

QUARTERLY FOCUS ISSUE: HEART FAILURE

# Impact of Mechanical Unloading on Microvasculature and Associated Central Remodeling Features of the Failing Human Heart

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- Objectives** This study investigates alterations in myocardial microvasculature, fibrosis, and hypertrophy before and after mechanical unloading of the failing human heart.
- Background** Recent studies demonstrated the pathophysiologic importance and significant mechanistic links among microvasculature, fibrosis, and hypertrophy during the cardiac remodeling process. The effect of left ventricular assist device (LVAD) unloading on cardiac endothelium and microvasculature is unknown, and its influence on fibrosis and hypertrophy regression to the point of atrophy is controversial.
- Methods** Hemodynamic data and left ventricular tissue were collected from patients with chronic heart failure at LVAD implant and explant (n = 15) and from normal donors (n = 8). New advances in digital microscopy provided a unique opportunity for comprehensive whole-field, endocardium-to-epicardium evaluation for microvascular density, fibrosis, cardiomyocyte size, and glycogen content. Ultrastructural assessment was done with electron microscopy.
- Results** Hemodynamic data revealed significant pressure unloading with LVAD. This was accompanied by a 33% increase in microvascular density (p = 0.001) and a 36% decrease in microvascular lumen area (p = 0.028). We also identified, in agreement with these findings, ultrastructural and immunohistochemical evidence of endothelial cell activation. In addition, LVAD unloading significantly increased interstitial and total collagen content without any associated structural, ultrastructural, or metabolic cardiomyocyte changes suggestive of hypertrophy regression to the point of atrophy and degeneration.
- Conclusions** The LVAD unloading resulted in increased microvascular density accompanied by increased fibrosis and no evidence of cardiomyocyte atrophy. These new insights into the effects of LVAD unloading on microvasculature and associated key remodeling features might guide future studies of unloading-induced reverse remodeling of the failing human heart. (J Am Coll Cardiol 2010;56:382–91) © 2010 by the American College of Cardiology Foundation

The number of patients who receive left ventricular assist devices (LVAD) is rapidly increasing (1), which offers a valuable opportunity for in-depth investigations into human cardiac biology (2,3). The examination of paired human

tissue before and after LVAD therapy enables one to correlate functional and structural effects of various therapies combined with LVAD. These advantages along with the safety platform LVAD offers make this patient popula-

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tion a precious “research vehicle” for investigating new remodeling and regenerative therapies for heart failure (HF). Yet, for these promises to be fulfilled, we must first understand the fundamental impact of mechanical unloading on the failing human heart.

Pathophysiologic models derived from basic science and animal studies focus on the role of excess load in driving a vicious cycle of cardiac remodeling (4). This mechanistic model led to the hypothesis that mechanical unloading would disrupt this cycle and improve function of the failing human heart (2,3,5). Despite the opportunity afforded by the use of LVAD in clinical practice, the effects of mechanical unloading on basic remodeling features are still poorly understood or unknown (2,3,5). It is noteworthy that the direct effect of mechanical unloading on myocardial endothelium and microvasculature is unknown, and its effect on the degree of hypertrophy regression, possibly to the point of atrophy, is controversial (2,5). Furthermore, reports of the LVAD unloading effects on cardiac fibrosis have been conflicting, with some showing reduction in fibrosis, and others showing an increase (2,3,5,6). The need for further understanding of the impact of unloading on specifically these key remodeling features is now even more acute, because there is increasing robust evidence demonstrating important mechanistic links among microvasculature, fibrosis, and hypertrophy during the cardiac remodeling process (7,8).

Here, we use whole field, endocardium-to-epicardium digital microscopy (9,10) coupled with ultrastructural and functional evaluation to examine the influence of mechanical unloading on these three central features of cardiac remodeling. The application of state-of-the-art digital histopathology for examination of left ventricular (LV) myocardial samples acquired from normal donors and from patients with end-stage chronic HF markedly increases the volume of tissue objectively and quantitatively analyzed and provides a new standard for future studies in the field of mechanical unloading and cardiac remodeling.

## Methods

**Study population.** The study group comprised 15 consecutive patients who received HeartMate I LVAD (Thoratec Corporation, Pleasanton, California) as a bridge to transplantation due to chronic end-stage HF. Patients who received LVAD support due to acute HF ( $n = 11$ ) were not included. Eight age-matched donors whose hearts were found to be functionally and structurally normal but were not suitable for transplantation for noncardiac reasons were used as normal control subjects.

**Clinical data collection.** We collected patient demographic data, data on comorbidities, echocardiography results, invasive hemodynamic data, and laboratory data within 24 h before LVAD implantation and again between weeks 2 and 4 after LVAD implantation.

## Histochemical stains and immunohistochemistry.

Myocardial tissue was prospectively obtained from the LV apical core at LVAD implantation. At the time of cardiac transplantation, the tissue was obtained from the surrounding apical area, at least 1 cm distant from the LVAD inflow cannula (to avoid inclusion of reactive tissue changes).

Tissue preparation and use of Masson’s trichrome stain for collagen content evaluation, periodic acid Schiff reaction (PAS) for cardiomyocyte size evaluation, the combination of PAS and periodic acid Schiff with diastase (PASD) for demonstration of glycogen content, and immunostaining for the endothelial cell protein CD34 and the endothelial activation marker major histocompatibility complex class 2 are detailed in the Online Appendix. To achieve a high degree of reproducibility we avoided manual staining, and both the histochemical stains and the immunohistochemistry experiments were performed with automatic staining systems (Online Appendix).

