Diastolic function in the heterotopic rat heart transplant model

Effects of edema, ischemia, and rejection

Decreased systolic ventricular function and compliance and increased left ventricular edema and mass have been demonstrated in cardiac allograft rejection. Whether decreased left ventricular compliance in rejection is caused by myocardial edema has not been examined, and compliance in the Ono-Lindsey model has not been reported. Heterotopic rat abdominal cardiac transplantation was performed in ACI isografts \( n = 24 \) and in ACI to Lewis allografts \( n = 24 \). Subgroups were studied on posttransplantation days 0, 1, 3, and 5 (each \( n = 6 \)). Both transplanted hearts and native hearts were arrested with potassium for the assessment of myocardial water content, heart weight, and the left ventricular pressure-volume relation. In transplanted hearts, myocardial water content did not change in isografts but increased on posttransplantation day 5 in allografts (81.1\% on posttransplantation day 5 versus 76.1\% on day 0, 77.2\% on day 1, and 77.5\% on day 3, \( p < 0.05 \)). Wet and dry heart weight also increased on posttransplantation day 5 in allografts \( (p < 0.05) \). The left ventricular pressure-volume relation in transplanted hearts shifted to the left when compared with that in native hearts in all subgroups; these volume differences were statistically significant \( (p < 0.01) \) for all pressures above 7.5 mm Hg. This pattern was similar in isografts and allografts on posttransplantation days 0, 1, and 3, and no significant differences between isografts and allografts were demonstrated. On posttransplantation day 5, however, the pressure after a 0.05 ml injection in allografts was greater in transplanted hearts than in native hearts \((24 \pm 3 \text{ mm Hg}, p < 0.01)\). The pressure difference between transplanted and native hearts was also significantly greater in allografts than in isografts \((22 \pm 2 \text{ mm Hg}, p < 0.01)\), indicating an increase in stiffness of allografts. Thus edema and impaired diastolic properties occur concurrently with allograft rejection. Left ventricular volume is abnormal from posttransplantation days 0 to 5 in transplanted hearts but not native hearts in the Ono-Lindsey model with current methods, apparently because of ischemic injury during transplantation. (J Thorac Cardiovasc Surg 1994;108:928-37)

Mehrdad M. R. Amirhamzeh, MD; Chao-Xiang Jia, MD, MS; Joanne P. Starr, MD; Robert Sciacca, EngSciD; Nepal C. Chowdhury, MD; Daphne T. Hsu, MD; and Henry M. Spotnitz, MD

From the Departments of Surgery, Medicine, and Pediatrics, Columbia University College of Physicians & Surgeons, New York, N.Y.

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Address for reprints: Henry M. Spotnitz, MD, Department of Surgery, Columbia University P&S 14-460, 630 West 168th St., New York, NY 10032.

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Cardiac transplantation is an accepted treatment modality for end-stage cardiomyopathy, intractable heart failure, or uncorrectable congenital heart disease. Cardiac allograft rejection has been extensively studied and is associated with decreased ventricular function. Increased left ventricular (LV) wall thickness and mass have also been reported in echocardiographic studies. Doppler echocardiographic studies revealed prolongation of diastolic filling indices, consistent with a reduction in LV compliance. Histologically, severe rejection is characterized by myocardial edema, perivascular and/or
interstitial lymphocytic and fibrinous infiltrates, followed by myocardial hemorrhage and necrosis.\(^8,9\)

In rats, acute cardiac allograft rejection is associated with increasing myocardial weight on posttransplantation days 3 to 13.\(^10\) Additionally, myocardial water content increases as early as 2 days after heterotopic cardiac allograft transplantation in rats without immunosuppression.\(^11\) In dogs with heterotopic cardiac allografts and no immunosuppression, increasing myocardial water content correlates with histologic rejection score.\(^12\)

Previous studies not involving transplantation have shown that edema from a variety of causes is associated with decreased LV compliance in working or nonworking hearts.\(^13-23\) In isolated pig hearts, myocardial edema induced by coronary perfusion with hypotonic cardioplegic solutions causes a decrease in LV filling volume\(^15,17\) and an increase in diastolic stiffness of the posterior papillary muscle.\(^24\) Although myocardial edema may be assumed to occur during cardiac allograft rejection, causing both an increase in LV mass and a decrease in LV compliance, little information supporting this view is available.

