Regression of pressure overload-induced left ventricular hypertrophy in mice

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Myocardial hypertrophy occurs in response to a variety of biomechanical stimuli. Although left ventricular (LV) hypertrophy (LVH) in response to increased afterload can be viewed as a compensatory mechanism to maintain cardiac output and normalize wall stress, LVH in the long term is an independent risk factor for a range of adverse consequences, such as myocardial ischemia, systolic and diastolic dysfunction, arrhythmias, and cardiac mortality (4, 9, 18, 19). Therefore, prevention or regression of LVH is a major therapeutic target whether achieved by pharmacological, mechanical, surgical, or genetic means (11, 21, 27, 35, 38). There has been clinical evidence that regression of LVH reduces morbidity and mortality and improves prognosis (9, 32). For example, antihypertensive treatment and aortic valvular replacement reverse LV structural, functional, and electrophysiological abnormalities (12, 26, 28). Similar benefits have also been observed after drug treatment in different animal models (3, 30). Although reversal of LVH occurs after control of etiological factors, some studies have revealed incomplete reversal of pathophysiological changes, such as interstitial fibrosis, ventricular remodeling and dysfunction, and aberrant electrophysiology (2, 3, 12). Although numerous clinical and experimental studies have investigated effects of antihypertensive drugs (1, 3), coronary structure and function (17, 29), and alteration in gene expression (7, 31, 36) during reversal of LVH, the pathophysiological features and the time course of LVH regression are less clearly defined.

Gene manipulation in the mouse has become a standard approach in heart research, providing new insights into molecular and signaling pathways of importance in the development of heart disease (10). Understanding the molecular mechanism of hypertrophy regression through the use of gene-targeted mouse strains requires a mouse model of LVH and regression. Although drug-induced hypertrophy-and-regression murine models have been reported (7, 31), a regression model of pressure overload-induced hypertrophy is far more appealing because of the common use of an aortic constriction model in the mouse. In particular, such a model is not confounded by the use of drugs, which may have pressure-independent effects. Our hypothesis is that the duration of pressure overload was a determinant of the degree of LVH regression. Therefore, the aim of this study was to characterize in mice the regression of established LVH induced by transverse aortic banding, the most common model. Specifically, we monitored the process of LVH development and regression, together with associated histological and molecular alterations, and also characterized factors that determine the rate of LVH regression.

METHODS

Animals and microsurgery. Male and female mice (4–5 mo of age) with a C57BL×SJL genetic background were used. All experimental protocols were approved by a local animal ethics committee. Animals were anesthetized with a mixture of ketamine, xylazine, and atropine (10, 2, and 0.12 mg/100 g, respectively, ip), intubated via the oral cavity, and ventilated. Through a sternotomy, the transverse aorta between the right innominate and left carotid arteries was dissected and banded with a 0.5-mm probe using a 0.5 silk suture. The probe was then removed. For the degree of aortic banding (AB), the aortic diameter in these mice was 1.0–1.1 mm, measured by M-mode echocardiography. With the use of a 0.5-mm probe, aortic diameter was reduced 50–55%, which leads to an ~75% reduction in cross-sectional area. A bioresorbable gel (ADCON-L, Gliatech, Cleveland, OH) was applied locally to limit postsurgical adhesion. Sham-operated mice underwent similar surgery without AB. The animals allocated to LVH reversal groups were subjected to the second operation to cut the silk suture surrounding the aortic arch [debanding (DB)].

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To examine the pressure gradient across the banding site, a group of nonoperated animals were anesthetized, and both carotid arteries were cannulated using 1.4-Fr Millar catheters. Pressure sensors were placed at the ascending and the descending aorta, respectively. Changes in arterial pressures were recorded simultaneously at proximal and distal sites, while the transverse AB was induced through a stenotomy. Data from eight mice showed that AB resulted in a gradient of +28 ± 4 mmHg, which was very close to the increment of pulse pressure (+30 ± 5 mmHg) measured at the proximal site (i.e., ascending aorta).

