Early left ventricular remodeling in acute severe aortic regurgitation: insights from an animal model.

Short title: LV remodeling and acute AR

Key words: aortic regurgitation, left ventricle, remodeling, rat

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Substantive abstract

Background and aim: Chronic aortic valve regurgitation (AR) induces left ventricular hypertrophy and eventually left ventricular dysfunction. While the effects of chronic AR on the left ventricle (LV) are well known, the effects of acute AR on the LV have not been adequately evaluated. We hypothesize that LV tissues will be rapidly remodelled by acute AR and that the renin-angiotensin system will be involved in that acute remodelling.

Method: Using a rat model, we evaluated serially over a period of 14 days the early LV adaptations to acute AR. LV were evaluated both in vivo by echocardiography and also on explanted heart tissue after 1, 2 or 14 days.

Results: After 14 days, the LV of AR rats were already significantly hypertrophied and dilated (end-diastolic diameter: +16% p<0.05 vs. sham; LV mass: +16% p<0.01 vs. sham). A drop in fractional shortening was detected after 14 days after a transient increase. Cardiomyocyte cross-sectional area and peri-vascular fibrosis were significantly increased after 14 days of AR. The number of fibronectin-positive cells in LV sections quickly increased as well as the fibronectin protein and mRNA content in LV crude homogenates. The expression of pro-matrix metalloproteinase 2 was clearly abnormal after 2 days. Significant shifts in the expression of angiotensin II receptors were also detected as early as after 1 day.

Conclusion: Significant macroscopic and microscopic abnormalities were present in the LV of rats with acute AR early after the induction of AR. Significant hypertrophy, peri-vascular fibrosis and extracellular matrix remodeling were present after only 14 days. These results suggest that the myocytes and extracellular matrix are affected significantly very early in the disease.
Short abstract:

The impact of acute severe aortic valve regurgitation (AR) on the left ventricle is unknown. We hypothesized that myocyte and extracellular matrix remodeling would occur early in the process and that the renin-angiotensin system would play a role in this acute remodeling. Therefore the effects of acute AR were examined on the left ventricles (LV) using echocardiography in a rat model. Animals were sacrificed after 1, 2 or 14 days of acute AR to obtain cardiac tissue. Significant LV dilatation and hypertrophy were detected very early after the procedure. Tissue analysis revealed an important cardiomyocyte hypertrophy and the presence of peri-vascular fibrosis. Increases in fibronectin and pro-matrix metalloproteinase 2 expressions were also noted. Angiotensin II receptor expression was abnormal compared to controls. These results suggest that the LV is significantly remodeled very early by acute AR. Some abnormalities became evident as early as 24 hours after the occurrence of the disease.
**Introduction**

Severe aortic regurgitation (AR) is a valve disease causing progressive left ventricular (LV) dilatation and hypertrophy that may eventually lead to heart failure [1]. Severe myocardial abnormalities (dilatation, hypertrophy, fibrosis, and decrease in contractility) occur in subjects with long-standing chronic AR. The renin-angiotensin and adrenergic systems have been incriminated in this remodeling process [2-6]. Although the chronic negative effects of AR on the left ventricle are known and accepted, the events occurring very early in the LV myocardium before the full-blown development of LV hypertrophy are less well understood. We do not know how the myocardium reacts to acute AR.

We previously reported a reliable method of producing AR in rats using echocardiographic guidance and have studied the evolution of LV hypertrophy associated with chronic AR [7,8]. This animal model has also been used to study potential treatment strategies [2,3,8].

Understanding the early adaptations of the LV to the stress of acute aortic valve regurgitation may help us to better understand the evolution towards the chronic phase of the disease. Consequently, the aim of this study was to assess for the first time the very early adaptations of the LV to severe but acute AR from 0 to 14 days in the same animal model. We hypothesized that significant myocyte as well as extracellular matrix remodeling would occur very early in the disease process and that the renin-angiotensin system would be involved in this acute remodeling. This topic has received little attention in any type of LV volume overload [9-11] and even less in acute aortic valve regurgitation [8,12,13].
Methods:

Animals: Adult male Wistar rats were randomly assigned to one of 4 study groups (n=10/gr): #1: sham-operated controls (sacrificed 14 days post-surgery), #2: AR sacrificed one day after surgical procedure (described below), #3: AR sacrificed two days after surgical procedure and #4: AR sacrificed 14 days after surgical procedure. This protocol was approved by the Laval University Animal Protection Committee and was consistent with the recommendations of the Canadian Council on Animal Care.