Accordingly, in this study the hypothesis that decreased LV compliance and diastolic filling volumes are associated with edema and increased heart weight during allograft cardiac rejection was tested by a modified Ono-Lindsey heterotopic rat heart transplant model.\(^25\) Results reveal not only an expected relation between edema and LV compliance changes during rejection, but also unexpected diastolic abnormalities of the transplanted heart, beginning at the time of reperfusion.

**Methods**

All animals received humane care in compliance with the “Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the “Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

ACI rats \((n = 72)\) averaging 214.0 ± 2.6 gm (standard error of the mean) in weight (range 164 to 273 gm) and Lewis rats \((n = 24)\) averaging 247.5 ± 6.9 gm (standard error of the mean) in weight (range 216 to 333 gm) (Harlan Sprague-Dawley, Inc., Indianapolis, Ind.) were used in this experiment. Rats were divided into two major groups. In one group ACI hearts were excised and transplanted into the abdomen of Lewis rats (allografts); in the other, ACI hearts were transplanted into the abdomen of the ACI rats (isografts). Each group was further subdivided into subgroups to examine the transplanted and native hearts at specific postoperative periods.

This model was chosen because ACI hearts transplanted into Lewis rats undergo fulminant rejection within 5 to 6 days after
transplantation, associated with complete arrest of the cardiac electrical activity by posttransplantation day 6, whereas ACI to ACI transplants should show no sign of rejection.

**Experimental preparation.** Donor rats were anesthetized with intraperitoneal ketamine (40 to 80 mg/kg) and xylazine (5 to 10 mg/kg). After tracheostomy, mechanical ventilation was initiated with a small-animal ventilator (Harvard Apparatus, Cambridge, Mass.). The abdomen was entered through a transverse incision just below the diaphragm. Two vertical thoracotomy incisions were made through the diaphragm, and the chest plate was reflected upward. The rat was heparinized (1000 IU/kg) and a 4-0 silk suture was placed around the great vessels in the transverse sinus and around the inferior vena cava (IVC). The abdominal aorta and IVC were cut to vent the heart and 4 ml of cold (0° to 4° C) high-potassium (40 mEq/L) lactated Ringer’s solution was injected into the je to arrest the heart at end-diastole. The pulmonary artery and aorta were cut distally just before branching had occurred. The 4-0 silk suture in the transverse sinus was tied to occlude the superior vena cava and pulmonary veins. The IVC was also ligated near the right atrium. The donor heart was subsequently excised and placed in cold (0° to 4° C) high-potassium (40 mEq/L) lactated Ringer’s solution. The excised heart was gently blotted dry, placed in a preweighed Petri dish, and weighed on an analytical balance (H16, Mettler Instruments Corp., Highston, N.J.) immediately after excision to obtain the initial wet weight.

The recipient rat was anesthetized in a manner similar to the donor rat. The abdomen was shaved and entered through a vertical incision. The abdominal great vessels were located and freed from surrounding adventitia. A bulldog clamp was placed on the abdominal vessels longitudinally to isolate small ventral segments of aorta and IVC for transplantation. The donor heart aorta and pulmonary artery were anastomosed to the recipient’s abdominal aorta and IVC, respectively, with 8-0 nylon suture. The total ischemic time of the transplanted hearts was no longer than 30 minutes. After completion of transplantation, the bulldog clamp was removed and the coronary arteries were reperfused to allow for restoration of sinus rhythm within 30 seconds. The anastomoses were checked for adequate hemostasis. In experimental subgroups 1, 3, and 5 the abdomen was closed with 2-0 chromic suture.

**Experimental groups**

Subgroup 0 (posttransplantation day 0 or 15 minutes). Subgroup 0 included 12 rats (isografts, n = 6; allografts, n = 6). The abdominal aorta and IVC were clamped momentarily on both sides of the anastomoses after blood reperfusion of the transplanted hearts had continued for 15 minutes. The transplanted hearts were arrested at end-diastole after the injection of 0.1 ml (2 mEq/ml) of potassium chloride directly into the aortic root proximal to the clamp. Hearts were rapidly excised and placed in cold (0° to 4° C) high-potassium (40 mEq/L) lactated Ringer’s solution. Wet heart weight, myocardial water content, and LV diastolic pressure-volume relation were determined as described later.