Echocardiography. Transthoracic echocardiography was performed utilizing an Agilent Sonos 5500 ultrasound machine (Hewlett-Packard) with a 15-MHz linear transducer, as described previously (8). Mice were anesthetized with one-half of the ketamine-xylazine-atropine mixture used for surgery. M-mode images were recorded onto a magnetic optical disk and analyzed offline by an operator unaware of study groups. The following parameters were derived from M-mode traces: heart rate, LV end-systolic and end-diastolic diameters (LVESd and LVEDd), external LV diastolic diameter (ExLVDd), LV wall thickness at diastole, LV mass index (i.e., [(ExLVDd) - LVEDd] × 0.053/ body wt), fractional shortening [FS% = (LVEDd - LVESd)/LVEDd], and the ratio of diastolic wall thickness to radius of the LV cavity (h/r).

Micromanometry and morphometric analyses. At the end of the study period, surviving animals were anesthetized with a mixture of pentobarbitone sodium and atropine (6 and 0.12 mg/100 g, respectively, ip) and breathed spontaneously. A 1.4-Fr Millar catheter was inserted into the right carotid artery and advanced into the ascending aorta and the LV. Arterial and LV pressures were recorded and analyzed to confirm pressure overload and the normalization of hemodynamic loading by DB.

After the mice were killed, body weight and LV, right ventricular, atrial, and lung weights were obtained. Pathological signs that indicated the presence of heart failure, including pleural effusion, lung congestion, and atrial thrombus, were monitored as described previously (6). The apical half of the LV was fixed and serially and transversely cut into 5-μm sections, which were stained with hematoxylin and eosin or 1% picrosirius red for quantification of myocyte size or collagen content as previously described (8).

Gene expression. Total RNA was extracted from LVs with the use of TRIzol. After DNase treatment, 1 μg of total RNA was reverse transcribed with the use of random primers and SuperScript III RNase H− reverse transcriptase (Invitrogen). Gene expression of α- myosin heavy chain (α-MHC), sarco(endo)plastic reticulum Ca2+-ATPase (SERCA2a), collagen I and collagen III, and matrix metalloproteinases (MMP-2, MMP-9, and MMP-13) was determined by real-time PCR using SYBR Green Master Mix with the ABI PRISM 7700 sequence detection system. Primers were designed from known mouse sequences or from the literature. Expression level was normalized to the reference gene 18S rRNA. Expression of atrial natriuretic peptide (ANP) and glyceraldehyde phosphate dehydrogenase was determined by RNase protection analysis, as previously described (33).

Statistics. Values are means ± SE, unless otherwise indicated. Comparison among groups was made by one- or two-way ANOVA. χ2 or Fisher’s exact test was used to compare percentages. The least-squares method was used for correlation between selected variables. P < 0.05 indicates statistical significance.

RESULTS

Grouping and protocols. Animals surviving AB surgery were allocated to the following groups: 10 and 14 wk of AB, early DB (4 wk of AB followed by 6 wk of DB), late DB (8 wk of AB followed by 6 wk of DB), and 14 wk after sham operation. Echocardiography was performed before surgery and 1, 2, 4, 8, 10, 12, and 14 wk after surgery for animals subjected to AB and 1, 4, and 6 wk after the second surgery for animals subjected to DB. One additional group of mice subjected to 4 wk of AB followed by 1 wk of DB was killed to obtain organ weights and tissues for gene expression analysis.

Features of establishment of LVH. AB resulted in a rapid increase in LV mass with a 79% increment by week 4 but without further increase between weeks 4 and 12. This was associated with a 37% increase in wall thickness (Fig. 1). From 12 to 14 wk after AB, there was a further 14% increase in LV mass without LV wall thickening (Fig. 1). FS was slightly elevated within the first 2 wk (38 ± 1% at week 2 vs. 35 ± 1% at baseline, P = 0.052) but progressively declined thereafter, together with a gradual enlargement in LV dimensions after AB (Fig. 1). The ratio h/r increased in the early stage, indicating concentric hypertrophy, and then progressively declined. All the above-described echocardiographic parameters remained unchanged in sham-operated mice during the 14-wk study period. There was no significant difference in heart rate among the groups over the study period (group mean = 360–420 beats/min).

