# Follow-up Study of Morphology and Cardiac Function in Rats Undergoing Induction of Supravalvular Aortic Stenosis

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***Objective -*** *To characterize the follow-up of an experimental model of left ventricular hypertrophy (LVH) induced by supravalvular ascending aortic stenosis in young rats.*

***Methods -*** *Wistar rats were submitted to thoracotomy and aortic stenosis was created by placing a clip on the ascending aorta (AoS group, n=12). Age-matched control animals underwent a sham operation (C group, n=12). Cardiac function was analysed by echocardiograms performed 6, 12, and 21 weeks after aortic banding. Myocardial morphological features and myocardial hydroxyproline concentration (HOP) were evaluated 2, 6, 12, and 21 weeks after surgery in additional animals.*

***Results -*** *Aortic banding promoted early concentric LVH and a progressive increase in HOP. Under light microscopy, we observed myocyte hypertrophy and wall thickening of the intramural branches of the coronary arteries due to medial hypertrophy. Cardiac function was supranormal after 6 weeks (percentage of fractional shortening - $EAo_6$: 70.3±10.8; $C_6$: 61.3±5.4; p<0.05), and depressed in the last period. Diastolic dysfunction was detected after 12 weeks (ratio of early-to-late filling velocity - $EAo_{12}$: 4.20±3.25; $C_{12}$: 1.61±0.16; p<0.05).*

***Conclusion*** *- Ascending aortic stenosis promotes concentric LVH with myocardial fibrosis and minimal histological changes. According to the period of evaluation, cardiac function may be improved, normal, or depressed. The model is suitable and useful for studies on pathophysiology and treatment of the different phases of cardiac hypertrophy.*

**Key words:** myocardial hypertrophy, echocardiogram, ventricular function, aortic stenosis and rats.

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Received 12/5/02
Accepted 2/23/03

Cardiac hypertrophy is an important adapting mechanism that occurs as a response to chronic hemodynamic overload, allowing the heart to maintain its basic functions during overloaded conditions [1,2]. However, left ventricular hypertrophy is a risk factor for the development of congestive heart failure and sudden death [3].

Several experimental models have been proposed for the study of left ventricular hypertrophy due to pressure overload. Among them, the induction of renovascular stenosis [4], of abdominal aorta stenosis [5,6], or thoracic aorta stenosis [7], or even the development of animals genetically modified, such as spontaneously hypertensive rats [8-12]. However, several of these animal models have limitations for use in experimental studies. For example, the induction of renovascular stenosis or abdominal aortic stenosis, in rats, in addition to leading to systemic blood hypertension and left ventricular hypertrophy, can also promote systemic activation of the adrenergic nervous system, and of the renin-angiotensin-aldosterone system [13-15]. The intense neuro-hormonal activation is associated with the presence of important cardiac lesions, such as myocardial necrosis, peripheral arteritis, and reparative fibrosis [4,6]. Thus, these models may be very aggressive in the myocardium and, therefore, less representative of the chronic lesions that develop gradually in humans with chronic pressure overload. On the other hand, spontaneously hypertensive rats develop a gradual increase in systemic blood hypertension at 1 month of age, and, at 3 months, they already have established left ventricular hypertrophy [16,17]. However, these rats have a long period of stable myocardial hypertrophy, without heart failure, which usually occurs by 20 months of age [9,11,18]. Therefore, although in this model cardiac alterations are similar to those occurring in human hypertensive heart disease [9,16], the prevention studies of heart failure are expensive, because the animals have to be kept in captivity for a long period.

More recently, the model of ascending aorta stenosis has been used to promote the gradual development of left ventricular hypertrophy in young rats [7,19-25]. The animals, 3

to 4 weeks after delivery, undergo median thoracotomy to place a band, with an internal diameter of approximately 0.6 mm, around the thoracic aorta, 2 to 3 mm above its root. Immediately after the thoracic banding, the diameter of the artery is maintained; with animal growth, the vessel diameter is maintained and aortic stenosis progressively appears. The rats develop left ventricular hypertrophy precociously, which is associated, in the short term, with improvement in the systolic function of the heart. Then, the animals start to experience depression of mechanical heart performance, and develop cardiac decompensation, 20 weeks after the induction of aortic stenosis [7,20,26-28]. In this model of left ventricular hypertrophy, no systemic activation of the sympathic nervous system or of the renin-angiotensin-aldosterone system occurs [7,21,26,29]. Probably because of this fact, histological evaluation, performed 21 weeks after aortic stenosis induction, did not demonstrate the presence of important cardiac lesions, such as peripheral arteritis, myocardial necrosis, or extensive fibrosis [7,26].

The advantages of this experimental model are the gradual development of left ventricular hypertrophy, absence of severe anatomical lesions in the myocardium, and the low cost of the maintenance of animals because of the short period necessary to develop left ventricular hypertrophy and heart failure. In the several studies performed with this model [7,19-21,26,30,31], different periods were used for the morphological or functional analysis of the heart, or both, and no studies exist regarding simultaneous and follow-up evaluations of myocardial histology and of cardiac function after induction of aortic stenosis.