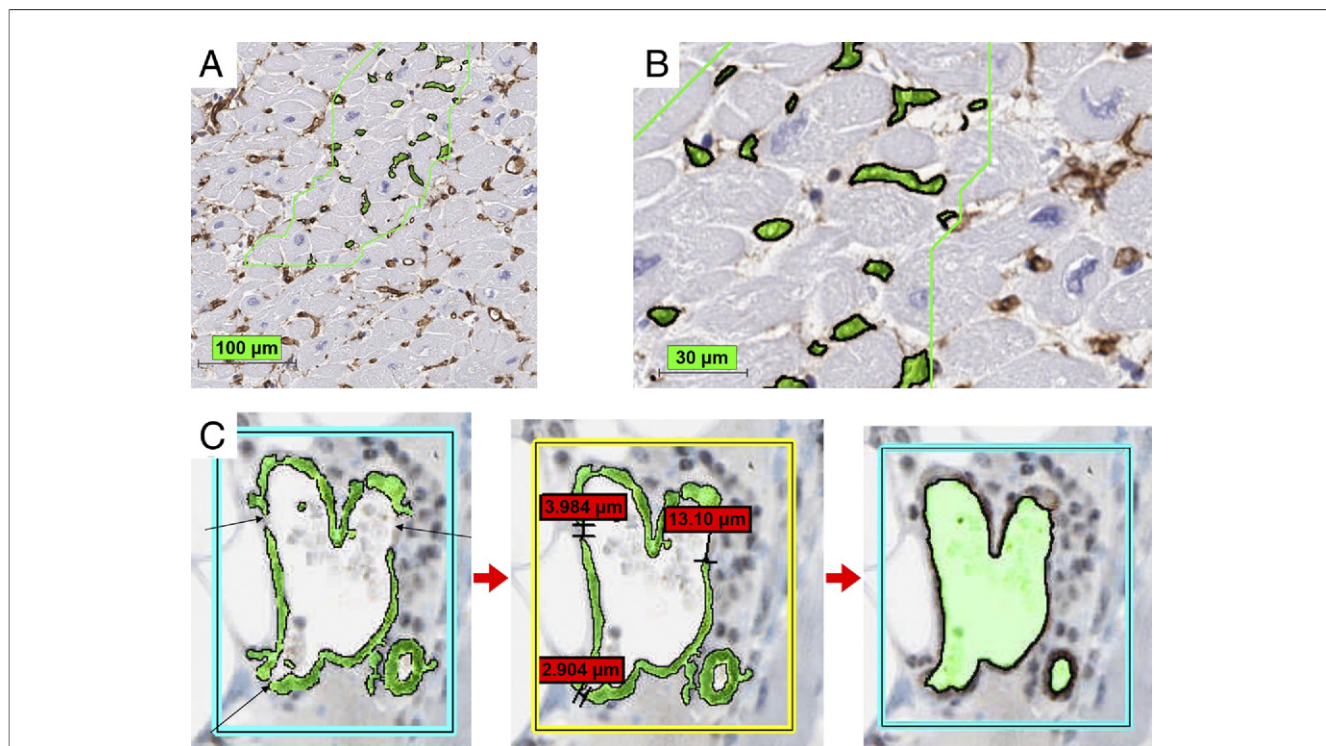
**Whole-field digital microscopy.** Advanced digital microscopy allowed examination of the entire heart tissue areas from the epicardium to the endocardium. Whole-slide images were analyzed with the ScanScope XT system equipped with the ImageScope 10.0 image analysis algorithms (Aperio Technologies, Vista, California) (9,10).

**MICROVASCULATURE EVALUATION.** We used the ImageScope 10.0 Microvessels analysis algorithm (10) to distinguish endothelial cells from nonspecific staining of surrounding tissue by applying appropriate dark and light thresholds (Fig. 1). Only myocardial tissue cuts oriented in cross section (epicardium to endocardium) were analyzed. Microvascular density was defined as “number of microvessels”/“total tissue analysis area.”

**COLLAGEN CONTENT EVALUATION.** We set the staining color threshold of the ImageScope 10.0 Colocalization analysis algorithm to accurately identify collagen on the basis of its blue color (Figs. 2A and 2B). “Interstitial fibrosis” was defined as the collagen content determined in the interstitial spaces and endomyrial/perimyrial spaces, including the collagen content around capillaries and small vessels (i.e., vessels with diameter  $<60 \mu\text{m}$ ) found within those spaces. The “total” collagen content (“total fibrosis”) was determined by including in our analysis the whole-field stained tissue without excluding any areas (Figs. 2C to 2E). The collagen content around medium and large vessels (i.e., vessels with diameter  $>60 \mu\text{m}$ ), “perivascular fibrosis,” was separately calculated by subtracting the “interstitial/perimyrial/endomyrial” collagen content from the determined “total” collagen content (i.e., “total fibrosis” minus “interstitial fibrosis”).

### Abbreviations and Acronyms

<b>HF</b>	= heart failure
<b>LV</b>	= left ventricular
<b>LVAD</b>	= left ventricular assist device
<b>PAS</b>	= periodic acid Schiff
<b>PASD</b>	= periodic acid Schiff with diastase



**Figure 1** Microvasculature Evaluation

(A, B) Left ventricular mid-myocardium immunohistochemically stained for the endothelial cell protein CD-34 (brown color). Algorithm thresholds were appropriately adjusted to allow microvascular evaluation within selected regions of interest. (A) 20× magnification; (B) 60× magnification. (C) Automatic completion of stained microvessels by the analysis algorithm enables measurements like vessel perimeter and lumen area.