Regression of LVH. Surgical DB 4 or 8 wk after AB led to a progressive reduction in LV mass during the following 6-wk period (Fig. 1). Absolute LV mass index was reduced dramatically at 1 wk after DB in early- and late-DB groups by 72 and 50% of the difference between the sham-operated control and AB values at 4 and 8 wk, respectively. Similar changes in LV wall thickness were observed (Fig. 1). Subsequent reduction in LV mass was more gradual. In sharp contrast, recovery of the enlarged LV dimensions and suppressed FS did not occur 1 wk after DB but was largely complete by 6 wk after early DB (Fig. 1). Reversal of increased LV mass, wall thickness, and LV dimension and reversal of decreased FS appeared to be a slower process in the late-DB than in the early-DB group. This was also reflected by the temporal change in h/r after DB (Fig. 1). Eventually, release of pressure overload for 6 wk resulted in a complete normalization of LV mass, LVEF, FS, and h/r in early-DB, but not late-DB, mice (Fig. 1).

Hemodynamic assessment. There was no significant difference in heart rate among the groups (Table 1). Compared with sham-operated controls, systolic arterial, LV, and pulse pressures were markedly elevated in AB mice, confirming pressure overload. After DB, these pressure parameters were normalized, and there were no statistical differences compared with sham-operated mice (Table 1). Echocardiographically derived LV mass correlated well with actual LV weight, systolic arterial pressure, and pulse pressure (Fig. 2).

Organ weights and pathological events. Body and organ weights and the incidence of pathological events in mice of various groups are displayed in Table 1. Body weights were similar among groups. LV, right ventricular, atrial, heart, and lung weight were markedly higher in AB than in sham-operated mice. There was a trend toward a greater degree of hypertrophy in 14-wk than in 10-wk AB mice. Autopsy-proven heart failure was apparent in AB mice, evidenced by higher incidences of pleural effusion, lung congestion, and atrial thrombus. Releasing pressure overload for 6 wk led to a restoration of increased LV and heart weights. However, the restoration of organ weights was incomplete in 8-wk AB mice. Although LV and heart weights were greater in 8-wk AB than in sham-operated mice, there was no significant difference in
LV and heart weights between the early-DB and sham-operated groups (Table 1).

Myocyte size and interstitial collagen content. Pressure overload doubled myocyte size and increased interstitial collagen by fivefold in AB mice compared with the sham-operated group (Fig. 3). Surgical unloading largely normalized cell size, but fibrosis remained ≥2.5-fold higher than the sham-operated values.

Gene expression. Expression of ANP increased by eight- to ninefold in the LV from the mice with pressure overload for 10 or 14 wk compared with the sham-operated controls, whereas expression of α-MHC and SERCA2a was significantly inhibited (Fig. 3). After the observation of a slow reversal of fibrosis, we further studied collagen turnover by determining gene expression of collagen I, collagen III, and MMPs. A progressive increase in the expression of collagens was observed in LVs with sustained pressure overload, which was more prominent in 14-wk than in 10-wk AB mice (Fig. 4). The ratio of collagen III to collagen I was increased significantly in 14-wk AB mice. Expression of the gelatinase MMP-2 was significantly enhanced in 14-wk, but not in 10-wk, AB mice (Fig. 4). Release of pressure overload for 1 wk rapidly restored gene expression pattern (Figs. 3 and 4). Expression of MMP-9 and MMP-13 was not significantly affected by AB or DB (data not shown).

**DISCUSSION**

In the present study, we determined the time course for development of LVH and, more importantly, the subsequent regression of established LVH. Pressure overload resulted in LVH, chamber dilatation, contractile dysfunction, interstitial collagen accumulation, and altered gene expression. Although release of pressure overload initiated reversal of established LVH, various structural, functional, and genetic components showed distinct time courses in their reversal toward the control levels. Moreover, early DB was associated with a more rapid and complete recovery of LV structure and function than

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**Table 1. Hemodynamics, body weight, normalized organ weights, and incidence of pathological events**