Aortic regurgitation (surgical procedure): Severe aortic regurgitation was induced in the anesthetized animals as previously published by puncturing the aortic leaflets via a retrograde right carotid approach under hemodynamic and echocardiographic guidance [7]. Briefly, the right internal carotid artery was exposed and canulated. Under continuous echocardiographic guidance, an 18-gauge epidural catheter was advanced from the carotid artery towards the aortic valve. The sonographer guided the position and the advance of the catheter in the aorta while it was pushed through one leaflet of the aortic valve into the LV inducing acute AR. Intra-ventricular as well as aortic pressures were recorded and compared to those recorded before the perforation. Leaflet perforation was repeated if the severity of the regurgitant jet was considered insufficient by hemodynamic and echocardiographic criteria. Animals were closely observed in the first hours after surgery for any sign of respiratory distress suggestive of acute heart failure. Severe AR was considered to be present if the following echographic criteria were found: Color-Doppler ratio of regurgitant jet width to left ventricular outflow tract diameter at its origin in the parasternal long axis view (>50% = severe); ratio of the area of regurgitant jet at its origin to the area of the aortic annulus (>25% = severe); presence
of significant retrograde holo-diastolic flow in the proximal descending thoracic aorta (holo-diastolic reversal with end-diastolic velocity \(>18\) cm/s = severe). These criteria are widely accepted in humans and routinely used in clinical practice to evaluate the severity of aortic regurgitation.

_Echocardiography:_ A complete M-Mode, 2D and Doppler echocardiogram was performed as previously published on the animals under anesthesia at the following time points: preoperatively, immediately postoperatively, 1, 2 and 14 days \([2,3,7,8]\). AR was graded semi-quantitatively at each echographic exam and animals not meeting the echocardiographic criteria of severe AR would be excluded from the protocol.

_Messenger RNA accumulation:_

Complementary DNA synthesis and RT-PCR analyses were carried total RNA from LV tissues as described previously \([2,3]\) using the following primer pairs: _glyceraldehyde phosphate dehydrogenase_ (GAPDH) 5\'-ATCCCATCACCATCTTCCAG-3' and 5\'-CCATCACGCCACAGTTTCC-3'; _collagen type 1_ (Col1): 5\'-TGTTCTGTTTCTCAGGGTAG-3' and 5\'-TTGTCGTCAGGTTTCTTTTCC-3'; _Col3:_ 5\'-CGAGGTAACAGAGGTAAAGA-3' and 5\'-AACCCAGTATTCTCGCTCTT-3' and _pro-matrix metalloprotease 2_ (pro-MMP2): 5\'-CTATTCTGTCAGCCTTTG-3' and 5\'-CAGACTTTTGTTCTCAAACCTTTG-3'; _fibronectin_ (FN) 5\'-GAGAGATCTGGAGGTCAT-3' and 5\'-GGGTGACACCTGAGTGAA-3'; _AT1a receptor_ 5\'-GCACACTGGCAATGTAATGC-3' and 5\'-GTTGAACAGAACAAGTGACC-3'; _AT1b_ 5\'-GCCTGCAAGTGAAGTGATTT-3' and 5\'-TTTAACAGTGCTTTGCTCC-3'; _AT2_ 5\'-GCATGAGTGTTGATAGGAT-3' and 5\'-
CCCATAGCTATTGGTCTTCAGCAGATG-3'; G-protein \( \alpha \)-subunit i (G\( \alpha \)) 5'-
ATGGCTACTCAGAGGAGGAGTG-3' and 5'-GTAAGTCTTTGAAGGTGAAGTGTGT-3';
G-protein \( \alpha \)-subunit q (G\( \alpha \)) 5'-CTGAGCGAGGAGGCCAAGGAAG-3' and 5'-
TTGTAGGGAAGCACAACGAC-3'. Denaturation, annealing and amplification
temperatures were 94, 60 (50 for Col3, 55 for MMP2) and 68\(^\circ\)C, respectively.
Amplification products were then separated by agarose gel electrophoresis, bands were
quantified using the Chemilmager Image analysis system (Alpha Innotech Corporation,
San Leandro, CA). Results are expressed as a ratio of the indicated mRNA on the band
intensity of GAPDH. The mean of the Sham group was fixed arbitrarily to 100.

