

Left ventricular response to sustained volume overload  
from chronic aortic valve regurgitation in rats

Short title: Chronic aortic regurgitation in rats

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## **Abstract**

**Objectives:** Aortic regurgitation (AR) induces left ventricular (LV) eccentric hypertrophy in response to chronic volume overload. Patients suffering from this disease often remain asymptomatic for decades before progressive LV dysfunction develops silently. Because of this slow evolution, large clinical trials with long term follow-up on subjects with chronic AR are hard to perform. To overcome this problem, animal models have been developed in the past but results were very heterogeneous.

**Methods:** Helped by echocardiography, we refined a known technique to induce homogenous degrees of severe AR in Wistar-Kyoto rats. The effects on LV function without treatment and with nifedipine (25 mg/kg daily) (a drug currently recommended in humans with chronic AR) were evaluated by echocardiography.

**Results:** Over six months, non-treated animals developed progressive LV dilatation and eccentric hypertrophy, characteristic of chronic LV volume overload. The animals also developed progressive LV systolic dysfunction mimicking closely the evolution of the disease in humans. Abnormal filling parameters were also detected in the majority of animals. Systolic and diastolic abnormalities were prevented but only partially in the group treated with nifedipine.

**Conclusion:** This model can be used to study chronic AR and LV dysfunction associated with the disease. Nifedipine seems to protect the LV against chronic volume overload but only partially. Treatment strategies currently used in humans deserve further investigation.

**Key Words**

Aortic valve, insufficiency, animal model, rat, echocardiography, volume overload, heart failure, remodeling

## **Introduction**

Severe aortic regurgitation (AR) is a chronic disease that results in progressive left ventricular (LV) dilatation and eccentric hypertrophy from chronic LV volume overload. Patients suffering from chronic AR often remain asymptomatic for decades before systolic dysfunction and, eventually, heart failure develops<sup>1-7</sup>.

Because of this slow and silent evolution there have been few clinical trials on chronic AR comparatively to other more prevalent cardiovascular diseases. Most studies were performed only on a limited number of subjects, often in a non-blinded manner without a placebo controlled-group or comparison with normal subjects. There is a clear lack of solid fundamental and clinical research focusing on chronic AR and volume overload cardiomyopathy.

Currently, treatment of chronic AR consists either of calcium channel blockers of the dihydropyridine family such as nifedipine or angiotensin converting enzyme inhibitors. There is very little data to guide physicians as to which drug should be given and at what dosage in order to maximize the protection against volume overload in those patients. To overcome this problem, other investigators have developed animal models of the disease<sup>8-24</sup>. Unfortunately, most animals in those experimental models either failed to develop significant left ventricular dysfunction over time thereby raising doubts as to the severity of their valvular dysfunction or developed some left ventricular dysfunction but only after a long follow-up period making the model less attractive to use. We reported recently a more reliable method to induce severe chronic AR in rats using echocardiographic guidance and monitoring<sup>25</sup>. In the present study, we describe the progressive deterioration of

left ventricular systolic function and diastolic filling pattern in those animals over a follow-up period of 26 weeks. We also evaluate the effects of nifedipine, a drug routinely used in the treatment of chronic AR in humans, on the progression of LV dysfunction in this animal model.

## **Methods**

*Animals:* 53 male Wistar rats (body weight 325-375g) were used in this protocol.

*Acute AR:* The animals were randomly divided in 3 study groups of 8 to 10 subjects as follows: #1: acute AR studied 24 hours after surgical procedure (described below), #2: acute AR studied 48 hours after surgical procedure and #3: AR studied 2 weeks after surgical procedure (transition phase from acute to chronic)

*Chronic AR:* The animals were divided in 3 study groups of 8-10 subjects as follows: #1: normal controls studied during 26 weeks (sham-operated), #2: chronic non-treated AR studied throughout 26 weeks following the surgical procedure and #3: chronic AR treated with 25 mg/kg/day of nifedipine in food for 24 weeks starting two weeks after AR induction (total follow-up of 26 weeks). This protocol was approved by the Université Laval's animal protection committee and was consistent with the recommendations of the Canadian Council on animal care.

*Surgical induction of severe aortic regurgitation:* The animals were put under general anesthesia with an intraperitoneal injection of 0,1mg/kg of ketamine and 0,75 mg/kg of xylazine. The technique for aortic valve perforation and

echocardiographic measurements have been described previously<sup>25</sup>. Briefly, the right internal carotid artery was exposed and cannulated. Continuous monitoring of systemic blood pressure was done via this route by standard fluid-filled catheter coupled to a pressure transducer. ECG was monitored via limb leads throughout the procedure. Then, under continuous echocardiographic guidance, an 18-gauge epidural catheter was advanced towards the aortic valve in a retrograde manner. The sonographer guided the position and the advance of the catheter in the aorta while it was pushed through a leaflet of the aortic valve into the LV. The resulting tear in one of the aortic valve leaflets induced 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. Significant aortic regurgitation was considered to be present when we obtained a 30% acute drop in diastolic pressure as previously published, combined with the echocardiographic criteria listed in the next section. Animals were closely observed in the first hours after surgery for any sign of respiratory distress suggestive of acute heart failure. They were weighed daily for detection of exaggerated weight gain again suggestive of pending heart failure.