Subsequently, the native hearts were exposed through thoracotomy incisions and arrested at end-diastole by the technique described earlier. Hearts were rapidly excised and kept cold for evaluation of wet heart weight, myocardial water content, and LV pressure-volume relation.

Subgroups 1, 3, and 5. Each subgroup included 12 rats (isografts, n = 6; allografts, n = 6). On posttransplantation days 1, 3, and 5, both transplanted and native hearts were arrested...
Transplanted Rat Heart
n=6
*p<0.05 vs. PTD-0, 1, and 3 (ALLO)
†p<0.01 vs. PTD-5 (ISO)

Fig. 3. Changes in myocardial water content of the transplanted hearts versus posttransplantation day in the isograft (ISO) and allograft (ALLO) groups. The large increase in myocardial water content in the allograft group on posttransplantation day 5 is statistically significant both versus the isograft group on day 5 and versus posttransplantation days 0, 1, and 3 in the allograft group.

and rapidly excised for evaluation of their wet heart weight, myocardial water content, and LV pressure-volume relation.

Heart weights and myocardial water contents. Excised hearts were gently blotted dry, placed in a preweighed Petri dish, and weighed on an analytical balance immediately after excision to obtain the initial wet heart weight. Hearts were again weighed after LV pressure-volume relation measurements. Each specimen was then dried to a constant dry heart weight in an oven at 60°C for 48 hours. Myocardial water content was calculated by equation 1:

$$\text{MWC (\%)} = \frac{\text{WHW} - \text{DHW}}{\text{WHW}} \times 100$$ (1)

where MWC is myocardial water content, WHW is wet heart weight, and DHW is dry heart weight.

LV pressure-volume relation. Excised hearts were submerged in cold (0° to 4°C) high-potassium (40 mEq/L) lactated Ringer's solution. A 16-gauge angiocatheter connected to a three-way stopcock was placed into the LV through the aortic valve. The aortic root was occluded around the catheter at the level of the coronary ostia with 4-0 silk suture. LV pressures were measured with a 5F micromanometer (Millar Instruments, Inc., Houston, Tex.) connected to the three-way stopcock. The third port of the stopcock was used for volume infusion. Before pressure measurements, all air was eliminated from the system. The LV was sealed by placing a small clamp approximately 1 mm on the atrial side of the mitral anulus, minimizing annular compression. Volume was infused into the LV in 0.05 ml increments while pressure was recorded with an analog to digital conversion and recording system (MacLab, MacLab Inc., Milford, Mass.) until an LV pressure of 20 mm Hg was reached. Pressure-volume relation measurements were obtained in duplicate. Only pressure-volume relation measurements in which greater than 95% of injected volume was recovered were used for data analysis. All pressure-volume relation measurements were done within 15 minutes of the onset of ischemia to avoid the onset of rigor.27

Data analysis
LV pressure-volume relation. Methods used were similar to previous studies from this laboratory.26 A mean pressure-volume relation was calculated for each animal by averaging ventricular pressure corresponding to the volume injected. Pressure was plotted versus volume by means of interpolated line segments on a digital computer (Apple Macintosh Quadra 950, Apple Computer, Cupertino, Calif.) with commercial software (Cricket Graph, Computer Associates International, Inc., Garden City, N.Y.).

For purposes of analysis, volume measurements were grouped into five pressure intervals: -2.5 to 2.4, 2.5 to 7.4, 7.5 to 12.4, 12.5 to 17.4, and 17.5 to 22.5 mm Hg. Volumes were averaged within each pressure range. LV pressure-volume relations were compared among isograft and allograft subgroups by repeated-measures analysis of variance (ANOVA) with statistical significance defined as $p < 0.05$. Differences in mean volumes among the subgroups at each pressure interval were tested for significance by a Bonferroni procedure.

