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Heart Failure

The Impact of Angiotensin-Converting Enzyme Inhibitor Therapy on the Extracellular Collagen Matrix During Left Ventricular Assist Device Support in Patients With End-Stage Heart Failure

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Objectives   We hypothesized that angiotensin-converting enzyme inhibition (ACE-I) during left ventricular assist device (LVAD) support in patients with end-stage heart failure prevents potentially deleterious effects on the extracellular matrix.

Background   Left ventricular assist device-induced mechanical unloading increases myocardial collagen and stiffness and may contribute to the low rate of recovery.

Methods   Heart samples obtained before and after LVAD implantation were divided into groups depending on whether the patients received (n = 7) or did not receive (control; n = 15) ACE-I. At transplant, ex vivo pressure-volume relationships were measured and chamber and myocardial stiffness constants determined. Myocardial tissue content of angiotensin (Ang) I and II, matrix metalloproteinase (MMP)-1, tissue inhibitor of MMPs (TIMP)-1, and total and cross-linked collagen was measured.

Results   Duration of support was comparable between ACE-I and control subjects (96 ± 65 days vs. 109 ± 22 days). Pre-LVAD Ang I and II and total and cross-linked collagen were similar between groups. Post-LVAD, Ang II was reduced in the ACE-I group but increased in control subjects (181 ± 7 fmol/g vs. 262 ± 41 fmol/g; p < 0.05). Similarly, cross-linked collagen decreased during LVAD support in the ACE-I group. Left ventricular (LV) mass and myocardial stiffness were lower in the ACE-I group. ACE-I normalized the LV and right ventricular (RV) MMP-1/TIMP-1 ratio. Collagen content and characteristics of the RV were not affected by ACE-I.

Conclusions   ACE-I therapy was associated with decreased Ang II, myocardial collagen content, and myocardial stiffness during LVAD support. This is the first demonstration of a pharmacologic therapy that can impact myocardial properties during mechanical unloading, and it could foster new lines of investigation in strategies of enhancing myocardial recovery during LVAD support.   (J Am Coll Cardiol 2007;49:1166–74) © 2007 by the American College of Cardiology Foundation

It is widely appreciated that hemodynamic support and unloading of the heart provided by left ventricular assist devices (LVADs) have profound broad-based effects on fundamental properties of cardiac myocytes of end-stage failing human hearts (1). Myocardial cell size and left ventricular (LV) size and mass approach normal, calcium cycling properties, and isolated myocardial contractility are improved, and

gene expression patterns and signaling pathways are normalized. These impressive effects have been broadly termed reverse structural, functional, and molecular remodeling, respectively (2,3). However, despite these positive effects, LVAD placement does not induce changes in ventricular function that permit LVAD removal with sustained cardiac recovery in a majority of patients (4,5). Thus far, the only patient groups in which promising outcomes are observed are those with myocarditis or acute cardiogenic shock (6).

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We recently showed that prolonged mechanical hemodynamic unloading increases myocardial tissue levels of angiotensin (Ang) II with concomitant increases in collagen cross-linking and elevation in myocardial stiffness (7). This led us to hypothesize that increased fibrosis occurring during mechanical unloading could be a factor in explaining the lack of correlation between cellular and whole heart recovery during LVAD support (8,9).

It is known that elevated serum levels of Ang II could be reduced by blocking the renin-angiotensin-aldosterone system with an angiotensin-converting enzyme inhibitor (ACE-I) (10). This leads to improved cardiac performance with reduced cardiovascular death and myocardial infarction, as shown by the HOPE (Heart Outcome Prevention Evaluation) study (11). Therefore, the purpose of the present study was to test the hypothesis that ACE-I therapy during LVAD support decreases tissue levels of Ang II, reduces myocardial fibrosis, and thus improves myocardial function. To understand mechanisms of changes in extracellular matrix properties, the impact of ACE-I on tissue inhibitor of matrix metalloproteinases (TIMP) and matrix metalloproteinase (MMP) activity was evaluated.

Methods

Myocardial tissue samples were obtained from the LV apex at the time of LVAD implantation (LVAD core) and subsequently at the time of cardiac transplantation from 22 patients after LVAD support (HeartMate VE LVAD, Thoratec Corp., Pleasanton, California). Seven of these patients were receiving ACE-I during LVAD support compared with 15 who were not. This was a retrospective study, and the use of ACE-I treatment was based exclusively on the clinical decisions of the primary care physicians. At the time of transplantation, myocardial samples were collected from the LV free wall at a region without macroscopic signs of fibrosis. In addition, LV samples were collected from end-stage heart failure patients who were transplanted without LVAD support (n = 26). To help differentiate the effect of LVAD unloading from the effects of ACE-inhibition on extracellular matrix (ECM) remodeling, myocardial samples were also collected from the right ventricle (RV) at the time of cardiac transplantation from end-stage failing hearts with (n = 15) and without (n = 16) LVAD support. In addition LV and RV samples were collected from nonfailing hearts not suitable for cardiac transplantation (LV: n = 5; RV: n = 3). Samples from nonfailing hearts were without ACE-I medication. All patients from which non-LVAD end-stage failing LV and RV samples were obtained were taking ACE-I. All samples were snap-frozen in liquid nitrogen and kept at −80°C for later analysis.

