Volume Overload Influence on Hypertrophied Myocardium Function

Antonio Carlos Cicogna,1 MD, Beatriz Bojikian Matsubara,1 MD, Luiz Shiguero Matsubara,1 MD, Katashi Okoshi,1 MD, Ana Lúcia Gut,1 MD, Carlos Roberto Padovani,1 PhD, Mariana Monteiro Meyer1, and Marina Politi Okoshi,1 MD

SUMMARY

The aim of this study was to demonstrate that hypertrophied cardiac muscle is more sensitive to volume-overload than normal cardiac muscle. We assessed the mechanical function of isolated left ventricular papillary muscle from male spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY) submitted to volume overload caused by aortocaval fistula (ACF) for 30 days. Muscles were perfused with Krebs-Henseleit solution at 28°C and studied isometrically at a stimulation rate of 0.2 Hz. The ACF increased the right and left ventricular weight-to-body weight ratio in WKY rats; it also promoted right ventricular hypertrophy and further increased the basal hypertrophy in the left ventricle from SHR. The arterial systolic pressure was greater in SHR than in WKY rats, and decreased with ACF in both groups. Developed tension (DT) and maximum rate of DT (+dT/dt) were greater in the SHR-control than in the WKY-control (P<0.05); the time from peak tension to 50% relaxation (RT1/2) was similar in these animals. ACF did not change any parameters in the SHR group and increased the resting tension in the WKY group. However, the significant difference observed between myocardial contraction performance in WKY-controls and SHR-controls disappeared when the SHR-ACF and WKY-controls were compared. Furthermore, RT1/2 increased significantly in the SHR-ACF in relation to the WKY-controls. In conclusion, the data lead us to infer that volume-overload for 30 days promotes more mechanical functional changes in hypertrophied muscle than in normal cardiac muscle. (Jpn Heart J 2002; 43: 689-695)

Key words: Spontaneously hypertensive rats, Volume overload, Myocardium function, Isolated muscle

CARDIAC hypertrophy is a compensatory response to a sustained mechanical stress of the heart that allows it to meet the demands of an increased workload. The spontaneously hypertensive rat (SHR) is a well established model of genetic...
hypertension that leads to an increase in cardiac mass, which often initially maintains cardiac performance despite the elevation of systemic arterial pressure.

Aortocaval fistula (ACF) is an effective method of developing volume-overload cardiac hypertrophy. Although this method can decrease intrinsic myocardial contractility and promote heart failure, several experimental studies have shown that the heart function is well preserved.3-7)

The aim of this study was to evaluate the mechanical function of stable concentric hypertrophied heart from SHR submitted to volume-overload caused by ACF for 4 weeks. We have attempted to demonstrate that concentric hypertrophied muscles are more sensitive to volume-overload than the normal cardiac muscle. Since ACF may promote a transition from concentric to eccentric hypertrophy, we hypothesized that the cardiac remodeling would be accompanied by myocardial functional impairment. Preliminary studies in our laboratory have shown that volume-overload for 4 weeks did not change myocardial function in Wistar-Kyoto rats analysed by examining isolated papillary muscle (unpublished data).

**MATERIALS AND METHODS**

**Animal model and experimental protocol:** Male 5-month-old SHR and normotensive Wistar-Kyoto (WKY) rats were studied. Left ventricular papillary muscle preparations were examined from 4 groups of rats: WKY without ACF \( (n=12, \text{WKY}^-C) \), WKY with ACF \( (n=8, \text{WKY}^+ACF) \), SHR without ACF \( (n=9, \text{SHR}^-C) \) and SHR with ACF \( (n=9, \text{SHR}^+ACF) \).

The aortocaval fistula was prepared according to the Garcia and Diebold technique,8) the shunt was produced using a 16-gauge disposable needle under anesthesia (pentobarbital sodium; 50 mg/kg, IP). The animals were killed by decapitation 30 days after creation of the ACF. Arterial systolic blood pressure (ASBP) was measured before the animal underwent the procedure and at the end of experiments, using the indirect tail-cuff technique.9)

All experiments and procedures conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and were approved by the ethics committee of the Faculdade de Medicina de Botucatu, UNESP, São Paulo, Brazil.

**Functional study:** After sacrifice, the hearts were quickly removed and placed in oxygenated Krebs- Henseleit solution at 28°C. Trabecular carnea or papillary muscle was dissected carefully from the left ventricle, mounted between two spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution at 28°C and oxygenated with a mixture of 95% O₂ and 5% CO₂ (pH 7.38). The composition of the Krebs-Henseleit solution in mMol/L was as follows:
118.5 NaCl, 4.69 KCl, 2.52 CaCl₂, 1.16 MgSO₄, 1.18 KH₂PO₄, 5.50 glucose, and 25.88 NaHCO₃.