On posttransplantation day 5, transplanted hearts in the allograft group had pressures exceeding 22.5 mm Hg at the smallest infused volume (0.05 ml). Therefore, it was not possible to use the standard pressure intervals to "construct" LV pressure-volume curves. As an alternative, a separate ANOVA
Fig. 4. Wet weight of the transplanted hearts (TxH) versus posttransplantation day in both the isograft (ISO) and allograft (ALLO) groups. Wet weight is expressed as a percentage of donor wet heart weight to account for variability in the weight of the excised donor heart before transplantation. The large increase in wet weight in the allograft group on posttransplantation day 5 is statistically significant both versus the isograft group on posttransplantation day 5 and versus posttransplantation days 0, 1, and 3 in the allograft group. WHW, Wet heart weight.

Table 1. LV Pressure (mm Hg) in native and transplanted hearts for isografts and allografts after 0.05 ml injection on posttransplantation day 5

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>TxH (ISO)</th>
<th>NH (ISO)</th>
<th>Δ (ISO)</th>
<th>TxH (ALLO)</th>
<th>NH (ALLO)</th>
<th>Δ (ALLO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>27</td>
<td>4</td>
<td>23</td>
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<td>3</td>
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<td>6</td>
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<td>9</td>
<td>4</td>
<td>5</td>
<td>25</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Avg ± SEM</td>
<td>10 ± 1*</td>
<td>4 ± 1</td>
<td>6 ± 1</td>
<td>24 ± 3†‡</td>
<td>3 ± 1</td>
<td>22 ± 2§</td>
</tr>
</tbody>
</table>

TxH, Transplanted heart (abdomen); NH, native heart (chest); ISO, ACI to ACI transplant; ALLO, ACI to Lewis transplant; Δ, TxH and NH difference; Avg, average; SEM, standard error of mean.

*p < 0.01 versus NH (ISO).
†p < 0.01 versus NH (ALLO).
‡p < 0.01 versus TxH (ISO).
§p < 0.01 versus Δ (ISO).

was performed for posttransplantation day 5 subgroups comparing the LV pressures at the infused volume of 0.05 ml between isograft and allograft groups. In essence, this amounts to performing a one-point compliance measurement using a fixed volume of 0.05 ml. In this analysis, significant increases in pressure are used to assess decreases in compliance.

Dry heart weight, wet heart weight, and myocardial water content. Averages were calculated for each experimental subgroup. Differences in dry heart weight, wet heart weight, and myocardial water content between subgroups were compared by three-way repeated-measures ANOVA with post hoc comparisons performed by a Bonferroni procedure. Differences between the wet heart weight measured immediately after excision and after determination of the LV pressure-volume relation were compared by Student’s t test. Statistical significance was defined as p < 0.05.
Results

Percent change in recipient rat body weight over time is illustrated in Fig. 1. On posttransplantation day 5, the weight loss in the isograft group was 5% ± 1% versus 13% ± 4% in the allograft group. This difference was statistically significant \( (p < 0.01, \text{ANOVA}) \). Comparison of the changes in body weights showed no significant difference between posttransplantation days 1, 3, and 5 in the allograft or isograft group. Body weight of transplanted rats measured when put to death on posttransplantation days 1, 3, and 5 decreased significantly \( (p < 0.05, \text{ paired } t \text{ test}) \) compared with the time of transplantation in both allograft and isograft groups. Differences in body weight of the donor or recipient rats between isograft and allograft in subgroups 0 through 5 were not statistically significant.

Wet heart weight compared immediately after excision and after determination of the LV pressure-volume relation did not change significantly in any of the isograft or allograft subgroups. On posttransplantation day 0 the myocardial water content of the transplanted hearts was significantly greater \( (p < 0.01, \text{ANOVA}) \) than that of the native hearts in the isograft group (77.0% versus 73.6%). A similar difference was observed in the allograft group (76.1% versus 74.9%, \( p < 0.01, \text{ANOVA} \)). As illustrated in Fig. 2, myocardial water content of the native hearts decreased on posttransplantation day 1 and then increased, becoming significantly greater \( (p < 0.05, \text{ANOVA}) \) on posttransplantation day 5 than on day 1 in both the allograft and isograft groups. Myocardial water content tended to be higher in the allograft group, but this difference was not statistically significant. In the transplanted hearts in the allograft group (Fig. 3), myocardial water content was significantly greater in subgroup 5 than in subgroup 5 in the isograft group \( (p < 0.01, \text{ANOVA}) \), and it was greater than in subgroups 0, 1, and 3 in the allograft group \( (p < 0.05, \text{ANOVA}) \).