In addition, the whole hearts were obtained from these patients at the time of cardiac transplantation after LVAD support for measurement of the ex vivo pressure-volume relationship and estimation of chamber and myocardial stiffness. The LV trabeculae were isolated from a subset of these hearts and contractile performance studied in a muscle bath as detailed in the subsequent text.

This study was performed according to the guidelines of the Declaration of Helsinki. All procedures involving human tissue use were approved by the institutional review board of the New York Presbyterian Medical Center and Erasmus Medical Center.
Angiotensin I and II myocardial tissue levels. Tissue Ang I and II levels were measured after SepPak extraction and high-performance liquid chromatography (HPLC) separation by radioimmunoassay (12). A known amount of ^{125}I-Ang I was added as an internal standard before the extraction procedure, and the recovery of ^{125}I-Ang I after HPLC separation was used to correct for losses (maximally 20% to 30%) that occurred during extraction and separation.
MMP-1, MMP-9, and TIMP-1 protein content. Heart samples were homogenized in 1-ml lysis buffer (0.05-mol/l Tris-Cl, 150-mmol/l NaCl, and 1% Triton, pH 8.0). The homogenate was centrifuged at 3000 g for 20 min at 4°C and the supernatant collected. One hundred milliliters of the supernatant was tested in duplicate for total MMP-1 (free and MMP-1/TIMP-1 complexed), MMP-9 (free and TIMP-1–complexed proMMP-9), and TIMP-1 using enzyme-linked immunosorbent assay kits (Amersham Biotrak, Piscataway, New Jersey) following the manufacturer's instructions. Values of MMP-1, MMP-9, and TIMP-1 were standardized per mg of heart tissue protein.
Collagen characterization. Myocardial collagen can be fractioned into pepsin-soluble and pepsin-insoluble collagens, the latter reflecting primarily cross-linked collagen. The myocardial pepsin-soluble collagens were extracted overnight with 5-mg/ml pepsin in 0.5-mol/l acetic acid. The soluble and insoluble collagens were separated by centrifugation at 3,000 g for 10 min at 4°C. The soluble collagens included both denatured and undenatured collagens, with the latter quantified by the Sircol collagen assay kit with soluble type I collagen as a standard (Accurate Chemicals, Westbury, New York).

Total myocardial collagen content was assessed by measuring hydroxyproline content using a modified Stegemann method in hydrolyzed total soluble and insoluble collagens (13). The extent of collagen denaturation was assessed by the content of undenatured collagen and the ratio of undenatured to total soluble collagens. The ratio of insolu-

Abbreviations and Acronyms

ACE-I = angiotensin-converting enzyme inhibition

Ang = angiotensin

CHF = chronic heart failure

ECM = extracellular matrix

EDPVR = passive end-diastolic pressure-volume relationship

LV = left ventricle/ventricular

LVAD = left ventricular assist device

MMP = matrix metalloproteinase

RV = right ventricle/ventricular

TIMP = tissue inhibitor of matrix metalloproteinases

ble to total soluble collagen was calculated and used as a measure of cross-linking.

Passive pressure-volume relationship and estimation of chamber and myocardial stiffness. The passive LV end-diastolic pressure-volume relationship (EDPVR) of each arrested heart was measured as described previously (7). In brief, all hearts were perfused with cold hypocalcemic hyperkalemic cardioplegic solution at explantation. The aortic root and the LVAD inflow cannula were clamp-occluded. A metal adapter was attached to the mitral annulus and a compliant water-filled latex balloon was placed within the LV chamber. Pressure within the balloon was measured with a high-fidelity micromanometer as volume was progressively increased. The pressure was then plotted as a function of volume at each step, resulting in a passive pressure-volume relationship equivalent to the end-diastolic pressure-volume relationship of the beating heart. While measuring this relationship in the LV chamber, the RV chamber was emptied. The size of the LV chamber was indexed by the volume at which pressure within the ventricle reached 30 mm Hg (LVV_{30}).

Ventricular chamber stiffness (α) was calculated from the ex vivo EDPVRs according to the following equation, as described by Mirsky and Pasipoularides (14):

$$P = \beta \cdot e^{[\alpha \cdot (V/V_w)]} \qquad [1]$$

where P is pressure, V is volume, V_w is myocardial wall volume (calculated from measured LV mass and assumed density of 1.05 g/ml), α is the chamber stiffness, and β is a stiffness constant.

Myocardial stiffness (κ) is the slope of the Ln(stress) versus Ln(strain) relationship, for which myocardial stress and strain are estimated assuming a spherical geometry of the heart with an internal radius "a" and an external radius "b" (14). Midwall stress (σ_m) and midwall strain (ϵ_m) were calculated according to the following equations:

$$\sigma_m = 2.03 \cdot P \cdot V / V_w \cdot [2 \cdot (b/a)/(1 + (b/a))]^3 \qquad [2]$$

$$\epsilon_m = (1/2) \cdot [(3/4 \cdot \pi) \cdot V]^{(1/3)} \cdot (1 + b/a) \qquad [3]$$

$$(b/a) = (1 + V_w/V)^{1/3} \qquad [4]$$

Because there is no accepted simple analytical approach for estimating RV stresses and strains, no attempt was made to estimate RV myocardial stiffness.