The lower spring clip was attached to a Kyowa model 12OT-20B force transducer by a thin steel wire, which passed through a mercury seal at the bottom 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 from 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 which were approximately 10% greater than the minimum required to produce a maximal mechanical response.

After a 60-minute period during which the preparations were permitted to shorten while carrying light loads, the muscles were loaded to contract isometrically and stretched to the apices of their length-tension curves.

After a 5-minute period during which preparations performed afterloaded isotonic contractions, muscles were again placed under isometric conditions, and the apex of the length-tension curve (L_max) was carefully determined. A 15-minute period of stable isometric contraction was imposed prior to the experimental period. One isometric contraction was then recorded for later analysis.

The following parameters were measured from the isometric contractions: 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 from peak tension to 50% relaxation (RT₁/₂, ms). At the end of each experiment the muscle length at L_max was measured and the muscle between the two clips was blotted dry and weighed. Cross-sectional area (CSA) was calculated from the muscle weight and length by assuming cylindrical uniformity and a specific gravity of 1.0. All force data were normalized for the muscle CSA.

Morphological study: Body weight (BW, g), left ventricular weight (LVW, g), right ventricular weight (RVW, g), LVW-to-BW ratio (LVW/BW), and RVW-to-BW ratio (RVW/BW) were measured for all groups of animals. The LVW/BW, RVW/BW, and LV myocyte cross-sectional area (MCSA, µm²) were used to characterize left and right ventricular hypertrophy. MCSA was determined for at least 100 myocytes per slide stained with hematoxylin-eosin. The measurements were performed using a Leica microscope (×40 magnification lens) attached to a video camera and connected to a personal computer equipped with image analyzer software (Image-Pro Plus 3.0, Media Cybernetics, Silver Spring, MD USA). MCSA was measured with a digitizing pad, and the selected cells were transversely cut with the nucleus clearly identified in the center of the myocyte.

Statistical analysis: Values are shown as mean ± SD. Comparisons between
groups were conducted by analysis of variance and the post hoc Tukey test. The level of significance was $P<0.05$.

**RESULTS**

Table I shows the general characteristics of the animals. BW was greater in WKY-C rats than in SHR-C rats ($P<0.05$). ACF did not change BW in either group. RVW and RVW/BW were the same in WKY-C and SHR-C; ACF increased these in both groups ($P<0.05$). LVW/BW was greater in SHR-C than in WKY-C ($P<0.05$). ACF significantly increased LVW/BW in WKY and SHR rats ($P<0.05$). ASBP was greater in SHR-C than WKY-C ($P<0.05$). ACF decreased ASBP in both groups ($P<0.05$). Myocyte CSA was greater in SHR-C than WKY-C ($P<0.05$); ACF significantly increased myocyte CSA in both groups ($P<0.05$). Muscle CSA was similar in both control groups and it was not changed by ACF.

Table II summarizes the data from isometric contractions. Myocardial performance was better in SHR-C than WKY-C because DT and $+dT/dt$ were higher in hypertrophied than in normal muscle ($P<0.05$); the other parameters were similar. ACF only increased RT in WKY-C ($P<0.05$); the other variables did not change significantly. Also, ACF did not cause significant variation in any parameters from the SHR group. However, the significant difference in cardiac function between SHR-C and WKY-C disappeared when we compared SHR-ACF and WKY-C ($P>0.05$). Also, the $RT_{1/2}$ values, equal in SHR-C and WKY-C, were significantly different between SHR-ACF and WKY-C.

**Table I.** General Characteristics of the Wistar-Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th></th>
<th>WKY C (n=12)</th>
<th>ACF C (n=8)</th>
<th>SHR C (n=9)</th>
<th>ACF SHR (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>383±40a</td>
<td>395±36a</td>
<td>325±22a</td>
<td>312±3b</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>0.25±0.05a</td>
<td>0.38±0.09b</td>
<td>0.22±0.02a</td>
<td>0.33±0.04b</td>
</tr>
<tr>
<td>RVW/BW (mg/g)</td>
<td>0.66±0.15ab</td>
<td>0.96±0.27b</td>
<td>0.68±0.05a</td>
<td>1.07±0.18b</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>0.71±0.12c</td>
<td>0.94±0.12c</td>
<td>0.92±0.09b</td>
<td>1.00±0.06b</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>1.85±0.18a</td>
<td>2.38±0.19b</td>
<td>2.83±0.22c</td>
<td>3.25±0.29d</td>
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<tr>
<td>IASP (mmHg)</td>
<td>128±8a</td>
<td>131±16a</td>
<td>174±9b</td>
<td>187±18b</td>
</tr>
<tr>
<td>FASP (mmHg)</td>
<td>129±4a</td>
<td>116±10b</td>
<td>186±6d</td>
<td>147±8c</td>
</tr>
<tr>
<td>MCSA (µm²)</td>
<td>351±68ab</td>
<td>541±57bc</td>
<td>507±13b</td>
<td>665±102c</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>1.04±0.17ab</td>
<td>1.26±0.16a</td>
<td>0.86±0.15a</td>
<td>1.07±0.25ab</td>
</tr>
</tbody>
</table>

