

Improved Systolic Ventricular Function With Normal Myocardial Mechanics in Compensated Cardiac Hypertrophy

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SUMMARY

There is still controversy about the relation between changes in myocardial contractile function and global left ventricular (LV) performance during stable concentric hypertrophy. To clarify this, we analyzed LV function *in vivo* and myocardial mechanics *in vitro* in rats with pressure overload-induced cardiac hypertrophy. Male Wistar rats (70 g) underwent ascending aortic stenosis for 8 weeks (group AAS, $n = 9$). LV performance was assessed by transthoracic echocardiography under anesthesia. Myocardial function was studied in isolated papillary muscle preparations during isometric contraction. The data were compared with age- and sex-matched sham-operated rats (group C, $n = 9$). LV weight-to-body weight ratio (C: 2.13 ± 0.14 mg/g; AAS: 3.24 ± 0.44 mg/g), LV relative wall thickness (C: 0.18 ± 0.02 ; AAS: 0.33 ± 0.09), and LV fractional shortening (C: $54 \pm 5\%$; AAS: $70 \pm 8\%$) were increased in group AAS ($P < 0.05$). Echocardiographic analysis also indicated a significant association ($r = 0.74$; $P < 0.001$) between the percent fractional shortening index and LV relative wall thickness. The performance of AAS isolated muscle revealed that active tension (C: 6.6 ± 1.7 g/mm²; AAS: 6.5 ± 1.5 g/mm²) and maximum rate of tension development (C: 69 ± 21 g/mm²/s; AAS: 69 ± 18 g/mm²/s) were not significantly different from group C ($P > 0.05$). In conclusion, compensated pressure-overload myocardial hypertrophy is associated with preserved myocardial function and increased ventricular performance. The improved LV function might be due to the ventricular remodeling characterized by an increased relative wall thickness. (Jpn Heart J 2004; 45: 647-656)

Key words: Aortic stenosis, Left ventricular hypertrophy, Echocardiography, Papillary muscle, Cardiac function

MYOCARDIAL hypertrophy is an adaptive response to ventricular overload. Although there has been considerable investigation of myocardial function with

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pressure overload hypertrophy, the functional features of the myocardium are not completely understood. In experimental studies with left ventricular (LV) hypertrophy and congestive heart failure, the myocardial function is depressed.¹⁻³⁾ However, myocardial performance remains controversial in animals with compensated cardiac hypertrophy as mechanical activity has been reported to be increased,⁴⁻⁶⁾ unchanged,⁷⁻¹⁰⁾ and depressed.¹¹⁻¹³⁾ Furthermore, there is disagreement about myocardial function and LV performance when cardiac hypertrophy is stable. For example, Pfeffer, *et al*^{14,15)} have documented impaired pumping ability in hypertrophied ventricles from spontaneously hypertensive rats (SHR), while Bing, *et al*,⁶⁾ Bing and Wiegner,¹⁶⁾ and Conrad, *et al*¹⁷⁾ have shown that myocardial active tension development is not depressed in SHR of the same age.

To further address this issue, we analyzed the relation between changes in global LV performance and myocardial contractile function during stable concentric cardiac hypertrophy in rats with ascending aortic stenosis. Cardiac evaluation was performed *in vivo* using transthoracic echocardiography. This technique is easy to perform and provides qualitative and quantitative information to assess rat cardiac structures and heart function. Myocardial performance was studied in an isolated papillary muscle preparation, which allows us to evaluate myocardial intrinsic contractile function independent of load conditions.

METHODS

Animals and groups: All experiments and procedures were performed in accordance with the US National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Botucatu Medical School Animal Ethics Committee.

Male Wistar weaning rats (3 to 4 weeks old) weighing 70 to 80 g were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg). Aortic constriction was created by placing a stainless-steel clip of 0.6 mm internal diameter on the ascending aorta via thoracic incision (group AAS, $n = 9$). Age-matched control animals underwent left thoracotomy without clip placement (group C, $n = 9$). All rats were housed in a temperature-controlled room (24°C) on a 12-h light/dark cycle, with food and water supplied *ad libitum*. The animals were studied eight weeks after surgery. The relation between the left and right ventricular (RV) weight and body weight (BW) were used as indexes of ventricular hypertrophy.