All calculations were performed using commercially available software (Igor Pro 4.01, WaveMetrics, Lake Oswego, Oregon).

Myocardial force generation. Myocardial force data were obtained from LV free wall trabeculae as described previously (15). In brief, immediately after cardiectomy, trabeculae <1 mm in diameter were excised and immersed in oxygenated (95% O_2, 5% CO_2) ice-cold Krebs-Ringer solution with 30-mmol/l 2,3-butanedione monoxime. For the mechanical measurements, the muscle strips were mounted in a bath with one end connected to a force transducer and the other connected to a length-adjustable micrometer gauge using fine steel hooks. During superperfusion with 37°C oxygenated Krebs-Henseleit buffer (rate 1 ml/l, bath volume 1 ml), trabeculae were stimulated at 1 Hz and allowed to equilibrate for at least 1 h at slack length. After the resting period, isometric twitches were evoked with stimulation voltage 20% above threshold (duration 5 ms). The trabeculae were then progressively stretched to the length of maximal force generation (L_{max}) at 1 Hz frequency. Adequate muscle oxygenation was confirmed as detailed previously (16). Myocardial force generation was calculated after normalization to cross-sectional area.

Statistics. Results are presented as mean ± SEM. One-way analysis of variance (ANOVA) was used to select differences between groups. A Student-Newman-Keuls test was used for multiple comparisons. A Student *t* test was used for comparison of 2 groups. A paired *t* test was used for comparing pre- and post-LVAD samples. The statistical analysis was conducted with commercially available statistical software (SPSS 11.5; SPSS, Chicago, Illinois). A p value of <0.05 was considered to be statistically significant.

Results

Patient population and ACE-I medication. Patient demographics of the ACE-I and control LVAD groups are summarized in Table 1. Both groups had comparable ages, gender distributions, heart failure etiologies, and durations of LVAD support. Except for a lower mean arterial pressure in the ACE-I group, baseline hemodynamic parameters before implant were also comparable. Reasons for LVAD implantation were similar in both groups and included

Table 1	Baseline Characteristics		
	CHF Without LVAD	**LVAD With ACE-I**	**LVAD Without ACE-I (Control)**
n	26	7	15
Age, yrs	51 ± 13	51 ± 17	55 ± 8
Male, %	66*	100	84
DCM, %	57	57	40
LVAD duration, days	N/A	109 ± 22	96 ± 65
ACE-I use before LVAD, %	88.7	71.4	73.3
MAP, mm Hg	78.1 ± 10.7	65.7 ± 6.2†	75.5 ± 7.5
PCWP, mm Hg	29.6 ± 12.7	28.0 ± 3.7	25.5 ± 7.0
RAP, mm Hg	13.7 ± 7.0	13.0 ± 6.6	11.8 ± 4.7
CO, l/min	2.84 ± 0.8	3.9 ± 1.1	4.2 ± 1.1
Reason for LVAD			
Deterioration on Tx wait list		6 (86%)	12 (80%)
Post-thoracotomy		1 (14%)	1 (7%)
Post-MI cardiogenic shock		0 (0%)	2 (13%)

*p < 0.05 CHF without LVAD versus ACE-I and control; †p < 0.05 versus CHF without LVAD and control (analysis of variance).

ACE-I = angiotensin-converting enzyme inhibition; CHF = congestive heart failure; CO = cardiac output; DCM = dilated cardiomyopathy; LVAD = left ventricular assist device; MAP = mean arterial blood pressure; MI = myocardial infarction; N/A = not applicable; PCWP = pulmonary capillary wedge pressure; RAP = right arterial pressure; Tx = cardiac transplant.

clinical deterioration on the transplant waiting list with need for increasing doses of inotropic support, cardiogenic shock after myocardial infarction, and low output syndrome after coronary artery bypass graft. The relatively high average values of cardiac output were in the setting of preoperative inotropic support in most of the patients.

Eleven of the 15 patients from the control group received ACE-I therapy before LVAD implantation, but this was never restarted after LVAD implantation. Five of the 7 patients in the ACE-I group were taking ACE-I before LVAD implantation. The ACE-I used was enalapril, captopril, or lisinopril and was started in this group of patients approximately 30 days after LVAD implantation and continued during LVAD support until transplantation (Table 2). Other cardiovascular medications used are summarized in Table 3.

Angiotensin I and II myocardial tissue levels. Compared with the pre-LVAD state, in which most patients were already receiving ACE-I treatment, myocardial tissue levels of Ang I significantly increased after LVAD support in both the ACE-I and the control group ($p < 0.05$) (Table 4). However, the increase tended to be lower in the ACE-I group. In RV samples (obtained only after LVAD support), the Ang I levels were nonsignificantly higher in the control than in the ACE-I group.

In contrast, tissue Ang II levels were significantly reduced during LVAD support in the ACE-I group, whereas they increased in the control group (Table 4). The Ang II levels from the post-LVAD RV myocardium showed a similar trend.

