Unloading-induced remodeling in the normal and hypertrophic left ventricle

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McGowan, Brian S., Christopher B. Scott, Anbin Mu, Richard J. McCormick, D. Paul Thomas, and Kenneth B. Margulies. Unloading-induced remodeling in the normal and hypertrophic left ventricle. Am J Physiol Heart Circ Physiol 284: H2061–H2068, 2003. First published February 6, 2003; 10.1152/ajpheart.00873.2002.—To date, no study has assessed the degree of similarity between left ventricular (LV) reverse remodeling and atrophic remodeling. Stable LV hypertrophy was induced by creation of an arteriovenous fistula (AVF) in Lewis rats (32 days). LV unloading was induced by heterotopic transplantation of normal (NL-HT) and/or hypertrophic (AVF-HT) hearts (7 days). We compared indexes of remodeling in AVF, NL-HT, and AVF-HT groups with those of normal controls. LV unloading induced decreases in cardiomyocyte size in NL-HT and AVF-HT hearts. NL-HT and AVF-HT LV were both characterized by relative increases in collagen concentration that were largely a reflection of decreases in myocyte volume. NL-HT and AVF-HT LV were associated with similar increases in matrix metalloproteinase (MMP-2 and -9) zymographic activity, without change in the abundance of the tissue inhibitors of the MMPs. In contrast, AVF-HT, but not NL-HT, was associated with a dramatic increase in collagen cross-linking. Our findings suggest an overall similarity in the response of the normal and hypertrophic LV to surgical unloading. However, the dramatic increase in collagen cross-linking after just 1 wk of unloading suggests a potential difference in the dynamics of collagen metabolism between the two models. Further studies will be required to determine the precise molecular mechanisms responsible for these differences in extracellular matrix regulation. However, with respect to these and related issues, heterotopic transplantation of hypertrophied hearts will be a useful small animal model for defining mechanisms of myocyte-matrix interactions during decreased loading conditions.

myocardial atrophy; reverse remodeling; extracellular matrix; volume overload

Surgical implantation of a left ventricular (LV) assist device (LVAD) has proven to be an effective therapeutic modality for medically refractory patients with class IV heart failure awaiting transplantation. Beyond this therapeutic role, recent studies demonstrate that LVAD support alters the structure and function of the failing heart toward a less pathologic phenotype. These changes have been referred to as “reverse remodeling.” In general, remodeling of LV myocardium is associated with alterations of both cellular (7) and extracellular components (6, 31). Levin et al. (20) observed a leftward shift in the passive tension curve of the LVAD-supported heart compared with non-LVAD-supported failing hearts, thus demonstrating the structural plasticity of the failing human heart. Other work has demonstrated that LVAD support is associated with reductions in LV mass (LVM), reductions in cardiomyocyte length and volume, and alterations in myocardial remodeling enzymes (1, 6, 22, 44). Function of the LVAD-supported heart may also be altered. Studies performed on isolated cardiomyocytes and cardiac trabeculae have demonstrated improved contractility, normalized electrophysiology, and improved adrenergic responsiveness in the failing heart (9, 14, 15, 34).

Although the structural and functional outcomes of LVAD-induced unloading are well described, clinical success of achieving sustained recovery of the end-stage failing heart has been poor. One possible explanation for insufficient improvement in organ physiology relates to the maladaptive responses of the cardiac extracellular matrix, including fibrosis and altered patterns of collagen cross-linking (3, 25, 29, 31, 32, 39). Another argument against the clinical efficacy of LVAD-induced recovery relates to the comparison of reverse remodeling to the phenomenon of myocardial atrophy. Unfortunately, patient-based studies do not permit appropriately timed and controlled comparisons of myocardial atrophy and reverse remodeling. Therefore, the broad goal of this study was to compare the structural adaptations induced by surgical unloading of both normal and hypertrophied rat hearts. We hypothesized that hypertrophy would alter the LV response to surgical unloading with respect to remodeling of the cardiomyocyte and noncellular fractions of the myocardium.
METHODS