**CARDIOMYOCYTE SIZE AND GLYCOGEN CONTENT EVALUATION.** Cardiomyocytes were accepted for size measurement if they met specific criteria detailed in the Online Appendix. Glycogen content was evaluated by adjusting the staining color thresholds of the algorithm to precisely capture the magenta PAS-positive stain. To exclude nonglycogen, magenta PAS-positive staining substances, a paired adjacent slide was analyzed after glycogen depolymerization with the PASD reaction (Online Appendix). Next, with our algorithm, glycogen stores could be evaluated by digital subtraction of the images derived from 2 paired adjacent slides stained with PAS and PASD each. Glycogen content was defined as the percentage of magenta stained area for the PAS stained slide minus the PASD stained slide for identical regions of interest.

**Electron microscopy studies.** Tissue was examined according to a classification scheme that was based on previous work in the field by our group (11) as well as according to our pre-specified research hypotheses for this study. The Online Appendix section describes the specific parameters included in this ultrastructural classification scheme to assess microvasculature and cardiomyocyte hypertrophy, degeneration, and atrophy.

**Statistical analysis.** An independent samples *t* test, a paired *t* test, and a Wilcoxon signed rank test were used to assess the statistical significance of differences observed.

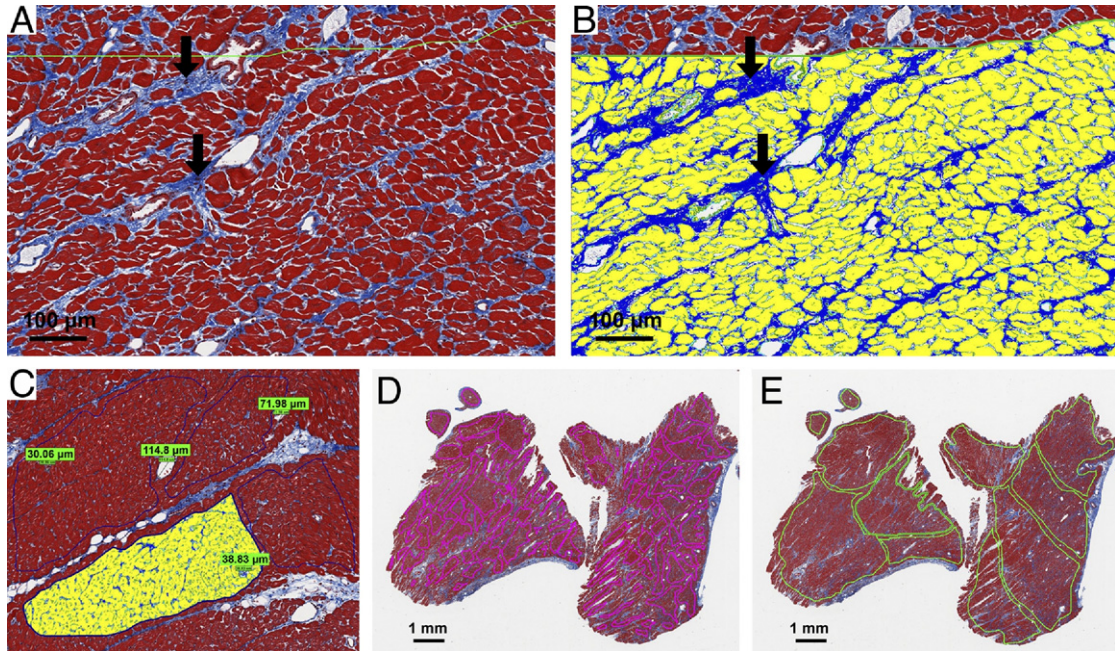
Our Institutional Review Board approved the study, and informed consent was obtained from patients before their enrollment.

## Results

**Clinical and hemodynamic data.** Patient demographic data and clinical and hemodynamic characteristics are shown in Table 1. Cardiac index and left and right sided pressures virtually normalized during LVAD support revealing significant LVAD-induced pressure unloading (Table 2). Medications used during LVAD support are listed in the Online Appendix.

**Structural, ultrastructural, and metabolic data.** The total tissue area under examination included all of the myocardial tissue sectioned and mounted on the slides (Figs. 2D and 2E). As such, the final average tissue analysis area/patient sample was  $74.9 \pm 49.5 \text{ mm}^2$ .

**MICROVASCULATURE STUDIES.** Microvascular density as assessed with digital microscopy was found to be significantly decreased in the failing hearts compared with the normal donor hearts. After LVAD unloading there was a 33% increase in microvascular density ( $p = 0.001$ ) (Fig. 3). Unloading effects on the microvasculature did not differ between ischemic and nonischemic chronic HF patients.



**Figure 2** Collagen Content Evaluation

Sections were stained with Masson's Trichrome stain—collagen stains blue. (A) Representative stained image from mid-myocardium before digital analysis was performed. (B) The section was digitally analyzed for collagen content on the basis of color thresholds. The analysis algorithm “highlighted” collagen as dark blue and was sufficiently sensitive and accurate to exclude even small nuclei (arrows) within the fibrous tissue. (C) “Interstitial fibrosis” was defined as the collagen content determined in manually selected regions of interest that excluded bands of perivascular fibrosis associated with any vessel with diameter >60 μm (i.e., medium/large vessels). (D) Whole slide, epicardium-to-endocardium image showing superimposed all the regions selected for assessment of “interstitial fibrosis” on the basis of the criterion described in C (0.5× magnification). (E) “Total” collagen content (“total fibrosis”) was determined by including in our analysis the whole-field stained tissue without excluding any areas (0.5× magnification).