Cardiomyocyte cross-sectional area (CSA) and evaluation of fibrosis:
Sections from paraffin-embedded mid-LV portions were stained using the Trichrome-
Masson staining. Three sections per slide from 10 animals per group were studied for
the evaluation of cross sectional area (CSA) of the cardiomyocytes as previously
described [3]. Total myocardial and sub-endocardial (inner third of the LV wall) fibrosis
were expressed as ratio (%) of blue staining (collagen fibers) over total staining (red and
blue) for each section using an image analysis software (SigmaScan, Systat Software,
Inc. Point Richmond, CA). Three sections per animal were used to evaluate the
proportion of LV sub-endocardial fibrosis. For each section, three pictures were made to
cover completely the inner contour of the LV. Peri-vascular fibrosis was evaluated
separately from the myocardial fibrosis from the same sections. The % of peri-vascular
fibrosis was evaluated similarly (fibrosis = blue) and is expressed as the ratio of total
area of blue staining minus area of the vessel lumen divided by the total fibrosis area plus the blood vessel area.

**Immunohistochemistry:**

The number of fibronectin-positive cells/field was evaluated in LV sections as previously described [3]. The mean results of sham controls were arbitrarily fixed to 100 and the results of the other groups are expressed relatively to these sham controls.

**Immunoblotting:**

Crude LV homogenates were separated by SDS-PAGE. Volumes of samples loaded on gel were corrected for the amount of protein. Immunoblotting was performed as described elsewhere [3]. Membranes were hybridized with primary antibodies directed against Stat 3, phospho-Stat 3, LIF and gp130 (Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA), fibronectin (Sigma Oakville Ont, Canada), phospho-Erk1/2 (Cell Signaling Technology, Beverly, MA). Horseradish peroxidase rabbit Anti-mouse immunoglobulins were used as secondary antibodies (Dako, Mississauga, Ont, Canada). Bands were visualized using a Chemilmager system (Alpha Innotech Corporation, San Leandro, CA).

**Angiotensin I converting enzyme (ACE) activity determination:**

At the time of sacrifice, the hearts were quickly removed, freed from connective tissue and the left ventricle dissected. A piece of the LV was immediately minced, snap-frozen in liquid nitrogen and then kept at -80°C until ACE activity measurements as described previously [2].
Nuclear expression of phospho-c-Jun and phospho-ATF2 in LV sections: Consecutive paraffin-mounted mid-ventricular sections of the left ventricle (5/group) were labeled with either an anti-phospho-c-jun rabbit polyclonal antibody, (1:1000) or an anti-phospho-ATF-2 (1:500) rabbit polyclonal antibody (Cell Signaling Technology, Beverly, MA, USA). Views (3/sections) of the LV wall were digitalized and assigned a random number. Analysis of the labeled sections was then done by two blinded observers using image analysis software (SigmaScan Pro, Version 4, Saugus, MA). Briefly, the number of positively-stained nucleus positive over the total number of nuclei was evaluated for each view and is expressed as a percentage.

Statistical analysis:
Results are presented as mean ± SEM unless specified otherwise. One-way analysis of variance was performed to compare serial data. Statistical significance was set at a p value of 0.05 or less using post-hoc Dunnett’s test. Data and statistical analysis were performed using GraphPad Prism (version 4.02, GraphPad Software, Inc., San Diego, CA).
Results

Hemodynamics (Table 1):

Aortic valve regurgitation severity was similar between groups (results not shown). 1 day post-AR, there was a significant increase in stroke volume and cardiac output that remained after 14 days. There was no significant change in heart rate although there seemed to be a trend towards an increased heart rate after 1 and 2 days. There was no significant difference in arterial blood pressure between groups (not shown).

Left ventricular hypertrophy (Tables 1 and 2)

LV weights were similar in the sham, 1 day and 2 days groups but were significantly increased 14 days post-AR as expected [7,8]. LV wall thickness remained unchanged in all groups. Echocardiographic data showed unchanged end-diastolic diameters (EDD) during the first 48 hours while LV chamber dilatation was clearly established at 14 days. The combination of increased LV weight, unchanged wall thickness and increased LV EDD is typical of eccentric LV hypertrophy. End-systolic diameters were smaller during the first 48 hours reflecting an increased contractile function and fractional shortening. Fractional shortening was back to sham control values after 14 days after a transient increase.