Overview

With the use of inbred Lewis rats, hypertrophy resulting from volume overload was produced by creation of an arteriovenous fistula (AVF) for 1 mo. Myocardial unloading was induced by heterotopic transplantation of normal (NL-HT) and/or hypertrophic (AVF-HT) hearts for 1 wk. In each of the four study groups [normal controls (NL), AVF, NL-HT, and AVF-HT], we assessed myocyte morphology, collagen and nonreducible collagen cross-linking (hydroxylsylpyridinoline (HP)) concentrations, and the abundance of matrix metalloproteinase (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). All experiments conformed to the “Guiding Principles in the Care and Use of Laboratory Animals” approved by the American Physiological Society and were approved by the Temple University Animal Care and Use Committee.

Creation of Volume Overload by AVF

Animals were sedated with 2% isoflurane and anesthetized by an intraperitoneal dose of a ketamine (50 mg/kg) and xylazine (10 mg/kg) mixture (KX bolus). Appropriate levels of anesthesia were assessed by the absence of toe-pinch and blink reflexes. Once anesthesia was confirmed, the abdomen was opened, and the inferior vena cava (IVC) and intra-abdominal aorta (IAA) were isolated and temporarily ligated. The IVC was opened, and the obturator from a 14-gauge angiocath was forced across the IVC-IAA common wall to create the shunt. The vessels were flushed with saline, and the IVC was repaired with 9.0 nylon suture. The abdomen was closed in layers, and animals awoke within 30 min of skin closure.

Heterotopic Cardiac Transplantation

Recipient preparation. Heterotopic cardiac transplantation was performed on hearts from normal rats (n = 11) or rats with previously established AVF (n = 11). Recipient rats were anesthetized with the KX bolus. A midline abdominal incision was made, and the IVC and IAA were isolated, temporarily ligated, opened, and flushed with saline. The surgical site was covered and kept moist with saline-soaked gauze for the duration of the donor organ harvest.

Allograft harvest. Donor rats were sedated with the KX bolus. After confirmation of anesthesia, a midline abdominal incision was created, and the skin, abdominal muscles, and bowels were retracted. The donor rat’s IAA was cannulated with a 22-gauge needle, the IVC was vented, and the donor heart was arrested with 50 ml ice-cold ViaSpan cardioplegia (DuPont). Adequate cardioplegic perfusion was determined by the cessation of contraction within 15–30 s of perfusion. The sternum was retracted, and the heart was freed of connective tissue. The pulmonary veins, cavae, and innominate artery were ligated with 4.0 silk sutures. A single 4.0 silk suture was secured on the ascending aorta, proximal to the innominate artery, before the donor animal’s arterial circulation was opened to prevent entrance of air in the coronary vasculature. The donor aorta was severed distal to the innominate artery, and the heart was excised, weighed, and stored in cardioplegia at 4°C.

Allograft transplantation. Nylon sutures (9.0) were used to create a two-part running stitch to complete anastomoses of the donor pulmonary artery to the recipient IVC and donor ascending aorta to recipient IAA. At the completion of the vascular anastomoses, the ligatures placed on the recipient’s IVC and IAA were removed. When the suture lines were free of leaks, the ligature on the donor heart’s ascending aorta was removed, allowing reperfusion of the donor organ and thus ending the ischemic time. The donor heart was kept moist with ice-cold saline for the duration of the transplantation. Cardiac cold ischemic time was consistently <30 min. The abdominal incision was closed in two layers, and the animals were monitored until standing, moving, and freely drinking.

Experimental End Points

Echocardiographic studies. Echocardiography was used to define the temporal pattern of cardiac remodeling after creation of the aortocaval fistula in the AVF and AVF-HT groups. Before all echocardiographic procedures, animals were sedated with a half-dose of the KX bolus and maintained with isoflurane via a nose cone. Heart rates for all animals studied were >275 beats/min. Echoes were performed using a 12-MHz transducer and a Hewlett-Packard Sonos 5500 ultrasound machine. An M-mode image was obtained and recorded for archiving and analysis of LV end-diastolic (LVEDD) and end-systolic dimensions (LVESD), interventricular septal thickness (IVST), and posterior wall thickness (PWT) in accordance to the conventions of the American Society of Echocardiography (38). These measurements, derived from the short-axis image, were used for offline calculations of LVM, relative wall thickness (RWT), and ejection fraction (EF) by the following formulas:

\[
\text{LVM} = 1.055 \times \left[ (\text{IVST} + \text{LVEDD} + \text{PWT})^2 - (\text{LVESD})^2 \right] + 0.6
\]

\[
\text{RWT} = 10 \times \frac{\text{PWT}}{\text{LVEDD}}
\]

\[
\text{EF} = 100 \times \frac{(\text{LVEDD} - \text{LVESD})}{\text{LVEDD}^2}
\]

Terminal organ harvest. Hearts used for all experimental end points were harvested in the same way. The animals were given KX bolus and 1,000 units of heparin via intraperitoneal injections. After confirmation of anesthesia, a midline abdominal incision was performed, and the donor or native hearts were dissected free of connective tissue, harvested, and weighed in cold Krebs-Henseleit buffer. Hearts were prepared for biochemical and molecular assays (n = 6/group) or prepared for cellular isolation (n = 5/group).

Inclusion criteria. Because of the technical demands of the heterotopic transplantation procedure, we established several means by which to exclude inappropriate hearts from clouding data interpretation. First, we developed a state-of the-art cardioprotection protocol by which the heart was arrested in vivo with ViaSpan (DuPont) cardioplegic solution. The heart was then harvested and maintained cold throughout the heterotopic transplantation with topical ViaSpan. For additional quality control, before inclusion in MMP/TIMP or collagen analyses, sections from each and every heart were prepared for histological examination. Hematoxylin and eosin-stained and Masson’s-stained sections were evaluated for the presence of inflammatory infiltrates or other signs of tissue damage. Any hearts found to have focal damage (necrosis or infiltrates) were excluded.

Cellular isolation. A subset of hearts from each of the four experimental groups was used for cellular isolation. In brief, after harvest, the ascending aorta was cannulated and perfused with the nonrecirculating rinse solution at 37°C for 5 min and at a constant flow of 12 ml/min (pressure at ~60 mmHg). The rinse solution was composed of a supplemented Krebs-Henseleit buffer. The perfusion was switched to a
recirculating, digestion solution supplemented with collagenase (type II, 177 U/ml; Worthington) for 10 min. The right ventricle and atrial tissues were removed, and the LV was minced and triturated to disaggregate the LV myocytes. Isolated myocytes were filtered and suspended in 1.5% glutaraldehyde solution for isosmotic fixation.

Myocyte morphological analysis. A Coulter Counter ZM 256 was used for measuring the volume of cells in suspension. The fixed myocytes were counted and measured as they passed across the aperture of the Coulter unit. A threshold of 256 was used for measuring the volume of cells in suspension.

Isolated myocytes were minced and triturated to disaggregate the LV myocytes. Ventricular and atrial tissues were removed, and the LV was minced and suspended in 1.5% collagenase (type II, 177 U/ml; Worthington) for 10 min. The right ventricle and atrial tissues were removed, and the LV was minced and suspended in 1.5% collagenase (type II, 177 U/ml; Worthington) for 10 min. The right

BX 40 microscope (×400). The system was calibrated with a cross-sectional area (CSA) was derived with the use of the least micrometer before each round of data collection. With the use of the following formula: CSA = MCV/MCL.

Hydroxyproline and hydroxylysylpyridinoline cross-link assays. Hydroxyproline concentration was measured in LV colorimetrically using the method of Woessner (42). Specifically, collagen concentration was calculated assuming that hydroxyproline represents 13.7% of collagen amino acid content. The degree of collagen cross-linking, as measured by hydroxylysylpyridinoline (HP) concentration expressed as mole HP per mole collagen, was assessed using the reverse-phase HPLC method of Eyre et al. (10) with modifications described previously (30).