Next, we examined the ultrastructural effects of LVAD unloading on microvasculature by electron microscopy studies in a randomly selected subset of HF patients (n = 5,

Patients #1, #2, #6, #8, and #14). We identified ultrastructural evidence of post-LVAD endothelial cell activation (pre-LVAD grading:  $0.4 \pm 0.5$  vs. post-LVAD:  $1.8 \pm 1.0$ ,

**Table 1** Individual Patient Characteristics

Patient #	Age (yrs)/ Sex	HF Etiology	DM	Before LVAD Implantation											Duration of Support (days)
				HR (beats/min)	SBP (mm Hg)	LVEF (%)	RA (mm Hg)	PAs (mm Hg)	PCWP (mm Hg)	CI (l/min/m <sup>2</sup> )	PVR (WU)	Na (mmol/l)	Cr (mg %)	Hgb (g/dl)	
1	57/M	IDC	No	106	90	20	12	55	30	1.7	2.7	131	1.0	11.0	183
2	55/M	IDC	No	120	95	10	13	70	26	1.4	3.4	133	1.2	11.2	63
3	39/M	IDC	No	115	85	10	12	53	32	1.8	3.5	124	2.1	10.0	24
4	59/M	IDC	No	95	90	10	9	55	23	1.7	4.8	128	1.0	13.2	33
5	50/M	IDC	Yes	129	90	15	10	62	30	1.8	3.0	148	3.0	10.6	70
6	51/M	IDC	No	120	85	10	9	60	25	2.0	3.4	144	1.7	9.0	28
7	17/M	IDC	No	120	110	20	20	56	37	1.5	2.8	135	1.6	14.0	212
8	62/M	ICM	No	118	80	5	8	50	25	1.8	2.5	131	1.2	13.6	86
9	49/M	ICM	No	101	95	15	5	39	22	2.0	1.5	135	1.3	10.1	283
10	64/M	ICM	Yes	80	95	15	11	102	31	2.8	6.5	135	1.9	10.0	187
11	48/M	ICM	No	91	105	20	8	88	32	2.8	6.0	133	1.6	11.0	113
12	49/M	ICM	No	125	95	22	15	40	23	3.2	0.6	132	2.5	13.0	79
13	58/M	ICM	No	95	80	25	12	61	29	2.2	3.7	131	1.9	12.0	22
14	57/M	ICM	No	124	95	10	17	45	30	2.8	2.5	135	0.9	13.0	21
15	57/M	ICM	No	101	80	20	7	47	20	2.8	2.6	132	2.6	10.8	21

CI = cardiac index; Cr = serum creatinine; DM = diabetes mellitus; Hgb = hemoglobin; HR = heart rate; ICM = ischemic cardiomyopathy; IDC = idiopathic dilated cardiomyopathy; LVAD = left ventricular assist device; LVEF = left ventricular ejection fraction; Na = serum sodium; PAs = systolic pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; RA = right atrial pressure; SBP = systolic blood pressure; WU = Wood unit.

**Table 2** Hemodynamic Parameters Before and After LVAD Unloading

	Before LVAD	After LVAD	p Value
Heart rate, beats/min	109 ± 15	82 ± 29	0.01
Systemic arterial blood pressure, mm Hg			
Systolic	91 ± 9	115 ± 21	<0.001
Diastolic	55 ± 8	63 ± 13	0.04
Right atrial pressure, mm Hg	11.2 ± 3.9	10.8 ± 5.3	0.776
Mean pulmonary artery pressure, mm Hg	41.9 ± 10.4	22.3 ± 4.4	<0.001
Diastolic pulmonary artery pressure, mm Hg	31.7 ± 5.6	16.1 ± 5.5	<0.001
Pulmonary capillary wedge pressure, mm Hg	27.7 ± 4.7	10.8 ± 4.4	<0.001
Cardiac index, l/m <sup>2</sup> /min	2.1 ± 0.6	2.9 ± 0.3	<0.001
Pulmonary vascular resistance, Wood units	3.3 ± 1.5	2.0 ± 0.5	0.008

N = 15. Values are mean ± SD.  
LVAD = left ventricular assist device.

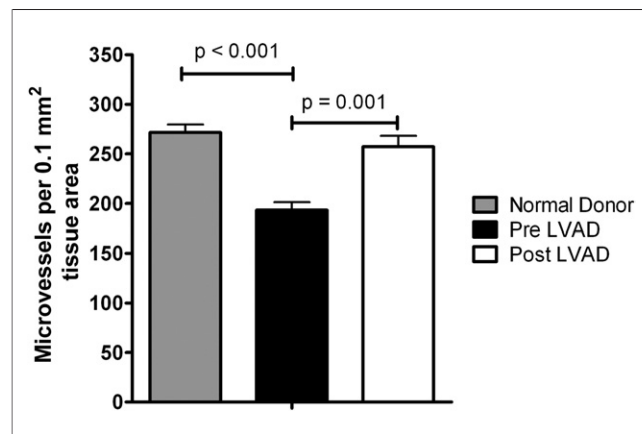
$p = 0.038$ ), in agreement with the digital histopathology data revealing post-LVAD increase in microvascular density. The most striking of these post-LVAD changes were: 1) basal lamina reduplication; 2) increase in the number of luminal cytoplasmic processes and irregular surface membrane projections; and 3) increased size of nuclei and increased number of cytoplasm organelles protruding into the capillary lumen, which significantly decreased the luminal area (Fig. 4) (12). In further agreement with these findings the post-LVAD expression of the endothelial activation marker major histocompatibility complex class 2 was consistently found to be increased in all of our patient samples (Online Appendix).