*Echocardiography:* All studies were performed with a transthoracic 12 MHz phased-array transducer coupled to a Sonos 5500 echographer (Philips Medical Imaging, Andover, MA). Studies were recorded on standard VHS videotapes for off-line analysis. Prior to the surgical procedure, a complete echocardiographic examination was performed on each animal while under sedation. Parasternal

long and short axis views as well as apical four chamber view were obtained. Aortic, left atrial, LV outflow tract diameter, LV diastolic and systolic dimensions, septal and posterior wall thickness were measured by M-mode and two-dimensional imaging. Color-Doppler evaluation of the four valves was done. Using pulsed-Doppler, aortic flow and stroke volume were calculated. During the procedure, the parasternal views were used to guide catheter location and movement and to determine which leaflet was perforated.

Severe AR was considered to be present if the following criteria were found on echocardiographic evaluation: 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); acute increase in left ventricular dimensions; 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. Ejection fraction was calculated by the method described by Quinones et al.<sup>26</sup>. Diastolic filling pattern was evaluated from the E wave/A wave ratio of the mitral pulsed Doppler flow at the tip of mitral leaflets. Normality of diastolic parameters was set at the mean  $\pm$  SD as derived from data previously calculated in a normal cohort of rats (i.e. an E/A ratio ranging from 1,4 to 2,7 was considered normal (Table 2)). This range is consistent with published data by other groups. Relative wall thickness was calculated as the ratio of the sum of diastolic septal and posterior wall

thickness to left ventricular diastolic diameter. Left ventricular mass was calculated with the following formula:

$$\text{LV mass} = 1.04 \times (\text{DD} + \text{PW} + \text{SW})^3 - \text{DD}^3$$

Where DD = diastolic diameter, PW = posterior wall thickness and SW = septal wall thickness.

All other standard echographic measurements were made according to the latest recommendations of the American Society of Echocardiography<sup>28-29</sup>. The data from five cardiac cycles were measured and averaged.

A complete echocardiogram as described above was performed under anesthesia at the following time points: preoperatively, immediately postoperatively, at 24 hours (acute study only), 48 hours (acute study only), 2 weeks, 8 weeks and 26 weeks after AR induction (total of 24 weeks of treatment for nifedipine-treated animals).

#### *Statistical analysis:*

Results are presented as mean  $\pm$  SEM unless specified otherwise. Comparisons between two samples were made using paired or unpaired *t* tests whenever appropriate. Linear regression analysis was performed to assess correlations and regression coefficients compared. Repeated measures analysis of variance was performed to compare serial data. Statistical significance was set at a *P* value of 0,05 or less. Data and statistical analysis were performed using GraphPad Prism version 3.02 for Windows, GraphPad Software, (San Diego California USA). Inter



and intra-observer variability for echocardiographic measurements was assessed on 10 randomly chosen echocardiographic studies.

## **Results**

### *Survival rate:*

All animals in the control group and in the non-treated AR group survived until the end of the protocol. One animal died of acute heart failure in the nifedipine-treated group. None of the surviving animals required medical treatment for heart failure.

### *Acute phase of aortic regurgitation (figure 1)*

Left ventricular dimensions: Figure 1 depicts the evolution of LV dimensions, mass and ejection fraction in the acute period up to 2 weeks. In the first 48 hours after surgery, the diastolic dimension (Fig. 1A) remains unchanged while the systolic diameter decreases (Fig 1B) resulting from a hypercontractile state also commonly seen in severe acute aortic regurgitation in humans. In fact, left ventricular ejection fraction abruptly increases in the acute phase up to 48 hours after surgery (Fig. 1E). Thereafter, this hypercontractile state recedes and, after 14 days, ejection fraction returns back to normal values. At 14 days, both diastolic and systolic dimensions start to increase above normal levels as a result of the sustained volume overload. Interestingly, at 24 hours of AR, an apparent and unexplained increase in wall thickness is observed (Fig. 1C and D). At 48 hours, this phenomenon recedes.

The evolution of LV mass is also summarized in Figure 1. At 14 days, the eccentric pattern of hypertrophy is already established as illustrated by the significant decrease in relative wall thickness (Fig. 1F). At sacrifice, the left ventricle of each animal was weighed and the value corrected for their body weight. As illustrated in panel H of Figure 1, the LV mass is significantly higher 2 weeks after surgery confirming that hypertrophy had developed.

*Chronic phase of aortic regurgitation (Figures 2, 3 and Table 1)*

*Hemodynamic parameters:* Stroke volume and cardiac output increases significantly in AR animals compared to controls (51% and 54% increase respectively,  $p < 0,05$ ). Pulse pressure also significantly increases as expected in chronic AR (controls:  $51 \pm 5$  mmHg vs AR:  $66 \pm 6$  mmHg,  $p < 0,05$ ). There is no significant change in heart rate.

*Left ventricular dimensions:* After the early rapid dilatation occurring in the first two weeks, the left ventricle continues to dilate at an intermediate rate over the following 6 weeks followed thereafter by a much slower rate of dilatation (Fig. 2B).

*Wall thickness:* Wall thickness increases as the animal age but nevertheless remain comparable to normal controls (Fig. 2C and table 1). The relative thickness however, compared to the diastolic diameter of the cavity, is strongly reduced (Fig. 2D). Since the dimensions of the LV cavity increase as described above, total indexed LV mass consequently increases (Fig. 2E) even if the animals gain weight during the course of the protocol (Fig. 2A). This is characteristic of eccentric hypertrophy seen in chronic volume overload such as in chronic aortic

regurgitation. Left ventricular mass measured by echocardiography correlates very well ( $r = 0.86$ ) with the anatomic measurement performed on the explanted hearts at the end of the protocol (Fig. 2F).