Protein extraction. Separate pieces of frozen LV tissue, collected from the same hearts, were homogenized in two volumes of lysis buffer. The homogenate was agitated on ice for 1 h and was spun at 800 × g for 10 min to extract protein and remove membranous and nuclear fragments. Samples were taken for Bradford protein analysis. Supernatants were separated into aliquots as total protein lysates and stored at 20°C until used.

Western immunoblotting. Frozen protein lysates were thawed on ice and mixed with Laemmli sample buffer, 1.5 M Tris (pH 8.8), and 2% β-mercaptoethanol to match a final protein concentration of 4 μg/μl. Importantly, sample preparation for zymography does not use reducing agents, and samples were not boiled. Lysates (20–50 μg) were electrophoresed in precast zymogram gels (NOVEX) containing gelatin substrate set in the polyacrylamide matrix. After electrophoresis, the gels were incubated two times in 2.5% Triton Renaturing Buffer (NOVEX) for 30 min. The gels were rinsed two times in deionized H2O for 10 min and moved to Developing Buffer (NOVEX) for 48 h at 37°C. To visualize zymographic bands, gels were fixed in methanol-acetone fixative and stained with Coomassie stain until the lytic bands were optimally contrasted. Gels were dried, scanned, and analyzed by densitometry using NIH Image software. Media collected from activated rat aortic smooth muscle cells were used as positive controls for MMP-2 and -9. Duplicate zymographic gels were developed in the presence of EDTA as a negative control.

Statistical analysis. Data within each experimental group were averaged and are expressed as means ± SE. The main effect to be addressed was myocardial unloading. For temporal studies, repeated-measures one-way ANOVA was used. For single comparisons of independent groups, an unpaired Student’s t-test was used. For all comparisons, P < 0.05 was considered statistically significant.

RESULTS

Assessment of LV Response to Load Alterations

The growth response of the LV to volume overload induced by creation of an AVF was demonstrated by echocardiographic evaluation and is shown in Table 1. Progressive cardiac dilatation and decreases in RWT were observed throughout a 1-mo period of observation. In as little as 13 days after induction of volume overload, LVM had increased by 48%. However, LVM had essentially stabilized by 4 wk (Table 1). We observed no reduction in LV EF during the 1-mo period after AVF creation. In AVF rats, the calculated 64% increase in LVM was reflected by a 102% increase in overall heart weight at 1 mo (1.14 ± 0.03 in NL vs. 2.3 ± 0.12 in AVF, P < 0.05). The difference between the changes seen in echocardiographically derived LVM and gravimetric total heart weight is presumably because of remodeling of the right ventricle and atria. In hearts unloaded by heterotopic transplantation, we could not obtain adequate echocardiographic windows.

Table 1. Echocardiographic evaluations after AVF

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>13 Days</th>
<th>24 Days</th>
<th>32 Days</th>
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<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>LVEDD</td>
<td>0.7414 ± 0.012</td>
<td>0.8538 ± 0.019*</td>
<td>0.88 ± 0.018*</td>
<td>0.9236 ± 0.04*</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>1.988 ± 0.066</td>
<td>1.914 ± 0.10</td>
<td>1.845 ± 0.051</td>
<td>1.5 ± 0.106†</td>
</tr>
<tr>
<td>LVM</td>
<td>0.7177 ± 0.014</td>
<td>1.065 ± 0.049*</td>
<td>1.175 ± 0.467†</td>
<td>1.167 ± 0.159*</td>
</tr>
<tr>
<td>EF</td>
<td>44.47 ± 5.06</td>
<td>58.34 ± 2.90*</td>
<td>59.35 ± 2.957*</td>
<td>52.07 ± 4.47</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. LVEDD, left ventricular end-diastolic dimension; LVM, left ventricular mass; EF, ejection fraction. P < 0.05 vs. baseline (*), 13 days (†), and 24 days (‡).
to permit noninvasive analysis of remodeling. Therefore, the remodeling response to surgical unloading was assessed only after organ harvest. Surgical unloading of the normal heart induced a 38% reduction in organ mass by day 7 (1.15 ± 0.03 NL vs. 0.72 ± 0.03 NL-HT). Surgical unloading of the hypertrophic heart induced a 43% reduction in organ mass by day 7 (2.3 ± 0.12 AVF vs. 1.3 ± 0.09 AVF-HT).