Protein levels of MMP-1, MMP-9, and TIMP-1. MMP-1 protein levels increased significantly in end-stage heart failure despite ACE-I treatment in both LV and RV ($p < 0.05$) (Fig. 1A). After LVAD support, MMP-1 protein levels trended toward lower levels in patients who received ACE-I. This trend was also observed with MMP-9 levels, but to a lesser extent (Fig. 1B). In contrast, TIMP-1 levels, which was reduced in end-stage heart failure, increased tremendously during LVAD support, especially in the patients receiving ACE-I ($p < 0.05$ vs. chronic heart failure [CHF]) (Fig. 1C). Thus, the MMP-1/TIMP-1 ratio was normalized during LVAD support by ACE-I treatment (Fig. 1D).

Table 3	General Medication in Both LVAD Groups	
Medication	**LVAD Without ACE-I, % (n = 15)**	**LVAD With ACE-I, % (n = 7)**
ACE-I*	0	100
Beta-blocker	0	0
Amiodarone	13	29
Ca++ channel antagonist	7	14
Digitalis	13	29
Diuretics	40	43
Mineralocorticoid receptor antagonist	13	0

*For individual ACE-I medications, see Table 2.
Abbreviations as in Table 1.

Collagen content. In the paired samples from the LV, myocardial tissue levels of total, soluble, and insoluble cross-linked collagen significantly increased during LVAD support in the control group ($p < 0.05$ vs. pre-LVAD) (Table 4). However, ACE-I therapy was associated with a significant reduction of cross-linked collagen ($p < 0.05$ vs. pre-LVAD) and preserved the increase in total and soluble collagen. This reverse remodeling process was not detectable in the RV samples from the ACE-I group, which showed trends similar to the control group.

EDPVR and stiffness. Figure 2 shows group-averaged ex vivo LV EDPVR from the ACE-I and control groups. Although the duration of LVAD support was similar, these curves shifted leftward toward more normal volumes in both LVAD groups. This trend was more prominent in the ACE-I group, and there was a nearly statistically significant reduction of LVV_{30} in the ACE-I group compared with the control group (140 ± 23 ml vs. 195 ± 13 ml; $p = 0.06$). Further, there was a significant reduction in LV mass in ACE-I compared with control patients (171 ± 54 g vs. 252 ± 48 g; $p < 0.05$) (Fig. 3). The ACE-I treatment had no significant effect on RV ex vivo EDPVR or mean RVV_{30}.

The dimensionless chamber stiffness constant α was not affected by ACE-I treatment in the LV or RV (Table 5). However, calculated LV myocardial tissue stiffness was significantly lower in the ACE-I group than in the control group ($p < 0.05$) (Table 5).

Myocardial force generation. To assess whether there was any functional consequence of differences in collagen content, isolated muscle strips were superfused at baseline conditions and during beta-adrenergic stimulation with isoproterenol. Figure 4 shows that developed force was similar in muscle strips after LVAD support with and without ACE-I treatment both in basal state and after beta-adrenergic stimulation.

Discussion

Left ventricular assist device-induced hemodynamic unloading in patients with end-stage heart failure induces reverse structural remodeling (leftward shift of the EDPVR and reduced LV mass) but is associated with increases in myocardial Ang II levels, total and cross-linked collagen,

Table 2	ACE-I Medication in LVAD Patients		
Patient #	**ACE-I***	**Max. Dose**	**Duration (Days)**
1	Enalapril	5 mg bid	33
2	Enalapril	12.5 mg bid	56
3	Lisinopril	5 mg qd	20
4	Captopril	25 mg tid	75
5	Lisinopril	10 mg qd	94
6	Captopril	50 mg tid	177
7	Captopril	50 mg tid	217

*Medication started around 30 days after implant.
Abbreviations as in Table 1.

Table 4	Angiotensin I and II Measurements and Collagen Characterization					
			LVAD Without ACE-I (Control)		LVAD With ACE-I	
	Normal (LV n = 5; RV n = 3)	CHF + ACE-I (LV n = 26; RV n = 16)	Pre-LVAD (n = 15)	Post-LVAD (n = 15)	Pre-LVAD (n = 7)	Post-LVAD (n = 7)
Ang I						
LV	97 ± 13	275 ± 74	49 ± 8	653 ± 310*	54 ± 9	261 ± 52*
RV	129 ± 6	181 ± 48	N/A	631 ± 260	N/A	444 ± 281
Ang II						
LV	106 ± 16	129 ± 31	109 ± 39	262 ± 41*	163 ± 64	81 ± 7*†
RV	161 ± 22	217 ± 51	N/A	212 ± 57	N/A	77 ± 12
Total collagen						
LV	4.17 ± 0.16	8.21 ± 3.4	8.3 ± 1.38	10.8 ± 1.49*	6.9 ± 0.71	5.3 ± 0.73†
RV	5.51 ± 0.59	6.15 ± 0.41	N/A	8.5 ± 0.79	N/A	8.3 ± 0.92
Soluble collagen						
LV	3.61 ± 0.15	6.61 ± 1.6	3.8 ± 0.71	5.3 ± 0.72*	4.0 ± 1.06	3.3 ± 0.63
RV	4.68 ± 0.28	5.63 ± 0.32	N/A	5.9 ± 0.52	N/A	5.5 ± 0.47
Cross-linked collagen						
LV	0.56 ± 0.15	2.68 ± 1.4	5.9 ± 1.05	8.4 ± 1.10*	6.4 ± 0.76	5.4 ± 0.63*
RV	1.37 ± 0.15	2.21 ± 0.41	N/A	5.5 ± 1.66	N/A	5.4 ± 1.90