Because we discovered these specific ultrastructural microvasculature changes, we further proceeded with application of the unique tools of digital microscopy at the structural level and estimated the average microvessel luminal area (defined as “total luminal area measured in the entire tissue analysis area”/“number of microvessels measured in the entire tissue analysis area”). In agreement with the ultrastructural findings, the average luminal area was found to be decreased post-LVAD unloading by 36% ( $p = 0.028$ ) (Fig. 5). However, the fact that we had concomitantly found increased microvessel density after LVAD raised the possibility that, even if the average microvascular luminal area (i.e., luminal area/microvessel) is decreased, the total microvessel luminal area (i.e., the sum of the luminal areas of all microvessels in the entire examined myocardial area) might not be decreased. For this reason we also assessed the “total luminal area measured in the entire tissue analysis area”/“entire tissue analysis area,” and this value was found to be decreased by 18% after LVAD.

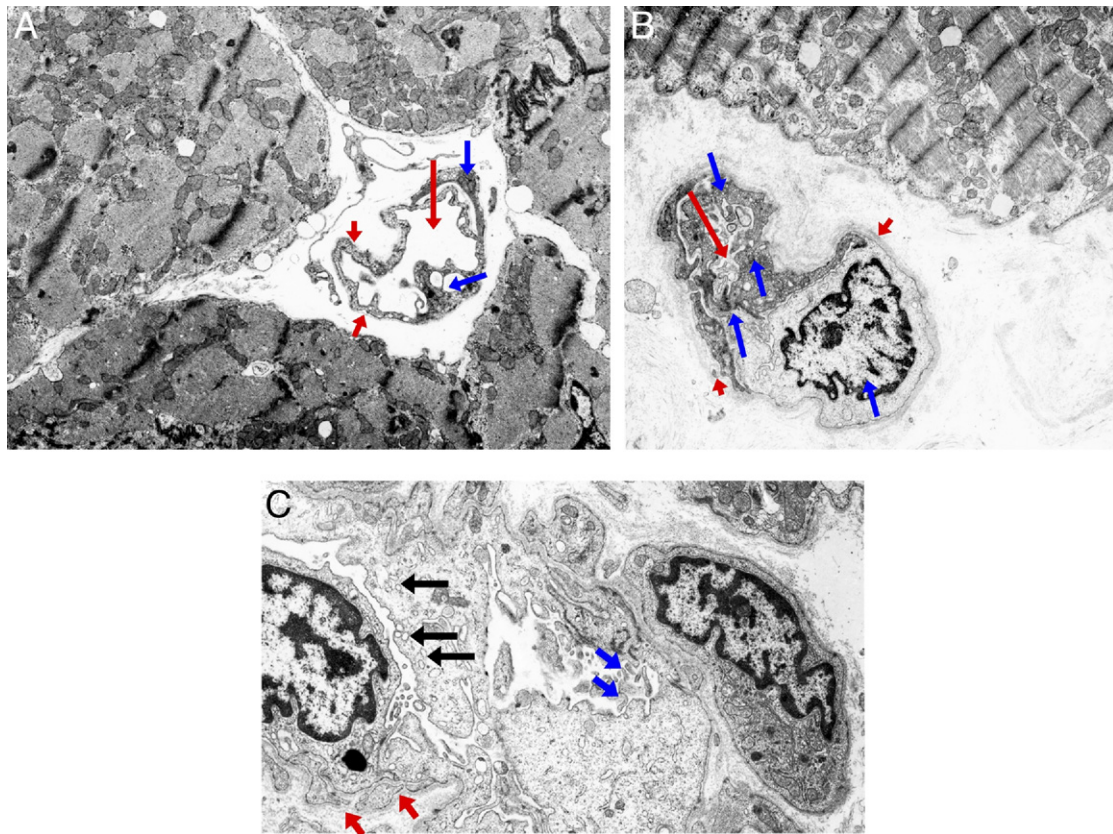
**FIBROSIS STUDIES.** “Interstitial” and “total fibrosis” were significantly increased in failing hearts compared with normal donor hearts and increased further after LVAD unload-

ing by 61% ( $p < 0.001$ ) and 35% ( $p = 0.001$ ), respectively (Fig. 6). The fibrosis around medium and large vessels (i.e., “perivascular fibrosis”) was found to be similar in pre- and post-LVAD samples. Therefore, the increase in “total fibrosis” was driven exclusively by increase of “interstitial fibrosis.” The effect on fibrosis was similar between the ischemic and the nonischemic chronic HF patients.

**CARDIOMYOCYTE STUDIES.** Cardiomyocyte size, as assessed by digital microscopy, decreased after unloading (pre-LVAD cardiomyocyte cross section area:  $923 \pm 336 \mu\text{m}^2$  vs. post-LVAD cross section area:  $733 \pm 194 \mu\text{m}^2$ ,  $p = 0.027$ ) but not beyond that of normal donor hearts (donor cross section area:  $543 \pm 119 \mu\text{m}^2$ ,  $p = 0.009$ )—a finding that might have suggested unloading induced atrophy (Fig. 7A). The results were similar for the subset of patients that underwent prolonged duration (>6 months) of mechanical unloading. Similarly, the comparison of the ultrastructural electron microscopy findings of the normal donor and pre-LVAD myocardial samples with the post-LVAD tissue revealed no evidence suggestive of hypertrophy regression to the point of cardiomyocyte degeneration and atrophy. Specifically, myofilament loss ( $1.0 \pm 0.8$  vs.  $1.0 \pm 1.0$ ,  $p = 0.65$ ); mitochondria abnormalities including changes in number, size, shape and cristae appearance ( $1.2 \pm 0.5$  vs.  $1.2 \pm 0.8$ ,  $p = 1.0$ ); and dilation of sarcotubular elements ( $1.6 \pm 0.5$  vs.  $1.0 \pm 0.8$ ,  $p = 0.18$ ) were all comparable before and after LVAD unloading. The rest of the ultrastructural parameters included in our electron microscopy classification scheme (described in the Online Appendix) were also found to be similar before and after LVAD unloading. In agreement with these findings, the cardiomyocyte glycogen content, as assessed by digital microscopy, was unchanged between pre- and post-LVAD unloading (Fig. 7B). Also, there was no difference between ischemic and nonischemic chronic HF patients.