The effect of time on wet weight for the transplanted hearts in the isograft and allograft groups is illustrated in Fig. 4. Data are presented as a percentage of pretransplantation donor wet heart weight. In the allograft group, wet heart weight on posttransplantation day 5 was significantly greater \( (p < 0.05, \text{ANOVA}) \) than in all other subgroups. Wet heart weight in the allograft group was also significantly greater \( (p < 0.01, \text{ANOVA}) \) than in the isograft group on posttransplantation day 5; differences on all other days were not significant (Fig. 4). Dry heart weight as a percentage of pretransplantation donor wet heart weight showed trends similar to the wet heart weight in the allograft and isograft groups.
Data comparing mean postmortem pressure-volume relations for the native and transplanted hearts in each subgroup are illustrated in Figs. 5 and 6. In the pressure intervals above 7.5 mm Hg, LV volume was significantly smaller ($p < 0.01, \text{ANOVA}$) for transplanted hearts than for native hearts. This effect was seen for the isografts on posttransplantation days 0, 1, 3, and 5 or for the allografts on posttransplantation days 0, 1, and 3. This magnitude of decrease in filling volume between native and transplanted hearts on posttransplantation days 0, 1, and 3 was not significantly different for the isograft and allograft groups. Furthermore, no significant differences were observed among native hearts from either group on posttransplantation days 0, 1, 3, and 5. Similarly, no significant differences were observed among transplanted hearts in the isograft group on posttransplantation days 0, 1, 3, and 5 or in the allograft group on posttransplantation days 0, 1, and 3. On posttransplantation day 5, comparisons were performed on LV pressures at filling volumes of 0.05 ml (Table I). LV pressure was significantly greater ($p < 0.01, \text{ANOVA}$) in transplanted hearts than in native hearts in both the isograft and allograft groups. However, this magnitude of increase in pressure for the transplanted hearts was significantly greater ($p < 0.01, \text{ANOVA}$) in the allograft group than in the isograft group.

**Discussion**

The present results identify increases in dry heart weight, wet heart weight, and myocardial water content on posttransplantation day 5 in the allograft group, concurrent with the onset of rejection. Previous studies have identified increases in LV mass during transplant rejection. The present data identify edema as an important cause, but not the only cause (see later), of this mass increase. The present results also identify an increase in LV pressure on posttransplantation day 5 in the allograft group, a time point associated with onset of severe rejection in previous studies.$^{15, 16}$ This last result supports the view that transplant rejection is associated with a decrease in LV diastolic compliance. Although additional experiments are needed to fully establish the hypothesis that edema causes reduced compliance in transplant rejection, the present results are the first of which we are aware to link edema, reduced compliance, and increased mass in a single study of transplant rejection.

The present results are also unique in identifying marked abnormalities of diastolic compliance in the...
Ono-Lindsey heterotopic heart transplant model beginning immediately after transplantation and persisting until rejection. Because these changes are observed immediately after reperfusion, ischemic injury and rigor complex formation are likely causes. Inflammatory adhesions, nonworking state of the LV, or procedure-related edema are alternative explanations, but these mechanisms should be associated with changes in compliance as a function of time in subgroups 1 and 3.

Griggs, Holt, and Case studied the effect of time of diastolic properties of the isolated, ischemic canine LV in 1960. In that study, more than 5 hours of ischemia at 5°C were necessary before the pressure-volume relation of the LV began to shift to the left, a process that appeared to reach completion in an extremely stiff, noncompliant chamber after 6 hours. This process was attributed by the authors to cross-linkage of actin and myosin after consumption of adenosine triphosphate by cellular metabolism. Studies in our laboratory demonstrated no change in the pressure-volume curve of the pig LV after 90 minutes of ischemia at 4°C. Abnormalities of diastolic compliance have been observed during cardiac operations, but those changes are usually small, even with 2 hours of ischemia. The onset of ischemic contracture in the rat is much faster, however, reaching completion in 17 minutes in the rat at 37°C. Studies currently in progress in our laboratory also suggest that stiffening of the LV pressure-volume relation of the rat may be observed after only 15 minutes of ischemia at 4°C, even without reperfusion (Starr and associates, unpublished data, used with permission).