Ang I and II measurements in fmol/g; all collagen measurements in μg/mg; *p < 0.05 versus pre-LVAD (same group, paired *t* test); †p < 0.05 versus post-LVAD (control, analysis of variance).
Ang = angiotensin; LV = left ventricle; RV = right ventricle; other abbreviations as in Table 1.

and myocardial tissue stiffness. These changes in ECM represent a further pathological progression beyond that observed in end-stage heart failure alone. Such changes are relatively unique in that almost all other aspects of myocardial properties that have been studied change toward normal during LVAD support. We have therefore speculated that
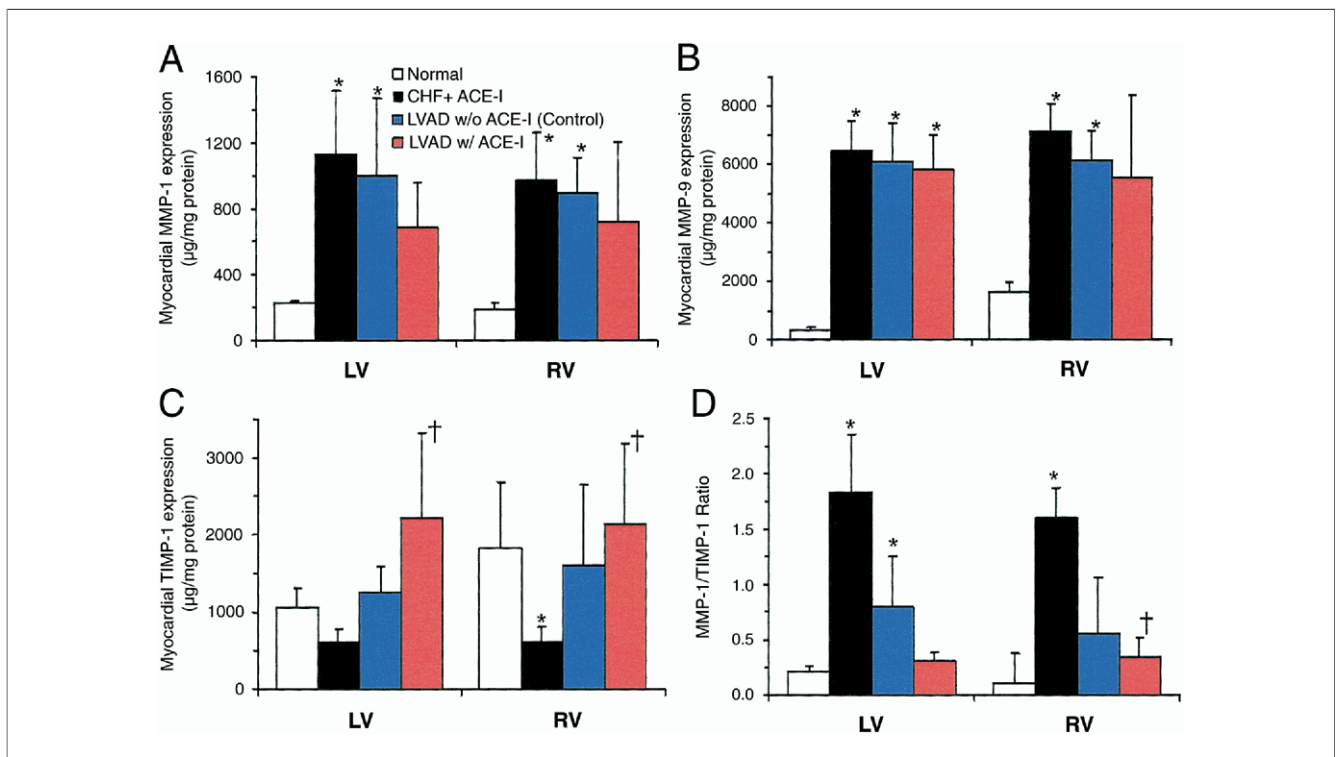