Assessment of Cardiomyocyte Volume and Shape

Figure 1 depicts the cellular morphometric findings from NL, AVF, AVF-HT, and NL-HT groups. As expected, volume overload induced profound hypertrophy of cardiomyocytes, including a 48% increase in median myocyte volume, a 26% increase in mean myocyte length, and a 20% increase in myocyte CSA. Surgical unloading induced profound remodeling of cardiomyocytes in both the normal and hypertrophic hearts by 7 days after heterotopic transplantation. MCV decreased by 38% in both the NL-HT and AVF-HT groups. However, the decrease in cell length associated with cardiac unloading tended to be more pronounced in the AVF-HT group compared with the NL-HT group (23 vs. 11%); therefore, the decrease in cell CSA tended to be less pronounced in the AVF-HT group compared with the NL-HT group (20 vs. 29%).

Assessment of LV Matrix Remodeling

Percent collagen and HP cross-linking concentration (mol HP/mol collagen) are presented in Fig. 2. In NL-HT, an increase in collagen from 2.58 ± 0.15 to 4.28 ± 0.25% (66% increase) was observed, whereas unloading in AVF-HT resulted in an increase in collagen from 2.2 ± 0.12 to 3.83 ± 0.15% (74% increase). Unloading had no effect on HP cross-linking in normal LV (0.31 ± 0.03 vs. 0.26 ± 0.03), whereas in AVF cross-linking increased significantly in the hypertrophied LV after unloading (0.29 ± 0.04 vs. 0.44 ± 0.02).

Assessment of Cardiac Remodeling Proteins

Figure 3 shows the changes in MMP and TIMP proteins in the four study groups. The levels of MMP activity and TIMP abundance in normal hearts and hearts hypertrophied because of AVF were unchanged. However, unloading normal hearts induced a 593% increase in MMP-2 and a 490% increase in MMP-9 zymographic activity. Similarly, unloading hypertrophied hearts induced a 480% increase in MMP-2 and a 758% increase in MMP-9 zymographic activity. There were no changes in the abundance of TIMP-2 or TIMP-4 proteins with unloading. Therefore, unloading of both normal and hypertrophic hearts was associated with profound increases in the ratio of MMP zymographic activity to TIMP protein abundance.

DISCUSSION

The goal of these studies was to compare the response of normal and hypertrophic LV tissue with reductions in myocardial load and to contrast the processes of atrophy and reverse remodeling. The major results can be summarized as follows. First, atrophy and reverse remodeling, induced by surgical unloading of normal or hypertrophic hearts, respectively, are both associated with rapid and dramatic reductions in cardiomyocyte volume and cardiac mass. Second, both atrophy and reverse remodeling are associated with relative increases in myocardial collagen content that can be most simply explained by the dramatic decreases in myocyte mass. However, this observation...
does not exclude simultaneously occurring changes in the extracellular matrix, as evidenced by altered collagen cross-linking in AVF-HT hearts. In addition, the increased relative collagen content associated with both atrophy and reverse remodeling is accompanied by increased MMP activity, without significant changes in the abundance of the endogenous inhibitor proteins (TIMPs). Taken together, these findings suggest that, as with myocardial adaptation to hemodynamic overload, remodeling after unloading likely requires activation of enzymes that regulate the extracellular matrix (MMPs), yet such activation need not result in reduced myocardial collagen content.

Confirming the work of Grossman et al. (13), sustained volume overload induced an eccentric pattern of remodeling with a 40% increase in LVEDD and a 40% increase in cardiomyocyte volume. We used hypertrophic hearts, 4 wk post-AVF, as donor organs for myocardial unloading. This time point was chosen based on the determination that the ventricular growth rate had slowed (stable LVM in Table 1); therefore, hearts harvested at this time point would provide a more consistent baseline for subsequent manipulations. Similar stabilization post-AVF have been previously reported elsewhere (5). In addition, hypertrophic remodeling, as assessed by the percent change in cell volume, ventricular volume, and organ weight after 4 wk of hypertrophic remodeling was similar to that previously reported in the study of hypertrophic and failing hearts (40, 20). However, AVF was not associated with marked changes in extracellular matrix structure or myofibrillar alignment; therefore, the model best represents a state of “compensated” volume overload-induced hypertrophy.