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$ (M/F)</th>
<th>HR, beats/min</th>
<th>SAP, mmHg</th>
<th>PP, mmHg</th>
<th>LVSP, mmHg</th>
<th>LVMI, mg/g</th>
<th>LVESd, mm</th>
<th>LVEDd, mm</th>
<th>Body wt, g</th>
<th>LV wt, mg/g</th>
<th>RV wt, mg/g</th>
<th>Atria wt, mg/g</th>
<th>Heart wt, mg/g</th>
<th>Lung wt, mg/g</th>
<th>PE, %</th>
<th>Ath, %</th>
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<tr>
<td>SH</td>
<td>6/8</td>
<td>341 ±13</td>
<td>103 ±3</td>
<td>35 ±2</td>
<td>105 ±5</td>
<td>26 ±1</td>
<td>3.4 ±0.1</td>
<td>0.9 ±0.2</td>
<td>0.4 ±0.2</td>
<td>4.7 ±0.1</td>
<td>5.9 ±0.2</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10 wk AB</td>
<td>6/4</td>
<td>365 ±13</td>
<td>146 ±11</td>
<td>89 ±9</td>
<td>152 ±9</td>
<td>28 ±2</td>
<td>6.1 ±0.4</td>
<td>1.1 ±0.12</td>
<td>0.7 ±0.06</td>
<td>7.9 ±0.6</td>
<td>8.9 ±1.6</td>
<td>0.00</td>
<td>0.00</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>14 wk AB</td>
<td>4/6</td>
<td>364 ±29</td>
<td>167 ±8*</td>
<td>89 ±5*</td>
<td>177 ±8*</td>
<td>25 ±1</td>
<td>6.5 ±0.4</td>
<td>1.2 ±0.05*</td>
<td>0.8 ±0.08*</td>
<td>8.7 ±0.4*</td>
<td>8.3 ±0.9*</td>
<td>0.00</td>
<td>0.00</td>
<td>60</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4 wk AB + 1 wk DB</td>
<td>3/4</td>
<td>349 ±12</td>
<td>112 ±11*</td>
<td>38 ±6</td>
<td>119 ±12*</td>
<td>28 ±1</td>
<td>3.6 ±0.1†</td>
<td>0.9 ±0.2</td>
<td>0.4 ±0.01†</td>
<td>4.8 ±0.1†</td>
<td>5.2 ±0.3*</td>
<td>0.00</td>
<td>0.00</td>
<td>80</td>
<td>10</td>
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<tr>
<td>4 wk AB + 6 wk DB</td>
<td>4/4</td>
<td>351 ±16</td>
<td>118 ±7*</td>
<td>40 ±5*</td>
<td>119 ±8*</td>
<td>27 ±1</td>
<td>3.8 ±0.1*</td>
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<td>5.2 ±0.2*</td>
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Values are means ± SE. AB, aortic banding; DB, aortic debanding; SH, sham operation; HR, heart rate; SAP, systolic arterial pressure; LVSP, left ventricular (LV) systolic pressure; PP, pulse pressure; RV, right ventricle; PE, plural effusion; Ath, atrial thrombosis. *$P < 0.05$ vs. sham. †$P < 0.05$ vs. 10 wk AB. ‡$P < 0.05$ vs. 14 wk AB.
was late DB, indicating that the process of LVH regression in this model is dependent on the duration of the preceding pressure overload.

During the first 2 wk of pressure overload, rapid increment in LV mass and wall thickness was observed, whereas LV dimension was reduced or maintained. Therefore, pressure overload-induced LVH at the early stage is concentric with the wall thickening as a major contributor to the increased LV mass. At this stage, FS was well maintained, indicating a full compensation for an augmented afterload. Several studies reported a similar alteration in mice at an early stage after AB (10, 20, 23, 25). At 4–12 wk after AB, there was no further increase in LV mass or wall thickness, but the LV was progressively dilated and FS declined, indicating an eccentric hypertrophy and decompensation. Taken together, these changes reflect the transition from compensatory LVH to heart failure in this model.