Echocardiographic study: Rats were anesthetized with an intramuscular mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). We assumed that these anesthetics would not promote any functional alteration in the heart. The chest was shaved, and the rats were positioned on their left side. Using a commercially available echocardiograph (Sonos 2000, Hewlett-Packard Medical Systems;

Andover, MA) equipped with a 7.5-MHz transducer, a two-dimensional short-axis view of the LV was obtained at the level of the papillary muscles. M-mode tracings were obtained from short-axis views of the LV at or just below the tip of the mitral-valve leaflets, and at the level of the aortic valve and left atrium.^{18,19} M-mode images of LV were recorded on a black-and-white thermal printer (SONY UP-890MD) at a sweep speed of 100 mm/s (Figure 1). All LV tracings were manually measured by the same observer, and according to the leading-edge method of the American Society of Echocardiography.²⁰

Measurements were the mean of at least five cardiac cycles on the M-mode tracings. The following variables were measured: heart rate (HR); LV end-diastolic dimension (LVEDD) and posterior wall thickness (PWD) at maximal diastolic dimension; end-systolic dimension (LVSD) at the point of maximal anterior motion of the posterior wall; relative wall thickness (RWT), which was obtained by dividing the PWD by LVEDD; LV ejection fraction (LVEF); and fractional shortening index [$\Delta D = (LVEDD - LVSD)/LVEDD \times 100$].

Myocardial mechanical study: After anesthesia with thiopental sodium, the animals underwent a thoracotomy and the hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. Trabecular carneae or papillary muscles were dissected from the LV, mounted between two spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution at 28°C and gassed with 95% O₂ and 5% CO₂. The composition of the Krebs-Henseleit solution was as follows: 118.5 mM NaCl, 4.69 mM KCl, 2.52 mM CaCl₂, 1.16 mM MgSO₄, 1.18 mM KH₂PO₄, 5.50 mM glucose, and 25.88 mM NaHCO₃. The lower spring clip was attached to a Kyowa model 120T-20B force transducer by a thin steel wire (1/15,000 inch), which passed through a mercury seal at the bot-

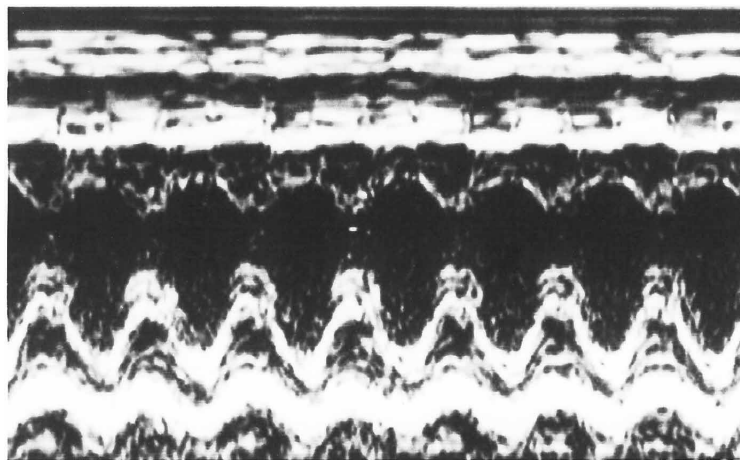


Figure 1. M-mode representative echocardiogram recorded in anesthetized rats eight weeks after ascending aortic banding.

tom of the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm above which a micrometer stop was mounted for the adjustment of muscle length. The lever arm was made of magnesium with a ball-bearing fulcrum and a lever arm ratio of 4:1. Preparations were stimulated 12 times/min with 5-ms square wave pulses through parallel platinum electrodes, at voltages 10% greater than the minimum required to produce a maximal mechanical response.

The muscles were kept contracting isotonically with light loads for 60 minutes and then loaded to contract isometrically and stretched to the maximum of their length-tension curves. After a 5-minute period in afterloaded isotonic contractions, muscles were again placed under isometric conditions, and the peak of the length-tension curve (Lmax) was carefully determined. A 15-minute period of stable isometric contraction was imposed prior to the experimental period and one isometric contraction was then recorded. The following parameters were measured: peak developed tension (DT; g/mm²), resting tension (RT; g/mm²), time to peak tension (TPT; ms), maximum rate of tension development (+dT/dt; g/mm²/s), maximum rate of tension decline (-dT/dt; g/mm²/s), and time for tension to fall from peak to 50% of peak tension (RT_{1/2}; ms).