| Figure 1 | MMP1- and TIMP-1 Protein Expression |

Matrix metalloproteinase (MMP)-1 **(A)**, MMP-9 **(B)**, and tissue inhibitor of MMP (TIMP)-1 **(C)** protein levels in normal **(open bars)**, chronic heart failure (CHF) + angiotensin-converting enzyme inhibition (ACE-I) **(solid bars)**, and left ventricular assist device (LVAD)-supported hearts without ACE-I **(blue bars)** and with ACE-I **(red bars)**. Whereas MMP-1 protein levels are slightly reduced in LV samples after LVAD support and ACE-I, TIMP-1 protein levels are significantly elevated in this group compared with CHF hearts without LVAD support. The MMP-1/TIMP-1 ratio **(D)** shows a trend toward better normalization after LVAD support if patients received ACE-I. *p < 0.05 versus normal hearts; †p < 0.05 versus CHF + ACE-I (analysis of variance). LV = left ventricle; RV = right ventricle.
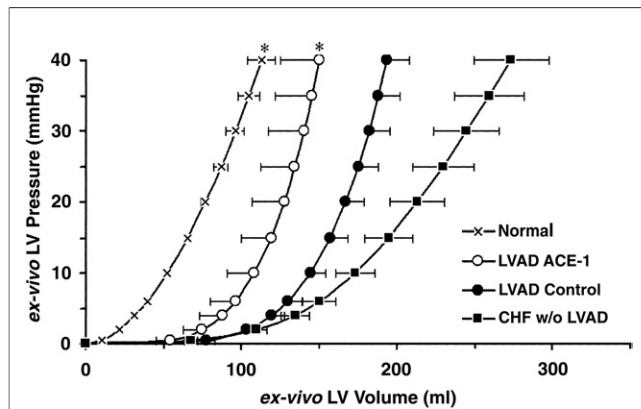
Figure 2   **EDPVRs With and Without ACE-I**

Grouped end-diastolic pressure-volume relationships (EDPVR) measured ex vivo from whole hearts. The average EDPVR from patients with LVAD support receiving ACE-I (LVAD ACE-I, **open circles**) tended to be shifted to the left toward lower volumes compared with the EDPVR from patients with LVAD support without ACE-I (LVAD Control, **solid circles**). X = nonfailing hearts; **solid squares** = failing hearts without LVAD support. *p < 0.05 versus CHF without LVAD (analysis of variance). Abbreviations as in Figure 1.

the ECM should be a target for improving the frequency and extent of recovery during LVAD support.

We demonstrated that ACE-I therapy during LVAD support enhances reverse structural remodeling in the LV (further shifts toward normal of the EDPVR and greater mass reductions) while reducing tissue Ang II concentration, normalizing the MMP-1/TIMP-1 ratio, and decreasing total and cross-linked collagen and myocardial stiffness. The data from the RV samples show that ACE-I also reduced myocardial tissue levels of Ang II and normalized the MMP-1/TIMP-1 ratio in that chamber as well, but RV collagen content remained elevated. Additionally, ACE-I therapy did not impact RV mass or chamber size. Combining this information from the LV and the RV leads to the conclusion that mechanical unloading combined with

ACE-I are required to normalize myocardial fibrosis, and myocardial mass regression is more prominent during LVAD support in the presence of ACE-I treatment. As such, these findings indicate that the regulation of the ECM during LVAD support is more complex than previously thought and involves a complex interaction of mechanical and neurohormonal factors. Additionally, it is observed that despite these substantial beneficial effects on the ECM, ACE-I therapy had no detectable effect on the myocardial force generation in isolated trabeculae.

It is noteworthy that our findings indicated that a reduction in load and reduction of tissue Ang II levels were both necessary to prevent fibrosis. This is provocative and deserves further study for verification; however we propose that, in addition to Ang II, other, potentially dual-regulated, upstream factors could be involved.

Although it is well documented that ACE-I therapy and other drugs can dramatically reduce cardiac fibrosis and impact on cardiac structure and function (17), the effects of such treatments in the setting of LVAD support are not previously understood. At a functional level, these changes were associated with a decrease in LV myocardial stiffness, reflecting the fundamental changes in myocardial material properties. The LV chamber stiffness, however, was not changed. This is not surprising, because chamber stiffness indexes lumped properties of the entire LV, which is determined not only by myocardial stiffness but also by myocardial mass, chamber size, and chamber geometry. In this case, LVAD-induced changes in LV mass and size counteract changes in myocardial stiffness such that net chamber stiffness is not affected.

There is a major gap between the overwhelmingly beneficial impact of prolonged LVAD support on myocardial cellular properties and the very low rate of recovery of cardiac function to the point where the native heart is once again able to support the circulation, thus obviating the need for transplantation. Even when such weaning is
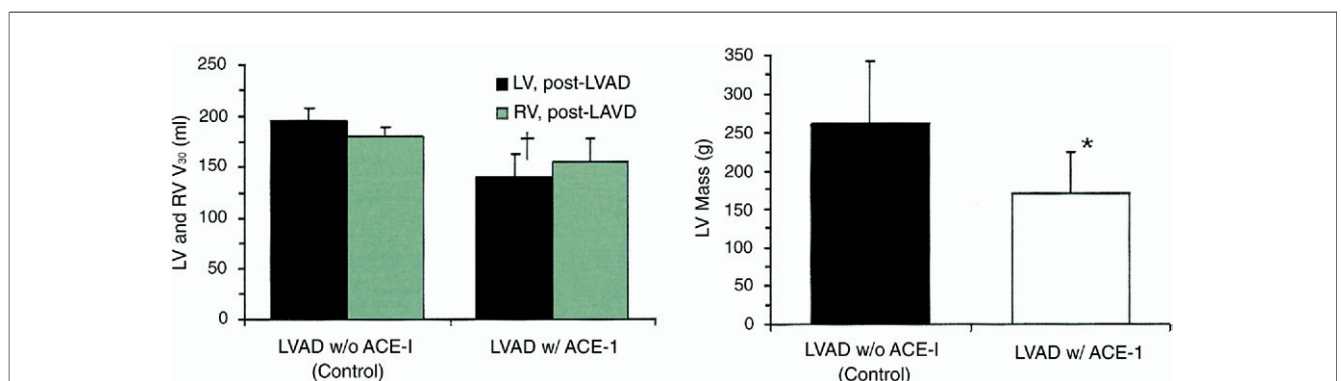