The remodeling after surgical unloading was very rapid. By 7 days, atrophy was associated with a 38% decrease in heart weight and a 38% decrease in myocyte volume. Likewise, reverse remodeling was associated with a 43% decrease in heart weight and a 38% decrease in myocyte volume. The changes in cardiomyocyte volume are similar to the decreases previously reported both in this unloading model and after LVAD support (7, 41, 44). These structural changes confirm the primary role served by hemodynamic load in maintaining cardiac size. Overall, the relationship of hemodynamic load and cardiac size seems balanced such that both increases and decreases in load can induce rapid cardiac remodeling and normalization of myocardial stress.

Structural remodeling induced by the mechanical unloading of the hypertrophic, failing heart by LVAD support has been the subject of numerous studies over the past several years. These reports demonstrate that LV volume, mass, and cardiomyocyte dimensions are consistently decreased after mechanical unloading (1, 15, 20, 44). However, studies that describe unloading-induced adaptations of the nonmyocyte fraction of the myocardium have been far less consistent in their findings. At least 10 studies have described changes in the extracellular matrix after LVAD support, with findings ranging from decreased fibrosis, to no change in extracellular matrix content, to increased fibrosis (3, 6, 16, 22, 25, 26, 29, 32, 39). The conflicting results may be because of clinical differences such as patient population, disease etiology, LVAD support duration, and/or type of medications. Conflicting results may also be because of analytical differences, such as disparate regional myocardial sampling or failure to interpret changes in the extracellular matrix relative to cardiomyocyte remodeling. For example, many of these earlier reports failed to relate changes in the extracellular matrix fractional area of the myocardium relative to the well-documented decreases in cardiomyocyte volume.

Coincident with a marked decrease in myocyte size, we observed a marked increase of MMP zymographic activity during surgical unloading, in association with little change in the abundance of TIMPs. It is important to point out that we chose the AVF model because our preliminary studies suggested that myocardial remodeling has stabilized by 28 days (Table 1) and MMP zymographic activity is unchanged. These findings have been confirmed by others (5). Brower et al. (5) examined LV remodeling and myocardial MMP zymographic activity in rats up to 56 days after AVF. Although MMP levels were increased in the first few days after AVF induction, by 28 days expression had nor-
malized and LVM had stabilized. With the methodology employed, MMP zymographic activity correlates directly to the abundance of the MMP, suggesting a profound increase in the abundance of these two MMP isoforms. In contrast, the process of atrophy and reverse remodeling was associated with no change in the abundance of TIMP-2 and TIMP-4 isoforms. Taken together, these results demonstrate a dramatic shift in the balance of myocardial MMPs and TIMPs. Given that TIMPs inhibit MMPs in a one-to-one stoichiometric fashion, the observed shifts in the MMP/TIMP balance during atrophy and reverse remodeling likely promote collagen turnover. Specifically, in the short-term, disproportionate MMP activation may facilitate cellular remodeling by mediating detachment of myocardial cells from the myocardial collagen and fibronectin matrixes. Indeed, previous studies have suggested that alterations in the ratio of MMPs to TIMPs have a permissive role in the hypertrophic remodeling of the myocardium during volume and pressure overload and that MMP inhibition may attenuate myocardial remodeling in such settings (21, 23, 37).