After the end of each experiment, the muscle length at Lmax was measured and the muscle between the two clips was blotted dry and weighed. Cross-sectional area was calculated from muscle weight and length by assuming cylindrical uniformity and a specific gravity of 1.0. All force data were normalized for the muscle cross-sectional area.

Data analysis: Data are expressed as the mean \pm SD. Statistical comparison between groups AAS and C was conducted using Student's *t*-test for independent samples with a significance level of 5%.

RESULTS

Morphologic data: Table I shows the morphological parameters for the group C and AAS rats. There were no significant differences in BW or RV weight between

Table I. Morphologic Data

| | C (<i>n</i> = 9) | AAS (<i>n</i> = 9) |
|--------------|-------------------|------------------------------|
| BW (g) | 384 \pm 62 | 353 \pm 39 |
| LV (mg) | 0.78 \pm 0.08 | 1.15 \pm 0.20 [#] |
| RV (mg) | 0.25 \pm 0.06 | 0.28 \pm 0.03 |
| LV/BW (mg/g) | 2.13 \pm 0.14 | 3.24 \pm 0.44 [#] |
| RV/BW (mg/g) | 0.69 \pm 0.17 | 0.79 \pm 0.12 |

Values expressed as mean \pm SD; *n* = number of animals; C = control group; AAS = supravalvular aortic stenosis group; BW = body weight; LV = left ventricular weight; RV = right ventricular weight; [#] *P* < 0.01 vs Control (Student's *t* test).

the two groups. LV weight absolute or normalized by BW was greater in the AAS group in relation to the C group. RV weight normalized to BW was the same in both groups (Figure 2A). Since the animals in both groups had no evidence of heart failure such as labored respiration, left atrial thrombi, pleural and pericardial effusions, or right ventricular hypertrophy,³⁾ we can assume that our animals had developed compensated LV hypertrophy.¹⁷⁾