Figure 3   **Impact of ACE-I on LV and RV Volume and LV Mass**

(Left) LV **(black bars)** and RV **(green bars)** ex vivo volume at the pressure of 30 mm Hg. With ACE-I therapy, LV volume was almost significantly lower compared with the control group. **(Right)** LV mass was significantly lower in the ACE-I group **(open bar)** compared with the control group **(solid bar)**. *p < 0.05 versus LV control group; †p < 0.062 versus control group (Student *t* test). Abbreviations as in Figure 1.

Table 5	Stiffness Measurements			
			LVAD Without ACE-I (Control)	LVAD With ACE-I
	Normal (LV n = 5; RV n = 3)	CHF + ACE-I (LV n = 26; RV n = 16)	Post-LVAD (n = 15)	Post-LVAD (n = 7)
Chamber stiffness, α				
LV	11.50 ± 4.22	6.52 ± 4.22	8.89 ± 2.99	7.54 ± 3.98
RV	6.39 ± 0.12	9.66 ± 2.87	8.33 ± 0.81	9.07 ± 0.53
Myocardial stiffness, κ (mm Hg · ml)				
LV	26.67 ± 3.80	30.30 ± 8.31	31.29 ± 5.84	23.02 ± 5.84*
RV	N/A	N/A	N/A	N/A

*$p < 0.05$ versus LVAD (control) and CHF + ACE-I (analysis of variance).
Abbreviations as in Tables 1 and 4.

possible, heart function frequently deteriorates and patients require transplant, reinsertion of another LVAD, or succumb to complications of heart failure (4,5,18).

In an attempt to enhance the rate and sustainability of cardiac functional recovery during LVAD support, investigators in Harefield employ a high dose of the selective β_2-adrenergic receptor agonist clenbuterol along with beta-blocker, ACE-I, angiotensin-receptor blockade, and aldosterone in combination with a special LVAD weaning protocol (the so-called Harefield protocol). With this approach, these investigators were able to successfully wean more than two-thirds of all LVAD-supported patients (19). The rationale underlying this approach was the intent to use clenbuterol to induce physiologic myocardial hypertrophy and thus improve cardiac contractility. However, other factors have been implicated as well. For example, Terracciano et al. (20) recently showed that specific changes in

Figure 4   Developed Force Measured From Isolated Myocardial Trabeculae

Developed force (mN/mm²) at baseline (**open bars**) and during β-stimulation with isoproterenol (**solid bars**) in the control and ACE-I groups. Whereas isoproterenol significantly increases developed force, there is no difference between the control and the ACE-I groups. *$p < 0.001$ versus baseline (Student t test). Abbreviations as in Figure 1.

excitation-contraction coupling appeared to be associated with this clinical recovery. In another recent publication, Barton et al. (21) showed that clenbuterol increased insulin-like growth factor I messenger RNA in cardiac myocytes in these patients and may limit atrophy and apoptosis during reverse remodeling. The fact that these patients are receiving a host of drugs with a multitude of effects renders it impossible to conclude that one drug or another is responsible for recovery or by which mechanism such treatments might be working.

Furthermore, long-term experience after LVAD removal in these patients is not yet available, so the sustainability of the recovery has yet to be confirmed. In addition, a similar protocol tested in LVAD patients at Columbia University was not able to show any improvement in cardiac function with this regimen, and no patients could be weaned from the LVAD (22,23).

Accumulating experimental evidence suggests that increased cardiac collagen concentration is coincident with a marked deterioration of systolic and diastolic function (24). Cross-linked collagen especially appeared to be involved in end-stage heart failure remodeling (25). Factors contributing to potentially deleterious ECM remodeling during LVAD support have recently been shown by our group (7) and in part confirmed by another group (26). Bruggink et al. (26) found that LVAD increased ECM volume in the first 200 days. Owing to changes in the ratio between MMP-1 and TIMP-1 and increased myocardial tissue Ang and angiotensinogen levels, collagen degradation is decreased, leading to these further abnormal ECM changes during LVAD support.