Both unchanged and increased myocardial collagen concentrations have been observed with this particular AVF model of hypertrophy (5, 33). In our study, collagen concentration remained static during the imposed loading and unloading conditions. Several observations indicate that the collagen fraction of the myocardium underwent dynamic changes. First, the increased MMP-to-TIMP ratio detected within the unloaded myocardium suggests that there may be an increased rate of matrix turnover with unloading. It is also likely that the rate of collagen synthesis was altered in response to the changes in myocardial load. Indeed, increased collagen synthesis is usually associated with enhanced turnover; a significant fraction of newly synthesized collagen is turned over rapidly either intracellularly or as nascent fibrils (4, 19, 28). Moreover, accompanying the dramatic loss of both mass and cellular volume within 1 wk of unloading, covalent collagen cross-linking increased by >50%. Thus one interpretation of increased cross-linking in the unloaded heart could be accelerated degradation of immature collagen during unloading. Thus it may be premature to assume that increased MMP activity reflects a net degradation of a mature collagen matrix; rather, increased degradative activity may simply parallel increased collagen synthesis to allow noncellular adaptation to the marked changes in myocyte size and shape. In other pathological models of LV hypertrophy (30, 31, 34, 45), increases in myocardial mass and collagen fraction are often associated with increased cross-linking. Indeed, in studies in which the stimulus for hypertrophy is infarction, significant increases in HP are first noted by 5 wk postmyocardial infarction. Thus the dramatic

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**Fig. 3. Assessments of matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) proteins.** A: representative zymogram depicting MMP-2 and MMP-9 lytic activity in myocardial samples. Molecular mass (in kDa) is listed on left. B: Ponceau stain demonstrating equal loading and autoradiograph showing TIMP-2 and TIMP-4 detection at 24 and 29 kDa, respectively. C: bar graph displaying the percent change in densitometric data from NL to NL-HT and from AVF to AVF-HT for MMP-2 zymographic activity (a), MMP-9 zymographic activity (b), TIMP-2 abundance (c), and TIMP-4 abundance (d). *P < 0.05 for comparison of NL-HT vs. NL or AVF-HT vs. AVF.
increase in cross-linking observed after just 1 wk of unloading that was preceded by 4 wk of hypertrophic stimulus may reflect the ongoing response to the initial stimulus rather that the effects of unloading per se.

Limitations

To elucidate the structural adaptations to myocardial unloading, we have employed the established technique of heterotopic transplantation in both normal and hypertrophied hearts. However, heterotopic transplantation necessitates an organ harvest, a short duration of cardiac ischemia, and surgical implantation in another animal. In the present work, an attempt to address concerns about confounding factors other than unloading was made by using highly inbred (Lewis) rats to assure recipient tolerance and avoid rejection. We also employed a state-of-the-art myocardial protection protocol to minimize ischemic damage and ischemia-reperfusion injury (as described in METHODS). An additional control group utilizing a loaded allograft would have allowed the effects of transplantation surgery to be further distinguished from the effects of hemodynamic unloading, but to date we and others (2, 11, 12, 17, 18, 27, 36, 43) have had limited success with this model. Another limitation, as briefly noted above, is that 4 wk of volume overload in the AVF model produces a state of compensated hypertrophy lacking several features of the end-stage, failing heart, including contractile abnormalities, myofibrillar misalignment, and interstitial fibrosis. It is reasonable to speculate that use of heterotopic transplantation in more severely failing hearts may have greater relevance to the biology of mechanical unloading of the medically refractory human hearts supported with circulatory assist devices.

In conclusion, the primary purpose of these studies was to compare the structural adaptations with surgical unloading in normal and hypertrophied rat hearts. Our findings indicate both similar adaptations and differences in the response of the normal LV compared with the hypertrophied LV to surgically induced unloading. The observed loss in myocyte volume and corresponding increase in collagen concentration was similar between NL-HT and AVF-HT groups. Additionally, similar increases in the MMP-to-TIMP ratios were observed after unloading of both normal and hypertrophied hearts. However, the dramatic increase in HP cross-linking after just 1 wk of unloading in only the hypertrophied hearts indicates differences in the dynamics of collagen metabolism between the two models. Further studies will be required to determine the precise molecular mechanisms responsible for the observed responses. Additional studies will also need to address whether decompensated, hypertrophic hearts exhibit responses similar in the degree and rate to those seen here. Finally, further studies will be needed to define functional outcomes of myocardial structural adaptations to hemodynamic unloading. With respect to these and related issues, we believe the present studies establish a useful small animal model of reverse remodeling that mimics myocardial adaptations induced by LVAD support of the end-stage failing heart.

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