*Comparison with normal controls (Table 1):* Results comparing normal animals to those with severe AR are illustrated in Table 1. Weight gain over 6 months is similar in the two groups. As expected, both LV end-diastolic (EDD) and end-systolic (ESD) diameters are clearly larger in AR rats while wall thickness is left unchanged. LV wet tissue weights corrected or not for body mass are increased in chronic AR rats as expected. Lung weight was used as an index for pulmonary congestion and thus of heart failure. Although this parameter tends to be increased in chronic AR rats, it does not reach statistical significance ( $p=0.06$ ).

In Figure 3, we have corrected the LV dimensions obtained by echocardiography for the body weight of the animal in order to discriminate between the changes due only to aging and body weight gain and those resulting from volume overload.

Even after this correction, end-diastolic diameter index (EDDi) and end-systolic diameter index (ESDi) are significantly higher than normal controls.

*Left ventricular systolic function:* A rapid decrease in ejection fraction and fractional shortening occurs between weeks 2 and 8 followed by a slower but still constant deterioration until 26 weeks of follow-up (Fig. 4). Overall, ejection fraction in chronic AR animals decreases by almost a third ( $31.5 \pm 2.7\%$ ) during the course of the protocol. Animals thus develop abnormal systolic function defined as an ejection fraction of less than 50% at the end of the protocol (see Table 1).

*Left ventricular diastolic filling pattern:* Table 2 depicts the evolution of left ventricular diastolic filling pattern in rats with severe AR. Rats were classified as shown in Table 2 and Figure 5. As soon as 2 weeks after AR induction, two subjects already display significant changes in their E/A ratio. After 24 weeks of AR, 5/9 rats have a severely abnormal diastolic filling pattern suggestive of restrictive filling of the ventricle and high filling pressures. Of the four remaining animals, only one has maintained normal diastolic parameters during the course of the protocol at every time point (Fig. 5, #7). The left atrial diameter significantly increases in untreated AR animals compared to normal controls (table 1). There is no significant mitral valve regurgitation to explain this finding. Animals treated with nifedipine display intermediate LA dimensions. Examples of normal and abnormal diastolic mitral flow pattern are given in Figure 6.

*Effect of nifedipine treatment:* As depicted in table 1, nifedipine treatment does not prevent LV dilatation nor hypertrophy. LV systolic diameter increases less but this trend does not reach statistical significance. LV ejection fraction and fractional shortening however are significantly better than in the non-treated group, although remaining lower than in normal animals. Diastolic filling pattern is also better at the end of the protocol in the nifedipine-treated group compared to normal and non-treated animals as shown in table 3. Indeed, 6/8 animals display a normal E/A wave ratio at the end of the protocol and only 2 develop a decrease in their ratio whereas all but one animal develop a diastolic abnormality in the non treated group. Up to week 8, diastolic parameters are normal in all nifedipine-treated rats.

Intra and inter-observer variability for echocardiographic measurements were 5% and 8% respectively.

## **Discussion**

We describe in this paper the natural and progressive evolution of volume overload cardiomyopathy in a rat model of chronic AR. With the help of echocardiographic guidance <sup>25</sup>, we were able to induce consistently severe AR and significant systolic dysfunction (defined as an ejection fraction below 50%) with eccentric hypertrophy after 26 weeks in our animals.

We used the same echocardiographic criteria that are routinely used clinically in humans to assess the severity of aortic regurgitation in our animals. Although these criteria were developed for humans, the significant increase in pulse pressure, stroke volume and cardiac output combined with progressive left ventricular dilatation in our rats confirms the severity of volume overload and the reliability of these echocardiographic criteria in our animal model.

This model has been used by others in the past to study volume overload in the rat but, unfortunately, those previous studies were mostly limited to the acute phase of the disease and protocols were rarely extended past 12 weeks of follow-up <sup>9;10;12-14;18-20</sup>. Left ventricular dysfunction was therefore rarely attained in those studies.

Results obtained from those studies on acute regurgitation are irrelevant to chronic AR. In the present study, echocardiographic guidance allowed us to obtain a reliable quantification of the degree of aortic regurgitation and, therefore, our study group was very homogeneous compared to previous studies. Other animals such

as rabbits and dogs have been used to try to reproduce AR volume overload cardiomyopathy but the level of LV dysfunction attained in these models was often mild or unpredictable<sup>11;13;15-17;21-24;30;31</sup>. Excessively long follow-up periods were needed before some degree of LV dysfunction occurred making these models unattractive to use for large protocols. For example, one of the most promising models of rabbit aortic regurgitation produced moderate degrees of LV dysfunction but only in 50% of the animals and after a relatively long follow-up period of more than two years<sup>15</sup>. In our study, we have reached significant levels of LV dysfunction in all our subjects and after a much shorter follow-up period (6 months) making our model much more attractive to use. Arterio-venous shunting has also been used for decades as another means of creating chronic volume overload in animals<sup>32-42</sup>. However, arterio-venous shunting results in bi-ventricular volume overload and does not reflect a similar pathophysiologic situation as seen in severe AR. Moreover, significant left ventricular systolic dysfunction rarely occurs in animal models using this type of volume overload<sup>32</sup>.

Interestingly, we noticed the occurrence of an abnormal filling pattern in all but one of the animals after 6 months of follow-up as depicted by the abnormal E/A ratio seen on the mitral Doppler flow as well as progressive left atrial dilatation. These findings are suggestive of elevated left ventricular filling pressures. In rats and mice, decreased E/A ratios as well as high (>2) E/A ratios have been well correlated with increased left ventricular filling pressures<sup>43-51</sup>. Compared to systolic function, diastolic function has received little attention in humans suffering from severe aortic regurgitation<sup>52,53</sup>. However, it has been suggested that

abnormalities in diastolic function may influence the symptomatic status as well as the prognosis of subjects with severe aortic regurgitation as in other cardiac diseases such as myocardial infarction, congestive heart failure, diabetes and hypertension<sup>54-59</sup>. The occurrence of left ventricular filling abnormalities in our animals therefore deserves further attention and investigation.