The data concerning effects on the RV show that the reduction in myocardial Ang II and the normalization of the MMP-1/TIMP-1 ratio was a global effect of oral ACE-I. However, the RV data further show that, although ACE-I therapy is able to prevent deleterious collagen increase in the LV during LVAD support, the collagen remodeling in the RV was not significantly affected. The impact of ACE-I therapy to reduce RV tissue Ang II levels but not RV collagen or RV size may be at odds with the current thinking that in experimental settings, nonhypotensive doses of ACE-I inhibitors can decrease collagen content.
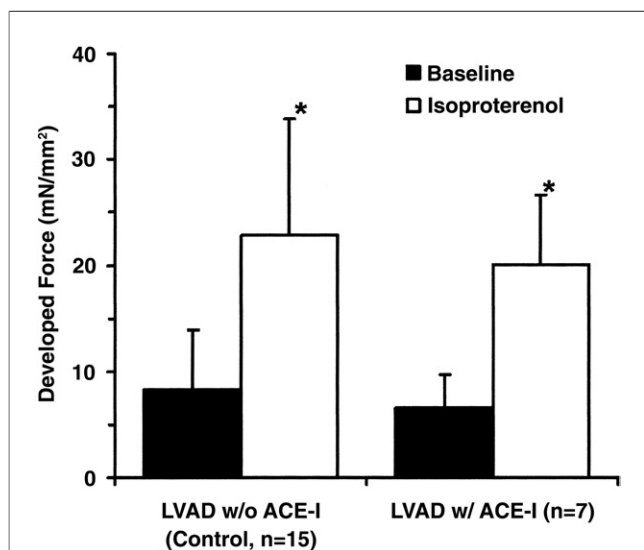
However, one important difference between those experimental settings (in which hemodynamic load is normal) and the RV of LVAD supported patients is that the RV of the LVAD patients is hemodynamically overloaded. Therefore, it is possible that the ongoing mechanical stimulation overrides the effect of ACE-I with regard to regulation of collagen metabolism.

In addition, despite changes in collagen content, there was no detectable difference in contractile performances of muscle strips isolated from post-LVAD hearts with or without ACE-I therapy. It is not certain whether such in vitro preparations are sensitive and reliable enough to detect what may be a subtle but clinically significant impact on myocardial function. If true, however, this finding would be in contrast to the popular notion that fibrosis by itself can independently impair myocardial function (27). Although myocardial force generation was unaltered by ACE-I treatment, this does not indicate that treatment had no effect on overall cardiac performance. As discussed in the preceding text, ACE-I treatment prevented dysfunctional matrix changes which can decrease the effectiveness of myocyte contraction. It is conceivable that myocyte alignment may have been modified in a manner that would permit more effective force generation in the hearts of LVAD patients receiving ACE-I.

Study limitations. This was a retrospective observational study, not a randomized interventional study, which results in several limitations. First, the decision of which patients received ACE-I treatment after LVAD implant was based on clinical grounds (i.e., development of hypertension) which could introduce a selection bias. It is significant that baseline clinical features, myocardial Ang content, and collagen characteristics were similar between the 2 groups. Furthermore, conventional wisdom would suggest that myocardial collagen content and cross-linking would be significantly increased in the setting of hypertension. The fundamental finding that collagen content and cross-linking were decreased in the LVAD ACE-I group suggests that this selection bias was not a factor, but this conclusion is not certain.

Second, it is generally assumed that abnormally increased collagen content and cross-linking which result in increased myocardial stiffness are in general detrimental to cardiac function. All patients in the present study were transplanted, and no functional assessment of recovery or attempt to wean LVAD support was performed. Although we demonstrate ACE-I–mediated reversal of ECM changes, no assessment of the impact of these changes on LV function in vivo has been made in these patients. Therefore, it cannot be concluded that these changes confer any benefit to pump function or, most importantly, patient hemodynamics. The findings of the lack of impact on myocardial function would suggest that if any such benefit were to be induced, they would more likely be mediated by beneficial effects on heart structure than on myocardial function, but at the present a definitive conclusion cannot be made.

Third, we make the assumption that the impacts of 3 different ACE-Is used in these patients (enalapril, captopril, and lisinopril) are equivalent with regard to effect on ECM remodeling.

Finally, in calculating myocardial stiffness, the same equation is applied to normal, failing, and post-LVAD hearts. The chamber geometry may be different between these conditions, and it is possible that the results could be influenced by such changes and therefore may not solely reflect changes in intrinsic myocardial material properties.

Conclusions

It is provocative that among the dozens of myocardial properties that have been examined after LVAD support (structural, cellular, molecular, and biochemical) the only major cardiac feature yet identified that does not return toward normal is the ECM (7,8,28,29). It is therefore important to understand whether such ECM changes contribute to the lack of global ventricular recovery that limits the ability to wean LVAD support in a vast majority of patients. Left ventricular assist device support is associated with increased myocardial content of Ang II, disruption of the MMP-1/TIMP-1 balance, and increased myocardial collagen cross-linking and myocardial stiffness. These effects are reversed by ACE-I therapy. This finding highlights the importance of understanding specific effects of different pharmacologic agents that may be used during LVAD support. Such understanding might lead to rational drug combinations that could optimize the chances of global myocardial recovery and facilitate the use of LVADs as a bridge to recovery.

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The Impact of Angiotensin-Converting Enzyme Inhibitor Therapy on the Extracellular Collagen Matrix During Left Ventricular Assist Device Support in Patients With End-Stage Heart Failure

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