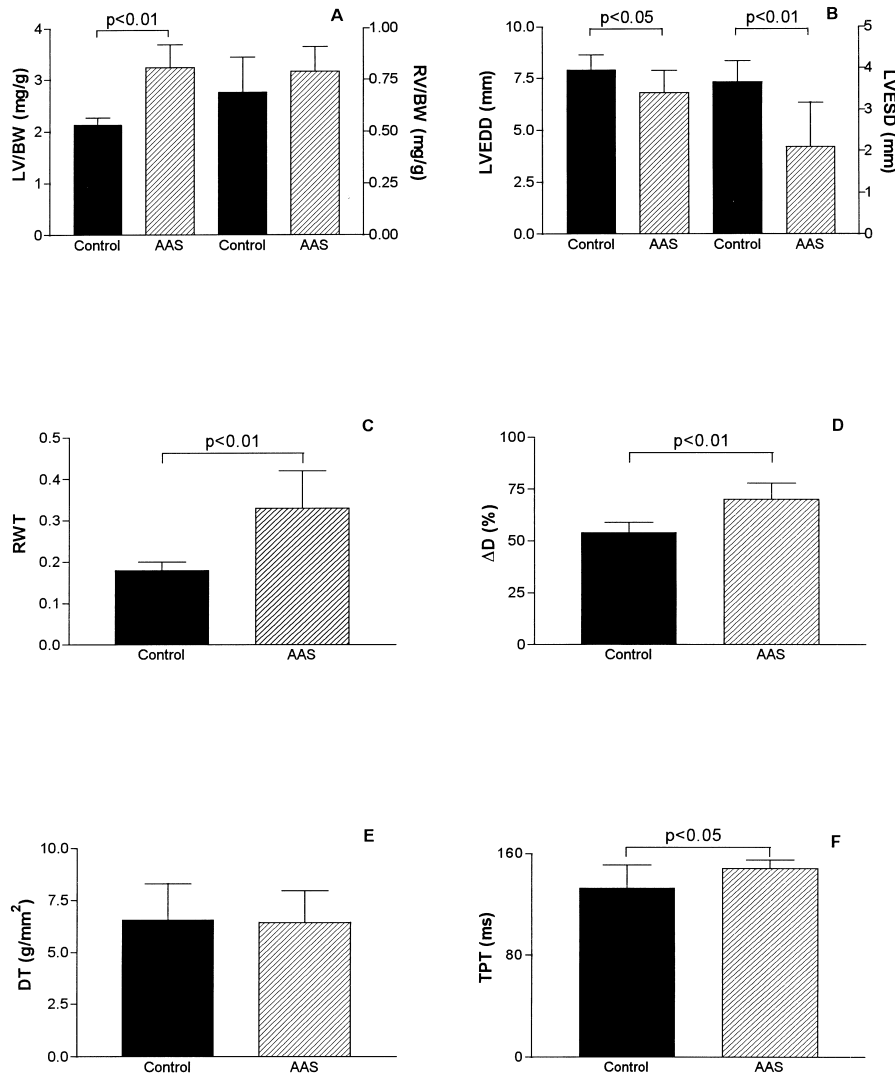


Figure 2. Effect of ascending aortic stenosis on morphological (A), echocardiographic (B, C, D), and isolated muscle parameters (E,F). AAS = ascending aortic stenosis; LV = left ventricular weight; BW = body weight; RV = right ventricular weight; LVEDD = left ventricular end-diastolic dimension; LVESD = left ventricular end-systolic dimension; RWT = Relative wall thickness; ΔD = percentage of fractional shortening index; DT = peak developed tension; TPT = time to peak tension (Student's *t* test).

Echocardiographic evaluation: Table II shows the average echocardiographic values. The heart rate was similar in both groups. The PWD was greater in the AAS group. LVEDD and LVESD were lower in the AAS group (Figure 2B). RWT was greater in the AAS group, demonstrating concentric LV hypertrophy (Figure 2C). The indexes of LV systolic function, ΔD (Figure 2D), and LVEF were greater in the AAS group. The correlation between RWT and ΔD was significant ($r = 0.74$; $P < 0.001$).

Isolated muscle mechanics: Table III presents the mean data for isometric contractions. There were no significant differences in DT (Figure 2E), RT, $+dT/dt$, $-dT/dt$, or $RT_{1/2}$ between the groups. Time to peak tension was greater in the AAS group muscles (Figure 2F).

Table II. Left Ventricular Monodimensional Echocardiographic Data

| | C ($n = 9$) | AAS ($n = 9$) |
|----------------|-----------------|----------------------|
| HR (beats/min) | 309 ± 60 | 282 ± 35 |
| PWD (mm) | 1.41 ± 0.19 | $2.19 \pm 0.24^*$ |
| LVEDD (mm) | 7.91 ± 0.74 | $6.80 \pm 1.08^*$ |
| LVESD (mm) | 3.65 ± 0.52 | $2.09 \pm 1.08^{\#}$ |
| RWT | 0.18 ± 0.02 | $0.33 \pm 0.09^{\#}$ |
| ΔD (%) | 54 ± 5 | $70 \pm 8^{\#}$ |
| LVEF | 0.90 ± 0.04 | $0.97 \pm 0.02^{\#}$ |

Values expressed as mean \pm SD; n = number of animals; C = control group; AAS = supra-ventricular aortic stenosis group; HR = heart rate; PWD = left ventricular posterior wall thickness; LVEDD = left ventricular end-diastolic dimension; LVESD = left ventricular end-systolic dimension; RWT = relative wall thickness; ΔD = percentage of fractional shortening; LVEF = left ventricular ejection fraction; * $P < 0.05$ vs Control; $\# P < 0.01$ vs Control (Student's t test).

Table III. Isolated Muscle Parameters

| | C ($n = 9$) | AAS ($n = 9$) |
|-------------------------|-----------------|-----------------|
| DT (g/mm^2) | 6.59 ± 1.71 | 6.45 ± 1.52 |
| RT (g/mm^2) | 0.87 ± 0.35 | 0.92 ± 0.38 |
| $+dT/dt$ ($g/mm^2/s$) | 69 ± 21 | 69 ± 18 |
| TPT (ms) | 133 ± 18 | $148 \pm 7^*$ |
| $-dT/dt$ ($g/mm^2/s$) | 27 ± 5 | 33 ± 8 |
| $RT_{1/2}$ (ms) | 157 ± 27 | 147 ± 16 |
| CSA (mm^2) | 1.02 ± 0.29 | 1.17 ± 0.23 |

Values expressed as mean \pm SD; n = number of animals; C = control group; AAS = supra-ventricular aortic stenosis group; DT = peak developed tension; RT = resting tension; $+dT/dt$ = maximum rate of tension development; TPT = time to peak tension; $-dT/dt$ = maximum rate of tension decline; $RT_{1/2}$ = time for tension to fall from peak to 50% of peak tension; CSA = cross sectional area; * $P < 0.05$ vs Control (Student's t test).