Nifedipine has been shown in humans with AR to help stop and even partially reverse LV dilatation and hypertrophy and help keep ejection fraction in the normal range<sup>60-63</sup>. In our model, nifedipine had little protective effects on LV dilatation or hypertrophy (except for a trend to preserve systolic diameter) but kept LV ejection fraction and diastolic function closer to normal range. Other vasodilators as well as higher doses of nifedipine need to be tested and compared in order to better understand this phenomenon.

Conclusion: Over the 6 months follow-up, we have encountered the whole spectrum of volume overload cardiomyopathy in our animals ranging from a normal sized, non hypertrophied hypercontractile left ventricle in the acute phase (also seen in humans with acute severe AR) to severely dilated, eccentrically hypertrophied and hypocontractile left ventricles in the end of the chronic phase (also seen in humans with decompensated AR volume overload cardiomyopathy). This model therefore allows the study of the disease in all its phases in a relatively short period of time compared to previously described experimental models. It can be used to study the development of volume overload cardiomyopathy that accompanies severe chronic aortic regurgitation as well as potential treatment strategies for patients suffering from this disease.

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### **Figure legends:**

Figure 1: Evolution of echocardiographic parameters after acute aortic regurgitation. In each table, left column depicts the results obtained in the animals 24 hours after acute aortic regurgitation, middle column depicts the results after 48 hours and right column after 14 days. Results are presented as mean  $\pm$  SEM (n=9-10). Panel A: % change in diastolic diameter from baseline; Panel B: % change in systolic diameter from baseline; Panel C: % change in septal wall thickness from baseline; Panel D: % change in posterior wall thickness from baseline; Panel E: % change in ejection fraction from baseline; Panel F: % change in relative wall thickness (RWT) from baseline; Panel G: % change in body weight and Panel H: % change in left ventricular wet tissue mass indexed for body weight. \*:  $P < 0.05$  and \*\*:  $P < 0.01$  vs before AR induction.

Figure 2: Evolution of echocardiographic parameters in the chronic phase of severe aortic regurgitation. In each graph, results are shown from baseline (left) until 26 weeks (right) after induction of aortic regurgitation. Panel A: Body weight; Panel B: Evolution of diastolic (circles) and systolic (squares) dimensions; Panel C: Evolution of septal (circles) and posterior wall (squares) thickness; Panel D: Evolution of relative wall thickness; Panel E: Evolution of left ventricular mass indexed for animal body weight and Panel F: Correlation between left ventricular

mass measured by echocardiography (Echo) and mass measured directly from explanted hearts (Real) in mg.

Figure 3: Comparison of the evolution of left ventricular dimensions indexed for body weight between sham-operated (squares) and chronic aortic regurgitation (circles) animals after 24 weeks of parallel follow-up. All results are expressed as the mean  $\pm$  SEM. Panel A: Indexed diastolic diameter (EDDi) Panel B: Indexed systolic diameter (ESDi). \*:  $P < 0.05$  and \*\*:  $P < 0.01$  vs Week 0. Indicated values of p are for comparison of between sham-operated and chronic AR animals at week 26.

Figure 4: Evolution of systolic function in the chronic phase of severe aortic regurgitation. All results are expressed as the mean  $\pm$  SEM. Ejection fraction and inset: fractional shortening (%).

Figure 5: Evolution of the diastolic function in untreated AR rats. E/A ratio was evaluated at the indicated time before and after AR induction for each rat (# 1-9).

Figure 6: Examples of diastolic mitral flow patterns in sham-operated or AR rats. Upper panel: normal or pseudo normal E/A (grade 2) ratio. Middle: E/A ratio above 2.7 with rapid E wave deceleration time also referred to as restrictive pattern or grade 3. Lower: Inverted E/A ratio (grade 1).

## Tables

Table 1. Comparison of echocardiographic parameters between normal control and chronic aortic regurgitation after 24 weeks of parallel follow-up. The effect of nifedipine treatment starting 2 weeks after AR induction is also presented. All results are expressed as the mean  $\pm$  SD. \*:  $P < 0.05$  vs sham-operated animals and †:  $P < 0.05$  vs untreated AR animals.