Release of AB, evident by the normalization of arterial and pulse pressures, resulted in regression of established LVH. In this model, LVH regression appears to have distinct early and late phases. The early phase, which occurred 1 wk after DB, is characterized by a dramatic decrease in LV mass, as deter-
minded by echocardiography and by weight, attributed to a significant reduction in LV wall thickness. Such a rapid regression of pressure overload-induced hypertrophy implies a high sensitivity of hypertrophy to the alteration of workload. During the late phase, a slow reversal in LV mass was observed without further change in wall thickness, but this phase was associated with a gradual decrease in LV dimensions over the subsequent 5-wk period. A similar degree of LVH and the same duration of DB restored LV mass in 4- and 8-wk AB groups. However, a complete normalization of LV weight was found in the 4-wk, but not the 8-wk, AB group. In contrast to the 4-wk AB mice, which showed a nearly complete normalization of LV wall thickness after 1 wk of DB, 8-wk AB animals displayed only a modest decrease in LV wall thickness at 1 wk of DB, and a further 5 wk of DB were required for complete normalization of wall thickness. These different time courses of regression in the early- vs. late-DB animals suggest that regression of LVH in this model is dependent on the duration of pressure overload.

Interestingly, functional restoration did not follow the same time course as hypertrophy regression but did parallel changes in chamber dimensions. Although LVH rapidly reversed after release of pressure overload, recovery of LV contractile function was not observed until later. A slow but complete recovery of FS was observed in the early-DB, but not late-DB, mice, again indicating that recovery of systolic dysfunction in the process of LVH regression was dependent on the duration of pressure overload. Pressure overload significantly increased myocyte size and interstitial collagen content. Collagen accumulation in the myocardium increases chamber stiffness and contributes to cardiac dysfunction (5, 37) and electrophysiological abnormalities (16). Release of pressure overload for 6 wk induced only a partial reversal of fibrosis, which might be related to the delayed function recovery.

Pressure overload also altered gene expression, with an increase in ANP and a suppression of α-MHC and SERCA2a. Decreased α-MHC expression in the myocardium may be related to the impaired contractile function, as seen in other heart failure models (8, 22, 34). SERCA2a is known to play a pivotal role in lowering intracellular Ca2+ levels during relaxation and maintenance of Ca2+ storage. Sustained pressure overload suppresses SERCA2a expression and subsequent elevation of intracellular Ca2+ concentration, which is known to mediate the onset and progression of hypertrophy (14). Moreover, enhanced expression of SERCA2a attenuates pressure overload-induced LVH (24) and deters onset of early heart failure (14). We also observed an enhanced expression and deposition of collagens by sustained pressure overload that is associated with increased transcription of MMP-2. Upregulation of MMPs may act to degrade increased collagen to maintain a balance between extracellular matrix synthesis and degradation. However, upregulated MMPs may also contribute to the transition from compensated hypertrophy to heart failure (15). In our study, release of pressure overload for 1 wk had already reversed the altered expression pattern of these genes, which was coupled with regression in LV mass and wall thickness but not restoration of systolic function and chamber size. Interestingly, rapid regression of hypertrophy and restoration of gene expression in our model were also reported in some pharmacological models (7, 31). A previous study showed that many weeks were required for the reversal of altered V3-MHC isoform in rats with pulmonary artery constriction followed by DB (13). Thus, although reversal of gene expression is rapid, the slow normalization of these molecules at the protein level might be partly responsible for a delayed function recovery, in conjunction with other factors such as ventricular remodeling and interstitial fibrosis. A slow and incomplete reversal of interstitial fibrosis 6 wk after DB is not due to continuous overexpression of collagens, but to a slow turnover rate of collagen together with a concomitant decrease in previously elevated expression of MMP.

In conclusion, we have established a regression model of LVH in mice by surgical intervention. Pressure overload-induced LVH was associated with LV dysfunction, remodeling, myocyte enlargement, interstitial fibrosis, and altered gene expression. These changes were restored partially or completely after 6 wk of surgical unloading. In this model, LVH regression involves various morphological, functional, and genetic components, and each of these displays a distinct time course. A longer period of pressure overload is associated with a slower rate of LVH regression.

Fig. 4. Expression of collagen I, collagen III, and matrix-metalloproteinase (MMP)-2 and ratio of collagen III to collagen I in LV from sham, AB, and DB groups. Values are means ± SE (n = 4–6). *P < 0.05 vs. sham.
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Surgical Technologies for the generous supply of ADCON-L gel.