DISCUSSION

The aim of this investigation was to study the relation between changes in global LV performance and myocardial contractile function, during stable concentric cardiac hypertrophy in rats with ascending aortic stenosis for eight weeks. Ventricular and myocardial functions were evaluated using a combination of a noninvasive method, echocardiogram, and papillary muscle preparation. This evaluation was performed in a state of compensated hypertrophy as there were no clinical signs of heart failure such as labored respiration, pleural and/or pericardial effusion, left atrial thrombi, or right ventricular hypertrophy.^{3,17)} In rats, gradual aortic constriction for eight weeks has been reported to produce stable LV hypertrophy.²¹⁾

Although left ventricular hypertrophy is the most important compensation mechanism in chronic pressure overload, it has frequently been associated with heart failure and death.²²⁾ It occurs because the hypertrophied myocardium presents structural changes that lead to cardiac remodeling, such as a disproportional increase in the myocyte component compared to vascular component. One of the consequences is an imbalance between oxygen supply and consumption, which causes myocyte necrosis, apoptosis, and abnormal collagen accumulation. Fibrosis also occurs as a direct consequence of activation of the renin-angiotensin system in the myocardium.²³⁻²⁶⁾

The stimuli for developing cardiac remodeling also include mechanical and biochemical factors which, acting on receptors, ionic channels, and integrin present in the sarcolemma membrane,²⁷⁾ trigger cytosolic biochemical signals provoking increased protein synthesis and changes in gene expression.²⁸⁻³⁰⁾ All these alterations lead to a worsening of cardiac function, associated with diminished contractility.

In this study, the unchanged developed tension and maximum rate of tension development indexes suggest preserved contractility, even in the presence of cardiac remodeling. One could argue that contractility is a very complex process and other methods should be used to determine its status. However, the isolated papillary muscle preparation reflects the mechanical properties of the myocardium with no interference from geometrical characteristics, load conditions, oxygen supply imbalance, or sympathetic tonus. It is therefore reasonable to assume that hypertrophy following an eight-week AAS does not jeopardize contractility.

Echocardiogram LV function analysis revealed that the AAS group had better systolic function than controls, since endocardial ΔD was greater in these animals. Ejection fraction results from factors such as contractility, preload, and afterload. In this study, contractility was found to be maintained, whereas load conditions were not assessed. Despite this, our data suggest that LVEDD was

decreased and wall thickness increased so that preload, which is largely influenced by these parameters, was preserved or even decreased. Decreased preload would lead to diminished LV ejection fraction; this was not found in our study. Therefore, the variation observed in ventricular systolic performance must be mainly due to changes in afterload.

Afterload is a mechanical parameter directly influenced by ventricular pressure and diameter, and inversely related to wall thickness. These geometrical changes were well documented in our study by increased RWT, indicating both reduced LVEDD and increased PWD. According to Laplace's law, with these geometrical characteristics, the ventricle presents an improved ability to develop pressure and shorten, even with preserved myocardial contractility. Thus, it is possible that afterload was normal or even subnormal, despite the pressure overload. That would explain the increased endocardial ΔD found in our data, reflecting a condition of overcompensated myocardial hypertrophy.

It should be pointed out that Ono, *et al*³¹⁾ preferred midwall ΔD to endocardial ΔD when ventricular concentric geometry is present, because the latter superestimates ejection fraction. On the contrary, Douglas, *et al*¹⁹⁾ studied male Wistar rats with AAS for 6 and 20 weeks, and using both indexes failed to find significant differences. Other authors analyzing systolic function in rats¹⁸⁾ and dogs³²⁾ with concentric LVH observed that midwall ΔD was more sensitive for detecting ventricular dysfunction, in relation to endocardial ΔD . In other words, in the presence of dysfunction, endocardial ΔD could be normal. Since in the present study this index was greater in AAS rats than in controls, it is improbable that ventricular dysfunction is present in hypertrophied heart.

In summary, compensated pressure-overload in rats with an eight-week AAS is associated with preserved myocardial function and increased or preserved ejection performance. These may be related to afterload conditions, and could be due to ventricular geometrical remodeling characterized by an increased relative wall thickness.

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