	Sham	AR	AR + nifedipine
Body weight, g	655.3 $\pm$ 69.1	711.4 $\pm$ 61.3	654.4 $\pm$ 92.0
Heart rate, bpm	246.0 $\pm$ 23.1	240.8 $\pm$ 27.4	258.6 $\pm$ 19.8
End-Diastolic Diameter, mm	8.8 $\pm$ 1.1	11.8 $\pm$ 1.0*	11.4 $\pm$ 1.5*
End-Systolic Diameter, mm	5.0 $\pm$ 0.9	8.5 $\pm$ 1.1*	7.5 $\pm$ 1.0*
Septal Wall, mm	1.8 $\pm$ 0.2	1.7 $\pm$ 0.2	1.6 $\pm$ 0.1
Posterior Wall, mm	1.9 $\pm$ 0.2	1.9 $\pm$ 0.3	1.8 $\pm$ 0.2
Relative Wall Thickness	0.41 $\pm$ 0.06	0.30 $\pm$ 0.04*	0.30 $\pm$ 0.04*
Left Atrial Diameter, mm	5.5 $\pm$ 0.8	7.4 $\pm$ 1.9*	6.8 $\pm$ 1.2
LV mass (echo), mg	1271 $\pm$ 261	2075 $\pm$ 118*	1845 $\pm$ 481*
LV mass index (echo), mg/g	1.95 $\pm$ 0.37	2.94 $\pm$ 0.55*	2.80 $\pm$ 0.86*
LV weight, mg	966 $\pm$ 288	1631 $\pm$ 341*	1616 $\pm$ 278*
LV weight index, mg/g	1.62 $\pm$ 0.17	2.32 $\pm$ 0.42*	2.52 $\pm$ 0.62*
Lungs weight, g	1.87 $\pm$ 0.20	2.44 $\pm$ 0.86	2.13 $\pm$ 0.64
Ejection fraction (%)	67.5 $\pm$ 7.6	48.4 $\pm$ 6.3*	56.4 $\pm$ 6.2*†
Fractional shortening	42.0 $\pm$ 6.5	28.3 $\pm$ 4.4*	34.3 $\pm$ 4.9*†

Table 2. Diastolic function in control rats. Diastolic function was evaluated as described in the Methods section. Results are the means of 9 normal rats prior AR induction.

PARAMETER	Mean $\pm$ SD
E wave	64.6 $\pm$ 6.62 cm/s
A wave	32.0 $\pm$ 6.54 cm/s
E/A	2.1 $\pm$ 0.64
E Slope	1284 $\pm$ 261.4

Table 3. Evolution of the diastolic function in sham-operated and AR rats. E/A ratio was evaluated at the indicated time before and after AR induction for each rat. We arbitrary fixed the normal range for E/A ratio from results of Table 1. Bold characters indicate normal range for E/A ratio.

E/A	Sham (n=9)		AR (n=9)		AR + nifedipine (n=8)	
	8	26	8	26	8	26
<1	0	0	1	0	0	0
1.0-1.4	0	0	3	2	0	2
<b>1.4-2.7</b>	<b>9</b>	<b>9</b>	<b>4</b>	<b>2</b>	<b>8</b>	<b>6</b>
>2.7	0	0	1	5	0	0



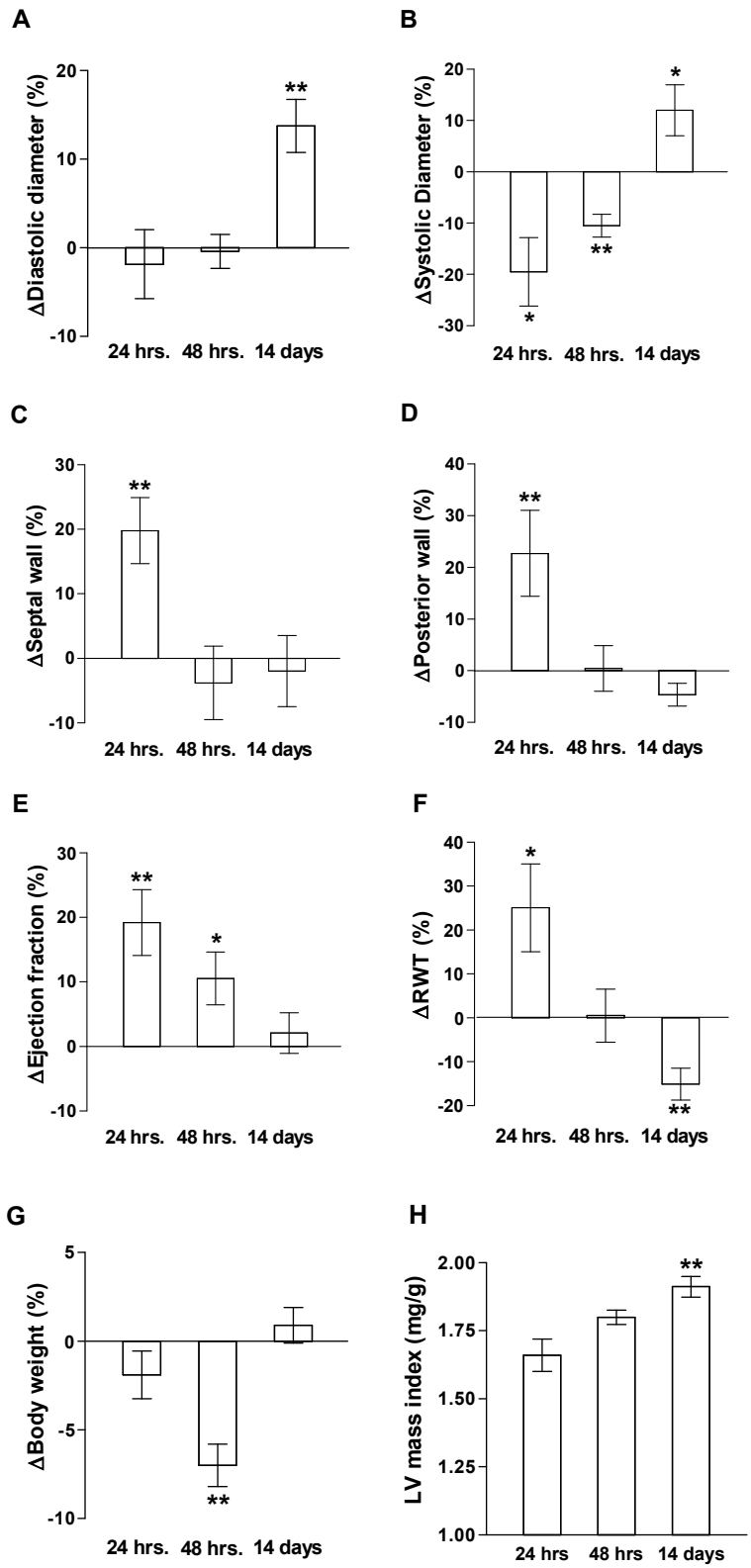


Figure 1, Plante et al.