Myocyte hypertrophy (Fig. 1)

The acute effects of AR on cardiomyocyte hypertrophy were evaluated by measuring cardiomyocyte cross-sectional area (CSA). CSA tended to increase after just 2 days of AR (p=0.08) and was significantly increased after 14 days compared to sham controls as shown in figure 1A.
Sub-endocardial and peri-vascular fibrosis (Figs. 1-3)

Sub-endocardial fibrosis tended to increase over the 14 days in AR rats compared to sham controls but without reaching statistical significance (Fig. 1B). Peri-vascular fibrosis was however significantly increased after 14 days (Fig. 1C). Most of the increase in peri-vascular fibrosis occurred in the first two days. Fibrosis in the LV of animals with chronic AR (rats and rabbits) is associated mainly with an increased fibronectin contents [2,14]. We therefore specifically studied this parameter in our animals with acute AR. As illustrated in Figure 2A and C, the number of fibronectin-positive cells/microscopic field was significantly increased 14 days post-AR. Fibronectin protein content evaluated by Western immunoblotting of crude LV homogenates increased sharply as early as 1 day after AR and remained increased afterwards (Fig. 2B and D). Messenger RNA levels of fibronectin were also quickly increased after AR induction (Fig. 3) but returned to normal levels after 14 days. Similar observations were made for the mRNA levels of collagen 1 and pro-MMP2 but not for collagen III.

Renin-angiotensin system (Fig. 4-5)

Angiotensin II (AT) receptors mRNA expression by semi-quantitative RT-PCR is shown in Fig. 4. All three AT receptor subtypes (1A, 1B and 2) mRNA levels were sharply increased in the LV of all AR animals as early as 1 day after the surgery and remained elevated after 14 days. We also studied the mRNA content encoding for the $G_{\alpha q}$ and $G_{\alpha i}$ proteins who are involved in signal transduction of these receptors in cardiomyocytes. We did not observe any significant changes for $G_{\alpha q}$ (not shown) but detected an increase in $G_{\alpha i}$ mRNA levels during the first two days post-AR. LV
angiotensin-converting enzyme (ACE) activity was measured in order to evaluate the capacity of the myocardium to form angiotensin II \textit{de novo}. There was also a trend towards an increase in LV ACE activity in AR animals (38.2, 36.4 and 44.6 nmol/min/mg protein at day 1, day 2 and day 14 respectively) compared to control sham animals (31.6 nmol/min/mg) but this trend did not reach statistical significance (p=0.06, 14 days vs. sham). Circulating ACE activity levels remained stable. We also evaluated the level of activation of downstream signaling pathways associated with the AT receptors in the myocardium namely the Erk, JNK and p35. We did not observe any change in the protein contents of the phosphorylated forms Erk 1/2 by Western blotting (Fig. 5A). However we noticed a sharp decrease as early as one day post-AR in the number of nuclei positively staining for the phosphorylated forms of c-Jun and ATF-2, two transcription factors associated with the stress-related JNK and p38 signaling pathways in LV sections from our animals (Fig. 5B and C).

**Activation of the cardiotrophin-1 signaling pathway in the LV of AR rats (Fig. 6)**

We analyzed another signaling pathway associated with LV hypertrophy namely the cardiotrophin-1 (CT-1) pathway. We did not notice any change in CT-1 protein levels in the LV of acute AR rats (not shown) by immunoblotting. However, we observed an increase in the protein content for the CT1 receptor subunits (gp130/LIF) as well as its downstream effector STAT3 as soon as one day after AR induction. More interestingly, the protein content of phospho-STAT3 was also increased suggesting the activation of this pathway.
Discussion

In the current study we observed rapidly developing macroscopic changes in the LV of our animals. In parallel to these macroscopic adaptations, the acute mechanical and hemodynamic stress associated with acute AR induced a rapid response in the extracellular matrix (ECM) and cardiomyocytes. These early acute macroscopic and microscopic adaptations precede the compensatory remodeling mechanisms that will become established in the chronic phase.

Significant changes were detectable in the LV as early as 24 hours after acute AR. The number of cells producing fibronectin (fibroblasts), fibronectin and collagen I expressions in the myocardium increase rapidly. Pro-matrix metalloprotease 2 (pro-MMP2) mRNA levels also quickly increased suggesting a significant and intense ECM remodeling. After two weeks of AR, chamber dilatation and LVH are well established and we observed a return to normal levels of gene expression of collagen 1 and fibronectin.

