstrated an improved force-frequency response compared with failing hearts. In addition, rates of force generation and decline increased in NF and HF-LVAD myocardium as stimulation frequency increased, contrary to HF myocardium. Overall, these findings confirm and extend previous studies in isolated LV myocytes (6) and studies in RV trabeculae after LVAD support (28).

Previous reports suggested that the negative force-frequency response in failing human hearts is a reflection of an alteration in the normal functional balance between SERCA and the NCX. Thus, increased dependency on the relatively slower NCX transport mechanism for the decay of the Ca^{2+} transport leads to reduced resequestration of Ca^{2+} into the sarcoplasmic reticulum during faster stimulation frequencies and a negative force-frequency response. In this context, our findings suggest that an isolated decrease in NCX protein abundance and transport capacity tends to ameliorate the negative force-frequency response by restoring a more favorable balance between SERCA and NCX, even without restoration of SERCA protein abundance to levels observed in NF hearts.

Finally, Pieske et al. have observed that $[\text{Na}^+]_i$ is elevated in muscle strips from failing human hearts, which promotes the maintenance of sarcoplasmic reticulum calcium load at low pacing rates (36). It is tempting to speculate that a reduction in the functional capacity of NCX after LVAD support along with a hypothetical decrease in $[\text{Na}^+]_i$ would further promote sarcoplasmic reticulum calcium uptake and a positive force-frequency relationship.

Study limitations. Myocyte studies are confounded by isolation procedure, regional variability in myocytes, and differences in disease as well as patient medications. In an effort to minimize variability in our myocyte preparations, we have employed an established, high-yield isolation protocol that is uniform for the different types of tissue studied. Trabeculae were isolated from the RV of the same hearts, and these multicellular preparations obviate some concerns about the isolation procedure while allowing more realistic measures of contractility in loaded preparations. Although our isolated trabeculae were from the RV, our studies suggest that the underlying pathology in the RV is similar to that reported in the LV. An advantage in using RV trabeculae is that this region is largely spared from infarction and ischemia. As in all studies employing human heart tissue, availability of control tissue is a challenge. Because truly "normal" tissue is rarely available, we use the terminology "non-failing" when describing control tissue. Indeed, the physiology and molecular characterization of this NF tissue does reflect what we assume to be normal based on animal studies, such as a positive force-frequency relationship and a rapid relaxation phase of both the calcium transient and force development. Because of practical limitations, not all assays were performed on all hearts. Nevertheless, for each functional and molecular assay, sufficient numbers of tissues from the appropriate groups were studied to ensure meaningful results.

Conclusion. Implantation of an LVAD removes, in part, the pathologic stimulus for hypertrophy caused by chronic hemodynamic overload of the failing heart (37). Our data show that functional improvement after LVAD support occurred without increases in SERCA and was associated with limited, but functionally significant, decreases in NCX. An isolated decrease in NCX restores a more favorable balance between SERCA and NCX, even without increases in SERCA. On a more general level, our findings also suggest that myocardial adaptations, including improved contractile responses, induced by LVAD support or other interventions, may involve selective, rather than global, normalization of the pathologic patterns associated with the failing heart.

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