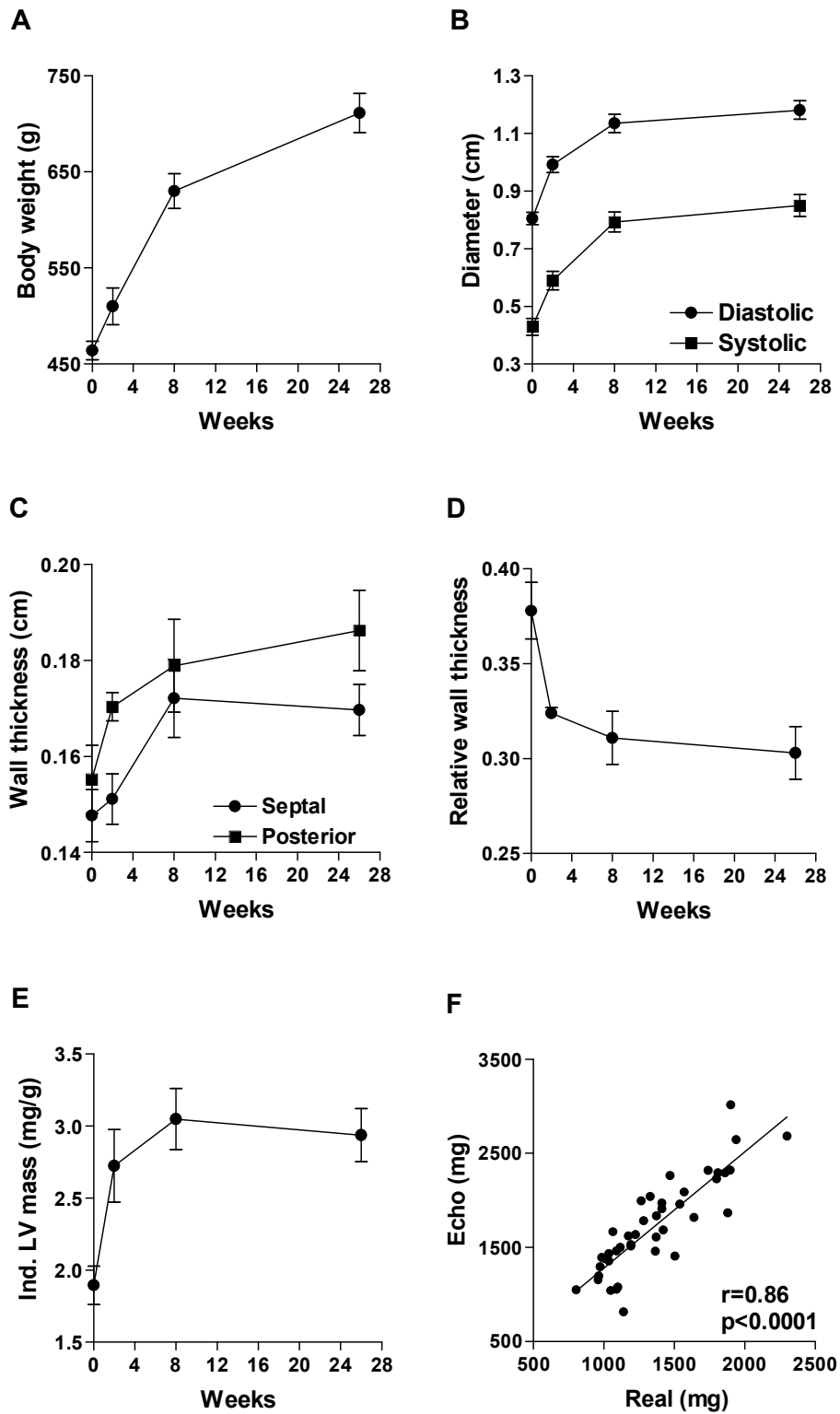
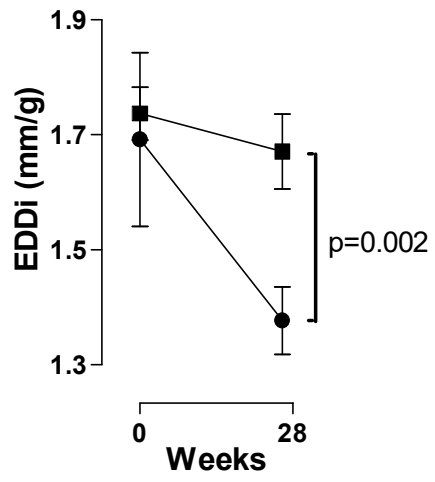


Figure 2.P Plante et al.

**A**



**B**

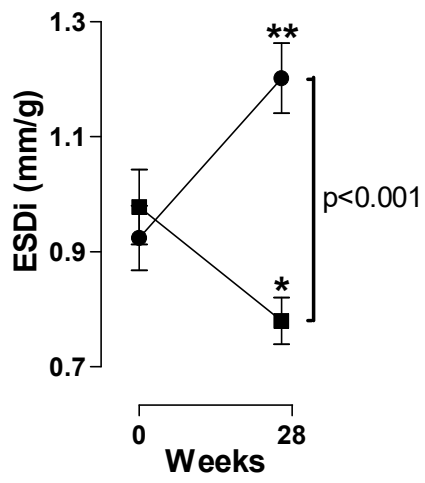


Figure 3, Plante et al.