Conventional histologic examination could be of value in these experiments. We will incorporate histologic methods when they have been improved to the point that edema is not minimized by dehydrating effects of tissue processing.

It appears that early alteration in diastolic properties of the surgically implanted hearts indicates ischemic injury, suggesting that improved myocardial protection is needed for the Ono-Lindsey model. Techniques that might improve myocardial protection for heterotopic transplantation would include intermittent or continuous antegrade or retrograde cardioplegia with crystalloid, hyperoxygenated crystalloid, or blood-based cardioplegic solutions. Intermittent perfusion during the transplantation process might also be beneficial. Finally, the ischemic time itself might be reduced to a small fraction of the present standard through the use of cuffed anastomoses.

Reduced LV filling volume in the transplanted hearts could also reflect myocardial edema, because myocardial water content of the transplanted hearts was significantly greater than for the native hearts in the isograft and allograft groups on posttransplantation day 0. This edema could reflect either initial coronary perfusion with lactated Ringer’s solution or reperfusion injury. Myocardial water content of the transplanted hearts remained increased in subgroups 1, 3, and 5 except on posttransplantation day 5 in the isograft group. This result contrasts with observations from our laboratory, which indicate that perfusion-induced edema resolves in less than 15 minutes in the absence of ischemic injury.

We interpret reduced myocardial water content in native hearts in the isograft and allograft groups on posttransplantation day 1 compared with day 5 (see Fig. 2) to reflect inadequate fluid replacement and dehydration at the time of the initial operation, aggravated by reduced oral fluid intake during the early recovery period. This hypothesis is supported by a trend toward decreased body weight on posttransplantation day 1 in both groups (see Fig. 1). We attribute a statistically significant decrease in body weight in the allograft group on posttransplantation day 5 to acute illness associated with transplant rejection (see Fig. 1).

Previous studies from our laboratory have emphasized the importance of myocardial water content as a measure of myocardial edema. If protein dry weight remains constant, changes in LV mass are predictable from changes in myocardial water content according to equation 1. If this equation is applied to the data presented in Fig. 3, the increase in myocardial water content of transplanted hearts in the allograft group between posttransplantation days 3 and 5 (77.5% to 81.1%) is not sufficient to explain the observed increase in heart weight during the same period (0.87 gm to 1.46 gm). Thus the increase in myocardial water content observed should increase heart weight from 0.87 gm to approximately 1.0 gm, whereas the actual increase was to nearly 1.5 gm. Because dry heart weight increased significantly from 0.19 to 0.27 gm between posttransplantation days 3 and 5 in the allograft group and was essentially unchanged (0.16 versus 0.17 gm) in the isograft group, the increase in wet heart weight reflects an increase in both dry heart weight and myocardial water content. This increase may reflect cellular infiltration and exudates. We plan in future experiments to determine whether the same processes that increase dry heart weight also contribute to decreased ventricular compliance.

We have used a simple approach to analyzing alterations in diastolic properties based on comparison of pressure and volume intervals. More sophisticated methods are available for this, including exponential curve-fitting and stress-strain analysis. In the present study, interval pressure-volume analysis was used because it does not require assumptions of the exponential curve-
fitting model used to derive the ventricular stiffness constant, $\beta$. Stress-strain analysis was not used because of unusual changes in ventricular geometry caused by edema: First, edema decreases the volume of the ventricle at a filling pressure of zero. $^{15,17}$ Second, edema increases mass with no alterations in the number of load-bearing elements in the tissue. In our view, it appears inappropriate to use calculations that would demonstrate a decrease in wall stress in edematous tissue when intuition suggests that forces on the load-bearing elements are minimally altered.

In conclusion, the present results support the view that cardiac allograft rejection is associated with a triad of increased mass, increased myocardial water content, and decreased LV compliance. A common cause for all of these could be myocardial edema in association with rejection. Other factors may also be involved, reflected in a large increase in dry weight during rejection. The results also demonstrate unexpected abnormalities of compliance of the transplanted hearts beginning from the time of reperfusion. This latter observation requires improved techniques of myocardial protection for the Ono-Lindsey model, both to improve relevance of study of this model to clinical transplantation and to allow the mechanism of reduced LV compliance during allograft rejection to be defined with greater precision.

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