Role of the renin-angiotensin-aldosterone system

The mechanisms involved in these adaptations to an acute hemodynamic stress are probably multiple and complex. The renin-angiotensin-aldosterone system has been suggested by others to be one of the significant actors involved in the development of LV hypertrophy caused volume overload [15-19]. In the current study we observed a strong induction in all AT receptors subtypes in our animals and this as early as 24 hours after AR. These increased levels of mRNA expression are sustained for at least the first two weeks. We did not observe such high levels of AT receptors mRNA expression in the more chronic phases (6 months) of the disease [2]. This suggests that the modulation of the AT receptors is more closely related to the early phases of
eccentric LV remodeling. Others have observed little changes in the AT receptors expression one month after the induction of volume overload in a model of aortocaval shunt [20]. We observed a trend (p=0.06) for higher levels of LV ACE activity while others had already noted an increased ACE mRNA expression [15]. Angiotensin II LV levels have also been shown to be increased in an aortocaval fistula model [21]. All these observations support a role for the renin-angiotensin-aldosterone system in the events leading to eccentric LV remodeling in acute volume overload including acute AR. A link between the renin-angiotensin system and LV ECM remodeling has also been previously suggested. An angiotensin II infusion for 24 hours in rats is enough to induce LV fibroblast proliferation as well as fibronectin and collagen I and IV production [22]. This link has also been established in humans bearing an aortic valve disease (aortic regurgitation or stenosis) in whom a rise in ACE activity was observed in LV biopsies as well as increases in mRNA expression of collagens I and III [23]. We and others have demonstrated the benefits of blocking the renin-angiotensin system to reduce LV remodeling and to preserve function in animal models of chronic AR [2,24,25]. The beneficial effects of ACE inhibitors in rats with chronic AR seemed to be less related to a load reduction than to a direct effect on LV renin-angiotensin system [2]. The effects of ACE inhibitors in humans with chronic AR are however still debated and remain controversial. More data will be necessary before a strong conclusion can be drawn from clinical studies.

**Pro and anti-hypertrophic pathways involved in acute AR**

Our results suggest that, at least during the acute phases of AR, both pro and anti-hypertrophic mechanisms compete as the LV tries to mitigate the effects of the pro-
hypertrophic stimuli to minimize permanent remodeling. Our results suggest that two stress-related signaling pathways (JNK and p38) could indeed be inhibited in the LV of acute AR animals. While it is accepted that the Erk1/2 pathway is linked to the pro-hypertrophic response, the respective role of the JNK and p38 pathways are more controversial [26]. Data from studies made in cultured cardiomyocytes support an action of ERK, JNK and p38 to promote hypertrophic growth [27-29]. Results obtained from animal models suggest that p38 signaling does not promote a hypertrophic response and could be anti-hypertrophic in adult hearts [30]. JNK signalling usually plays an anti-hypertrophic role [31]. Again, very little is known about the role of these pathways in eccentric hypertrophy from volume overload. The BMK1/Erk5 pathway would also have been interesting to study since this pathway has been associated with the serial rearrangement of cardiac myocytes as seen in AR [32]. Recently, this pathway has been associated with the transduction of cardiotrophin-1 in cultured cardiac myocytes [33]. In our study we observed an activation of the STAT3 pathway accompanied with an increase of the CT-1 receptor content. The cardiotrophin-1 pathway is believed to be protective while pro-hypertrophic [34]. Raised circulating levels of CT-1 have been observed in patients with valvular regurgitation [35]. Moreover, the CT-1 pathway is believed to promote the rearrangement of cardiac myocytes in series as seen in AR [36].

**Acute versus chronic AR**

The hemodynamics of acute and chronic AR are different. Acute AR in humans is a condition that may require urgent valve replacement surgery in order to keep the patient alive [37]. However, surgery in patients with severe acute AR can sometimes be delayed if AR is well tolerated. Although it is commonly believed that patients with chronic AR
have progressively and slowly evolved from mild to moderate to severe regurgitation, there exists no hard data to support this affirmation and the time-course of the evolution from mild to severe AR is unknown. In our rat model, the majority of the animals managed to pull through the acute phase and survive into the chronic phase of the disease without any active pharmacological treatment or hemodynamic support [7,8]. The data of the current study show that significant ECM and myocyte remodeling is already present as early as 24 hours after AR. Peri-vascular fibrosis is clearly present and established after only 2 weeks. These acute changes may be essential for the LV to adapt to AR and to maintain normal hemodynamics. However, a negative side may also be associated with this rapidly appearing LV hypertrophy, fibrosis and ECM remodeling. The potential reversibility of these cellular abnormalities is not known.

**Study limitations:**

Of course, the data obtained from an animal model may not perfectly reflect how the human heart will respond to acute AR. However, serial LV biopsies of humans with acute AR will never be obtained in clinical trials for obvious technical and ethical reasons. Animal data are therefore the only alternative and our best opportunity to learn more about the acute adaptations of the heart to AR.