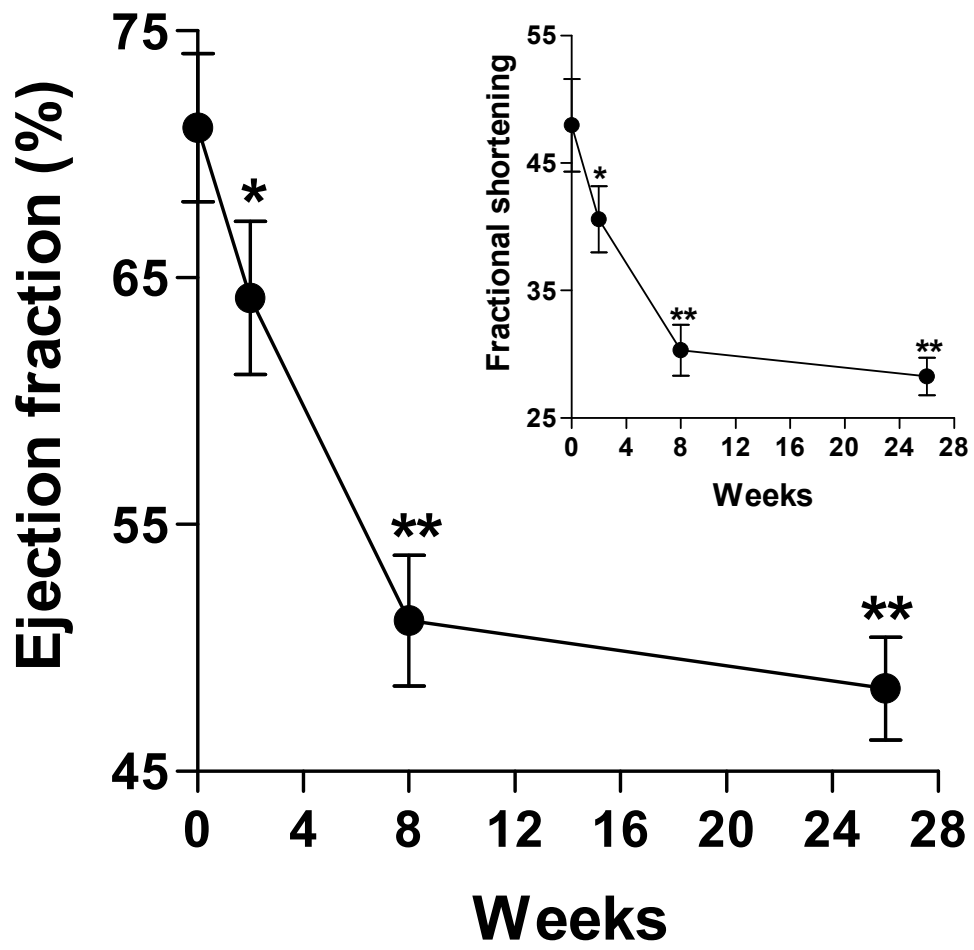
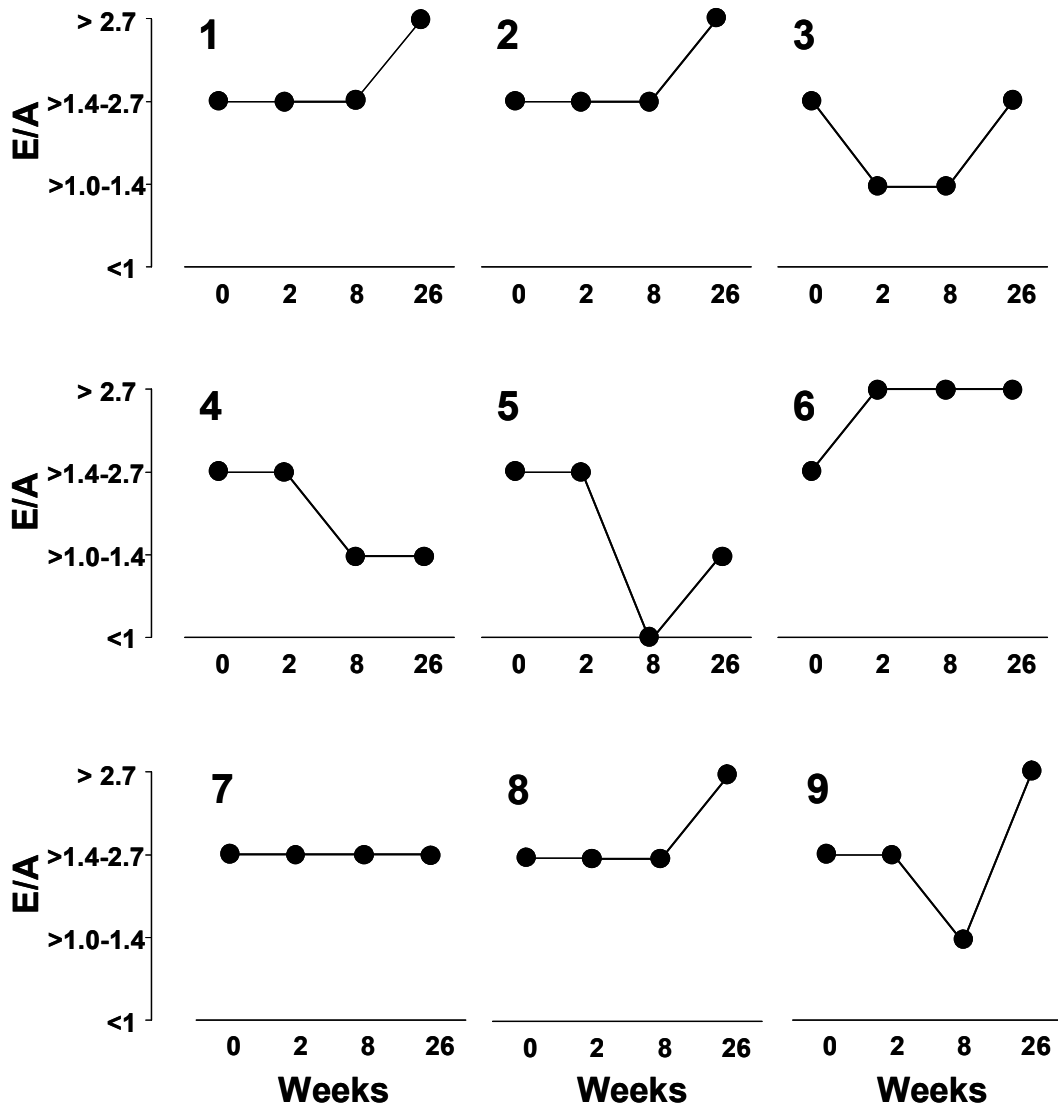