**Figure 3** Microvascular Density

Plots represent mean ± SEM. LVAD = left ventricular assist device.



**Figure 4** Endothelial Cell Activation

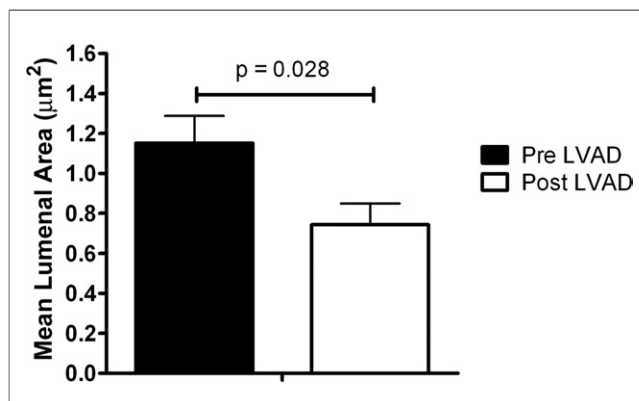
Ultrastructural appearance (10,000 $\times$  magnification, Patient #2) of capillaries before and after left ventricular assist device (LVAD) unloading revealed strong evidence of endothelial cell activation after LVAD. **(A)** Before LVAD. **Short red arrows** = basal lamina; **blue arrows** = cytoplasmic organelles and nuclei; **long red arrow** = capillary lumen. **(B)** After LVAD. **Short red arrows** = basal lamina reduplication; **blue arrows** = increased nuclei size and increased cytoplasmic organelles protruding into the capillary lumen—irregular luminal surface (**long red arrow**). **(C)** After LVAD. Basal lamina reduplication (**red arrows**); increased nuclei and cytoplasmic size with increased pinocytotic vesicles protruding into the capillary lumen (**black arrows**); and numerous irregular luminal and surface membrane projections (**blue arrows**); all indicative of endothelial activation.

## Discussion

This study demonstrates that pulsatile mechanical unloading of the failing heart increases microvascular density. In

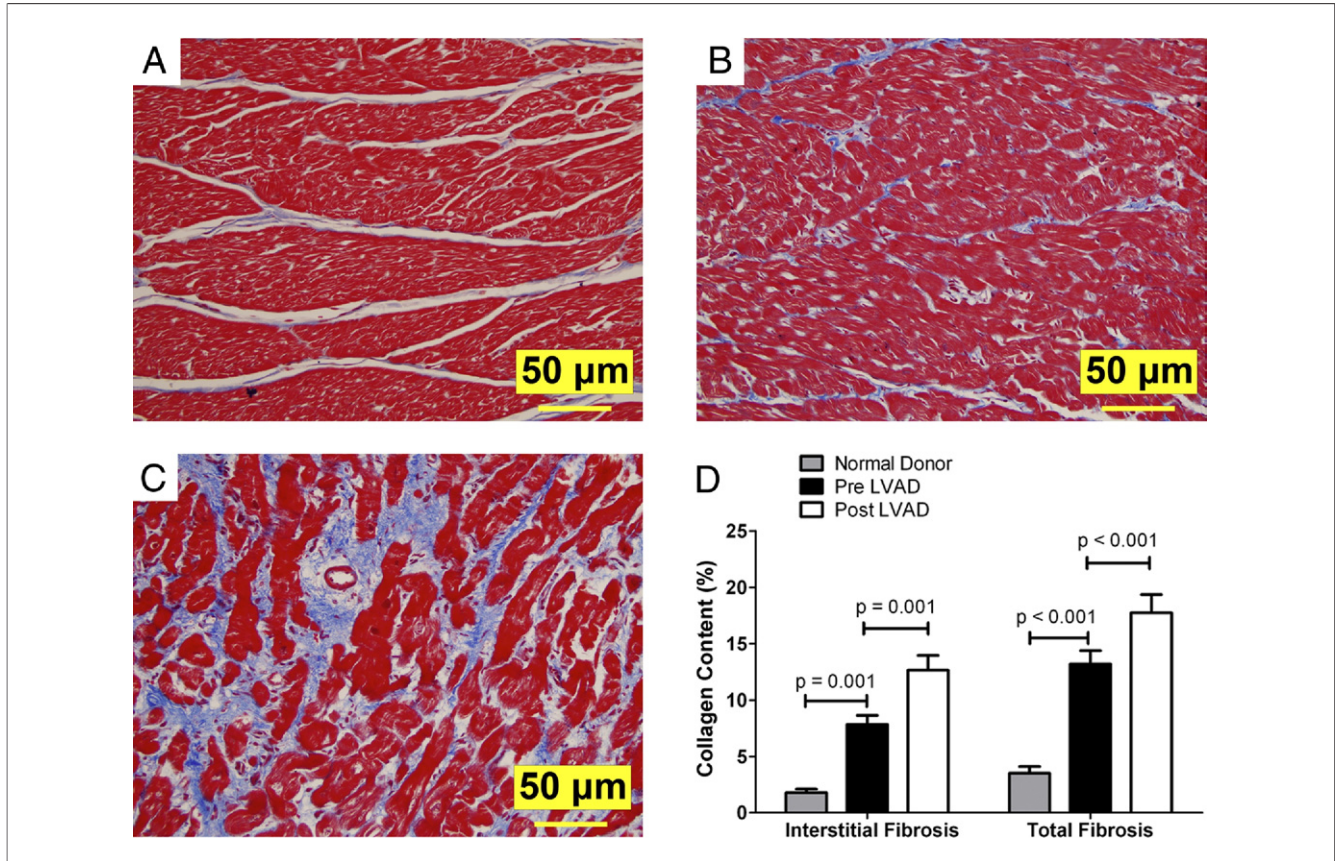
agreement with this finding, we found ultrastructural and immunohistochemical evidence of post-LVAD endothelial cell activation. The decrease of the microvascular luminal area observed by ultrastructural electron microscopy was confirmed by structural digital microscopy. The vascular changes were accompanied by increased fibrosis and reduced cardiomyocyte hypertrophy without any structural, ultrastructural, or metabolic evidence of outright degeneration and atrophy. To our knowledge, this is the first study that examines the direct effects of unloading on myocardial microvasculature and endothelium and simultaneously correlates the effects of LVAD unloading on microvasculature, fibrosis, and hypertrophy. Furthermore, the adoption of whole-field digital histopathology (9,10) allows comprehensive endocardium-to-epicardium examination and avoids bias intrinsic to the selection of random fields, the chief means of analysis used in previous studies.