**Conclusions:**

In conclusion, we demonstrated that significant changes are present in the LV as early as 1 day after acute AR occurs. We report an early activation of the renin-angiotensin system and of the CT-1/STAT3 pathway as well as the stimulation of cardiac myocyte hypertrophy and significant alterations of the ECM in response to acute AR in rats.
Subjects with acute AR endure an intense acute stress that does not only affect their hemodynamics but also induces acute adaptations in their myocardium very early in the process. Whether these adaptations are beneficial and/or reversible is unknown.
Acknowledgements

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Reference List


Figure legends:

**Figure 1:** LV myocyte cross-sectional area (CSA) (A), sub-endocardial (B) and peri-vascular fibrosis (C) in AR rats compared to sham controls. Results are expressed as mean ± SEM (n=10 animals per group). d = days post surgery for AR. *: p<0.05 vs. sham.

**Figure 2:** Number of fibronectin-positive cells (A, C) and fibronectin protein content (B, D) expressed in arbitrary units (AU) in the LV of AR rats compared to sham controls. Results are expressed as mean ± SEM (n=10 animals per group). *: p<0.05 and **: p<0.01 vs. sham.

**Figure 3:** Semi-quantitative evaluation of fibronectin, collagen type I (Coll), collagen Type III (Col III) and proMMP2 mRNA levels by RT-PCR. Results are expressed in arbitrary units (AU) as mean ± SEM (n=10/group). *: p<0.05 and **: p<0.01 vs. sham.

**Figure 4:** Semi-quantitative evaluation of AT1a, AT1b, AT2 receptors and Gαi protein mRNA levels by RT-PCR. Results are expressed in arbitrary units (AU) as mean ± SEM (n=10/group). *: p<0.05 and **: p<0.01 vs. sham.

**Figure 5:** Modulation of several MAP kinase pathways in the LV of AR rats compared to sham controls. (A) Erk 1/2 LV protein content. (B) Examples of p-c-Jun (left) and p-ATF2 (right) immunostaining in mid-wall LV sections from AR rats. Arrows: positively stained
nucleus. Magnification: x200. (C) Proportion of positively stained nuclei by field for p-c-Jun and p-ATF2 transcription factors in LV sections. Results are expressed mean ± SEM (n = 5/group). *: p<0.01 vs. sham.

**Figure 6:** Protein contents of members of the cardiotrophin-1 receptor (gp130/LIFr)/STAT3 signaling pathway in the LV of AR rats compared to sham controls as evaluated by immunoblotting.
Table 1. Hemodynamics and LV mass.

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<th>Parameters</th>
<th>Sham</th>
<th>24 hours</th>
<th>48 hours</th>
<th>14 days</th>
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<tr>
<td>Heart rate, bpm</td>
<td>240 ± 9</td>
<td>255 ± 8</td>
<td>253 ± 5.0</td>
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<td>Stroke volume, ml</td>
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<td>0.3 ± 0.01*</td>
<td>0.4 ± 0.02**</td>
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<tr>
<td>Cardiac output, ml/min</td>
<td>49 ± 5</td>
<td>71 ± 4**</td>
<td>74 ± 3**</td>
<td>90 ± 6**</td>
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<td>LV weight, mg</td>
<td>862 ± 16</td>
<td>871 ± 38</td>
<td>869 ± 30</td>
<td>999 ± 33*</td>
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</table>

Values are expressed as mean ± SEM (n=10). *: p<0.05 and **: p<0.01 vs. sham.
Table 2. Echocardiographic data.

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<th>48 hours</th>
<th>14 days</th>
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<td>EDD, mm</td>
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<td>8.4 ± 0.1</td>
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<tr>
<td>ESD, mm</td>
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<td>SW, mm</td>
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<td>1.6 ± 0.04</td>
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<tr>
<td>PW, mm</td>
<td>1.6 ± 0.05</td>
<td>1.7 ± 0.03</td>
<td>1.5 ± 0.05</td>
<td>1.7 ± 0.05</td>
</tr>
<tr>
<td>FS (%)</td>
<td>40.2 ± 1.2</td>
<td>50.0 ± 3.4*</td>
<td>48.0 ± 1.7*</td>
<td>42.0 ± 2.0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM (n=10). EDD: end-diastolic diameter; ESD: end-systolic diameter; SW: septal wall thickness; PW: posterior wall thickness; FS: fractional shortening *: p<0.05 vs. sham.