Figure 4, Plante et al.

Figure 5, Plante et al.



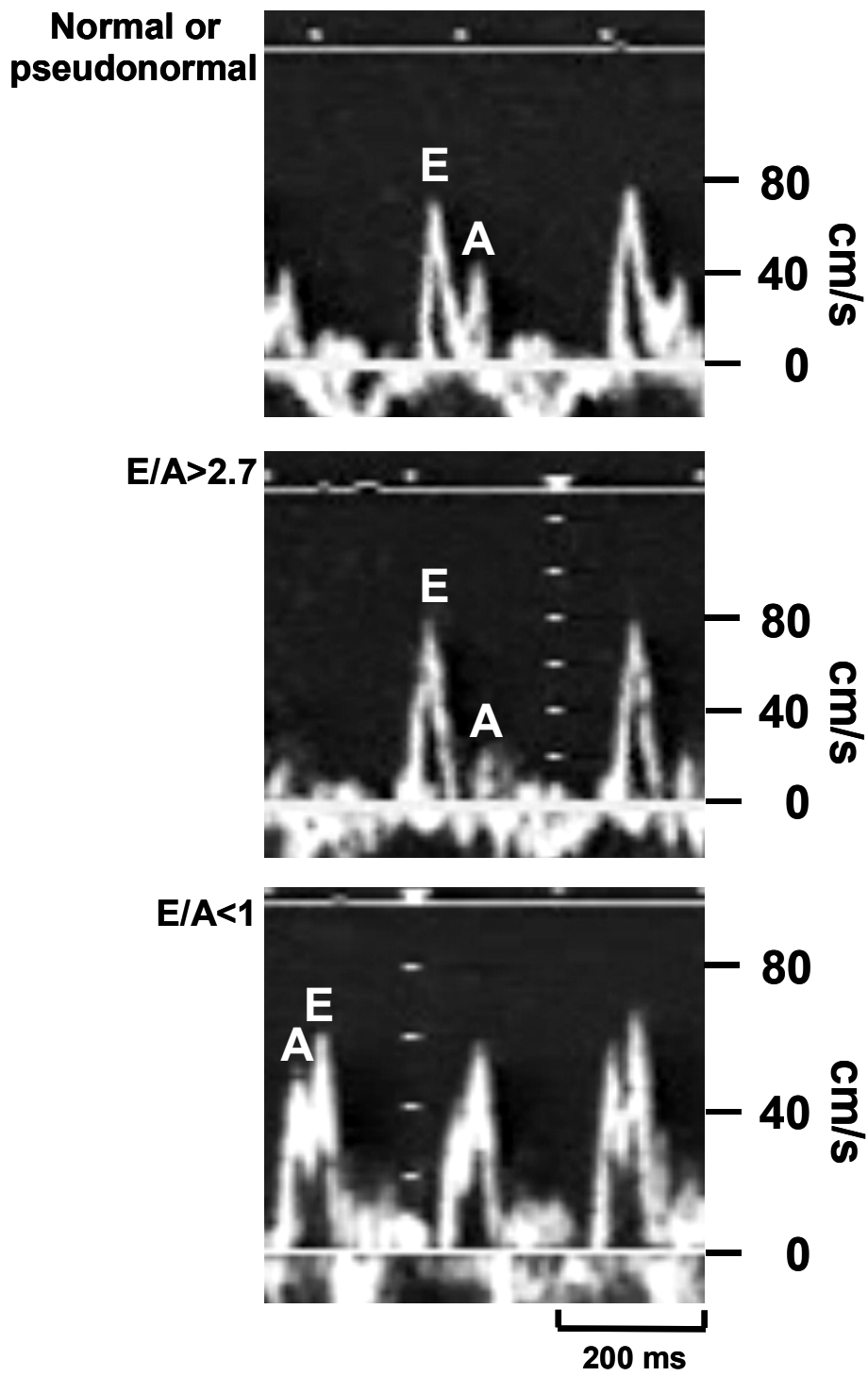


Figure 6, Plante et al.