Values are means±SD; n=number of preparations; C=control; ACF=aortocaval fistula; BW=body weight; RVW=right ventricular weight; LVW=left ventricular weight; IASP=initial arterial systolic pressure; FASP=final arterial systolic pressure; MCSA=myocyte cross-sectional area; CSA=muscle cross-sectional area; Groups that do not share a common letter are statistically different ($P<0.05$, ANOVA and Tukey).
The objective of this study was to evaluate whether hypertrophied muscles from SHR are more sensitive to a four-week volume overload than normal cardiac muscle. This experimental model, in a previous investigation in our laboratory, did not change WKY myocardial systolic function analyzed by examining isolated papillary muscle (unpublished data). This preparation allows us to evaluate the ability of the cardiac muscle to develop force and shorten, independent of influences that can change myocardial function in vivo such as heart rate, preload, and afterload. Furthermore, this preparation is stable when stimulated for many hours while bathed in a solution of appropriate composition.11,12)

This investigation showed that ACF increased RVW/BW and LVW/BW in the WKY and SHR groups. ACF also significantly increased the myocyte cross-sectional area in both groups (Table I). This allows us to conclude that ACF promoted hypertrophy in WKY and SHR right ventricle and in WKY left ventricle. ACF also increased SHR left ventricle hypertrophy.

This experiment demonstrated that papillary muscle function is better in SHR-C than WKY-C. This confirms previous observations by different authors.13) Our data show that volume overload did not significantly change isometric parameters in SHR hypertrophied muscle. But, if we take into account that DT and +dT/dt are greater in SHR-C than WKY-C and similar in SHR-ACF and WKY-C, and RT_{1/2} is equal in SHR-C and WKY-C but greater in SHR-ACF than WKY-C, it may be possible to infer that volume overload adversely affects the contraction and relaxation phases of hypertrophied myocardium. In WKY rats ACF only increases RT; it has no effect on systolic function, RT_{1/2}, or -dT/dt (Table II).

### DISCUSSION

Table II. Isometric Contraction Data

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT (g/mm²)</td>
<td>6.65±1.41a</td>
<td>7.28±1.28ab</td>
</tr>
<tr>
<td>RT (g/mm²)</td>
<td>0.80±0.34a</td>
<td>1.32±0.24a</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>200±25a</td>
<td>191±8a</td>
</tr>
<tr>
<td>+dT/dt (g/mm²/s)</td>
<td>57±14a</td>
<td>68±11ab</td>
</tr>
<tr>
<td>-dT/dt (g/mm²/s)</td>
<td>18±3a</td>
<td>21±5a</td>
</tr>
<tr>
<td>RT₁/₂ (ms)</td>
<td>254±36a</td>
<td>234±24a</td>
</tr>
</tbody>
</table>

Values are means±SD; n=number of preparations; C=control; ACF=aortocaval fistula; WKY=Wistar-Kyoto rats; SHR=spontaneously hypertensive rats; DT=peak developed tension; RT: resting tension; TPT=time to peak tension; +dT/dt=maximum rate of tension development; -dT/dt=maximum rate of tension decline; RT₁/₂=time from peak tension to 50% relaxation. Groups that do not share a common letter are statistically different (P<0.05, ANOVA and Tukey).
There are no data regarding isolated myocardial function in animals subjected to volume and chronic pressure overload. Noma, et al\textsuperscript{14} studied the effects of an arteriovenous shunt on ventricular function in rats with renovascular hypertension. These authors evaluated the rats when heart failure was present and found a significant fall in heart performance. Olivetti, et al\textsuperscript{15} studied the effects of nutritional anemia in SHR, and demonstrated that anemia provoked left ventricular dysfunction.

The mechanisms behind the functional changes induced by volume-overload in hypertrophied rat heart remain unknown. While Hisamatsu, et al\textsuperscript{16} found changes in cardiac sarcoplasmic reticulum function, Di Fusco, et al\textsuperscript{17} observed a decreased expression of myocardial G(s) alpha protein in rats with ACF. These latter authors also observed a diminished responsiveness of adenyl cyclase to guanosine 5’-O-(3-thiotriphosphate), isoproterenol, and forskolin in ACF rat hearts.

Our results showed that ACF in WKY rats provoked an increase in resting tension. Two mechanisms could be involved in this change: inadequate muscle oxygenation and increased cardiac collagen. Taking into account that papillary muscle cross-sectional areas were similar in both WKY groups, and that there was no increase in myocardial collagen concentration with volume overload,\textsuperscript{18-21} we are unable to provide an explanation for the resting tension elevation in the WKY-ACF rats.

In summary, this investigation shows that a 4-week period of volume overload causes more mechanical changes in hypertrophied rat myocardium than in normal myocardium.

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**REFERENCES**