**Mechanistic links among microvasculature, fibrosis, and hypertrophy.** A large body of work has shown that endothelial and epithelial cells can transition to fibroblast or



**Figure 5** Microvascular Luminal Area

Plots represent mean  $\pm$  SEM. LVAD = left ventricular assist device.

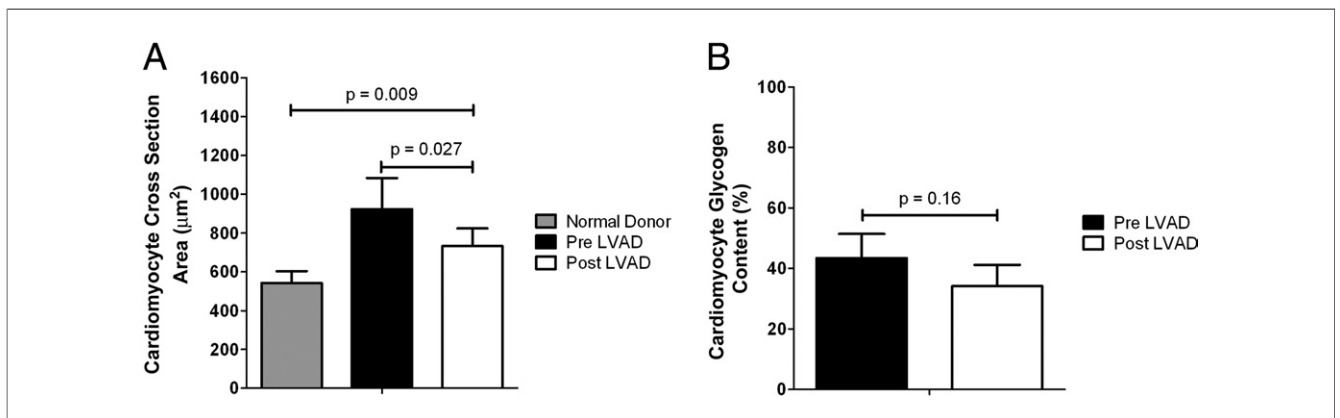


**Figure 6** Cardiac Fibrosis

(A) Normal donor heart; (B) before left ventricular assist device (LVAD); (C) after LVAD: increased fibrosis (Masson's stain—collagen content stains blue) after 63 days of LVAD-induced unloading (Patient #2); 20× magnification; (D) interstitial fibrosis and total fibrosis—see text for definitions. Plots represent mean ± SEM.

mesenchymal phenotypes and through this endothelial-to-mesenchymal transition contribute to fibrosis of many tissues (7,13). In a recent seminal article that used lineage tracing in a mouse model of aortic banding, Zeisberg et al. (7) showed that cardiac fibrosis was directly related to endothelial cells as those were activated and adopted a

mesenchymal or fibroblastic phenotype via pathways directly implicated in maladaptive cardiac hypertrophy. These findings were widely perceived in both the basic and clinical research arenas as the establishment of a significant pathophysiological link among myocardial microvasculature, fibrosis, and hypertrophy (8). Other



**Figure 7** Cardiomyocyte Studies

(A) Cardiomyocyte size evaluation; (B) cardiomyocyte glycogen stores evaluation. Plots represent mean ± SEM. LVAD = left ventricular assist device.

progenitor cells that could serve as fibroblast precursors include pericytes, fibrocytes derived from bone marrow, and myofibroblasts (alpha-smooth muscle actin-containing contractile cells). With regard to the latter, alpha-smooth muscle actin immunostaining was very rarely identified in cells other than contractile cells of the vessel walls and was not different between our pre- and post-LVAD samples (data not shown).

Our study of human patients revealed post-LVAD increase in microvascular density, a finding that is in agreement with experimental data of unloading by means of heterotopic transplantation (14). Our ultrastructural analysis provided mechanistic insights by revealing strong evidence of post-LVAD endothelial cell activation. Previous animal models of angiogenesis in myocardial and skeletal muscle revealed that ultrastructural endothelial cell activation represents 1 of the early stages of capillary growth and arteriogenesis (12,15). Furthermore, the increased microvascular density we identified was accompanied by increased fibrosis, and this suggests that the mechanistic link between the endothelium and cardiac fibrosis might apply to human myocardium. Obviously, direct proof for such a mechanism requires sophisticated lineage tracing possible only in genetically manipulated animal models (7,8). Of note, work done in our laboratory has increasingly shown that endothelial proliferation and migration, hallmarks of angiogenesis, must be balanced by mechanisms that stabilize the endothelium, so that a functional vascular network might be established and maintained (16–18). The findings of this study suggest an imbalance in these competing signals after LVAD implantation (19), which possibly results in increased microvascular density and endothelial activation that might contribute to cardiac fibrosis.

**Clinical research implications.** Future studies need to address not only the association of increased microvascular density, endothelial activation, and cardiac fibrosis but must also identify the primary triggers. One possibility is that the myocardial microvessel growth is stimulated by mechanical and hemodynamic factors associated with increased blood flow (20). However, although cardiac output is increased by LVAD (as also found in our study), previous positron emission tomography studies have shown that this is not accompanied by increased coronary flow (21,22). Of note, in a Harefield group study, even though all subjects showed varying degrees of LVAD-induced myocardial recovery, post-LVAD coronary flow reserve was impressively impaired, and the authors concluded that this finding required further investigation (21). This latter finding could be explained by the decreased microvasculature lumen area found in our study by both whole-field digital microscopy at the structural level and by electron microscopy at the ultrastructural level.

Whether LVAD unloading results in increase or decrease in fibrosis is controversial (2,3,5,6,23). The reasons for differing findings in various studies are unclear but might be related to the methodology used (2,3,5,23). Our use of

digital histopathology (9,10) enabled us to examine the whole field from endocardium to epicardium, which significantly reduced any selection or observer bias and removed the confounding effect of endocardial or epicardial sampling, known to be associated with different degrees of fibrosis (24). In addition, our results were derived from a total myocardial analysis area that greatly exceeds that of previous reports (2,5). Recently published human data demonstrated increase in post-LVAD cardiac content of angiotensin I, angiotensin II, and norepinephrine (6,25), findings compatible with the increased fibrosis observed in our study. Furthermore, experimental studies of heterotopic transplant also showed unloading-induced increase in myocardial fibrosis (26,27).

The hypertrophy regression observed in our study is in agreement with experimental heterotopic transplantation data (14,26,27) and also with other studies in humans showing that LVAD unloading reversed the altered cardiac expression and function of natriuretic peptides and receptors along with parallel reductions in myocardial mass and myocyte size (3). However, whether the hypertrophy of human failing hearts unloaded by means of LVAD regresses to the point of atrophy and degeneration is controversial and requires further investigation (2,3,5). In our study of failing human hearts, the absence of post-LVAD cardiomyocyte atrophy and degeneration was not based only on cardiomyocyte size data but was reinforced both by ultrastructural electron microscopy findings and by metabolic findings. Given that atrophy has been associated with significantly increased glycogen concentration (28,29), the lack of change of glycogen content after LVAD unloading observed in our study (with a trend to decrease) does not seem to be compatible with LVAD-induced cardiac atrophy. To our knowledge the question of LVAD-induced atrophy and degeneration has not been specifically addressed by previous LVAD clinical studies on ultrastructure or quantitative metabolic changes, and from this perspective our study adds to the published data.

**Study limitations.** A number of limitations of our study need to be addressed in future investigations. First, to focus on the role of mechanical unloading on the failing heart, drugs that are hypothesized to have direct effects on remodeling would not be employed or their use would be randomized. However, we do note that only 2 of our patients were treated with such medications, and their results were not significantly different from patients not given any such medications. Second, the post-LVAD decrease of myocyte size might have possibly affected other morphometric measurements that are based on the relative representation of other myocardial constituents. However, the structural changes of the nonmyocyte myocardial constituents in our study were of such magnitude that even demonstration of relative increases could potentially have significant functional myocardial consequences. While the variable duration of mechanical support is another limitation of our study, it offers advantages as well, given that investigating the effects



of LV unloading as a function of time is important and not well-studied (27). We found that the tissue changes were similar in all the patients despite differences in duration of LV unloading. Moreover, it is possible that reactive fibrous response could have been present in myocardial tissue obtained at LVAD explantation, even though in an attempt to avoid this phenomenon we obtained our samples at least 1 cm from the LVAD inflow cannula. We believe that this is not very likely, however, because we verified that no morphologic findings suggestive of reactive inflammatory response were present in the studied myocardium. Finally, we studied the effects of unloading induced by pulsatile LVAD, and this can be perceived as a limitation, because—due to mainly engineering reasons—continuous flow, non-pulsatile devices are increasingly used. However, whether the prospect of LVAD-induced reverse remodeling is better-served by pulsatile (30–32), nonpulsatile, or counterpulsation (3,33) devices or by full versus partial (34) unloading is unknown. Consequently, understanding the effects of pulsatile devices on remodeling becomes extremely important as the field is evolving. Our findings might be useful for future comparative investigations of various types and degrees of mechanical unloading.

## Conclusions

The LVAD-induced unloading of the failing human heart increased microvascular density and was accompanied by endothelial cell activation, increased fibrosis, and no structural, ultrastructural, or metabolic changes compatible with cardiomyocyte degeneration and atrophy. These clinical findings might guide future studies aimed at unloading-induced reverse remodeling of the failing human heart.


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- Key Words:** heart failure ■ left ventricular assist device ■ microvasculature ■ remodeling ■ unloading.
-  **APPENDIX**
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- For supplementary material, please see the online version of this article.**