The objective of this study was to characterize the follow-up of the experimental model of left ventricular hypertrophy because of supravalvular aortic stenosis, in young Wistar rats, using morphologic and functional evaluation of the heart. Myocardial morphology was analyzed, using optical microscopy, 2, 6, 12, and 21 weeks after the induction of aortic stenosis and cardiac function was assessed, *in vivo*, using echocardiographic study, performed 6, 12, and 21 weeks after banding of the ascending aorta. In addition to that, the collagen content was quantified using serial determination of hydroxyproline concentration in the left ventricle.

## Methods

Male Wistar rats, weighing 90 to 100g, were used from our biotery. Animals were kept in cages with 4 rats per box, at 23°C, with luminosity cycles of 12h and fed with Purina rat ration and water ad libitum. The protocol used was approved by the Ethical Committee for Animal Research of the Faculdade de Medicina de Botucatu, UNESP.

Rats underwent median thoracotomy after being anesthetized with ketamine hydrochloride (50 mg/kg intramuscular) and xylidine hydrochloride (10 mg/kg intramuscular). Next, the ascending aorta was dissected and a silver band with an internal diameter of 0.6 mm was placed at approximately 3 mm of the aortic root. During surgery, the rats were manually ventilated with positive pressure. Control animals underwent the same surgery; however, a band was not implanted.

Rats were divided into 2 groups: control (C) and aortic stenosis (AoS). For morphologic and biochemical evaluation (hydroxyproline dose), 5 to 7 animals from each group were sacrificed 2, 6, 12, and 21 weeks after AoS induction. The subgroups formed were given the following designations: $C_2$, $C_6$, $C_{12}$, $C_{21}$, and $AoS_2$, $AoS_6$, $AoS_{12}$, and $AoS_{21}$.

The other groups of rats (C and AoS) were formed only for functional study. Because this evaluation was performed in vivo and without later sacrifice of the animals, the same rats were assessed in 3 experimental periods: 6, 12, and 21 weeks after the induction of AoS (n= 12 for the C and AoS groups). Due to the difficulty in obtaining adequate echocardiographic images in low-weight rats, the functional study was not performed in the 2-week period after AoS induction.

Rats were weighed and anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal) then underwent median thoracotomy to remove the heart, which was rapidly washed in saline solution. Next, the right ventricle (RV) and left ventricle (LV) were dissected and weighed separately. From the central part of the left ventricle, a 2- to 3-mm thick annulus was cut, taking all the extension of its wall. The material was immersed in neutral and tamponade formalin 10% for 48 hours at 4°C. After this period, the tissue was washed, dehydrated, and imbedded in paraffin. The histological cuts 5- to 7-mm thick were dyed with hematoxylin-eosin and were analyzed with optical microscopy.

Morphometric analyses were performed using a VCR attached to a Leica microscope connected to the computer equipped with an analysis program (Image-Pro Plus 3.0, Media Cybernetics, Silver Spring, Maryland, USA). In left ventricle transverse sections, sectional areas (SA) of at least 50 cardiac fibers, whose nucleus was clearly identified in the center of the cell, were measured.

Myocardial concentration of hydroxyproline was assessed in tissue obtained at the tip of the left ventricle, according to the methods described by Switzer [32], using a previously described technique used in other studies at our laboratory [33-35].

The level of ventricular hypertrophy was assessed by the ratio between humid weight of the right and left ventricle and the body weight of the animals.

Rats received anesthesia with ketamine hydrochloride (50 mg/kg) and xylidine hydrochloride (1 mg/kg), administered intramuscularly. A trichotomy was performed in the anterior region of the thorax, and the animals were placed in the left lateral decubitus position for the performance of echocardiography [36] with the Sonos 2000 from Hewlett-Packard Co., equipped with an electronic transducer with a 7.5 MHz frequency.

To measure cardiac structures, M-mode images were obtained with an ultrasound beam guided by the bi-dimensional mode, with the transducer positioned at the smaller parasternal axis. An image of the left ventricle was obtained with the M-mode cursor positioned right below the mitral

valve plane at the papillary muscle level [22,37,38]. Aorta (AO) and left atrium (LA) images were obtained with the M-mode cursor positioned at the level of the aortic valve plane. Images obtained were recorded afterwards with the UP-890 printer from the Sony Co.; cardiac structures were measured manually with the help of a pachymeter.

Left ventricle diastolic diameter (LVDD) and systolic diameter (LVSD) were measured at the period of the cardiac cycle when their values were maximum and minimum, respectively. Diastolic thickness of the posterior left ventricle wall (LVDT) was measured at the same period of the cardiac cycle when left ventricle diastolic diameter was assessed. Aortic root diameter (AO) was obtained in the period immediately prior to the opening of the aortic valve. The left atrium (LA) was assessed when its diameter was at its maximum [22,38]. From the dimensions above described, we obtained: relative left ventricle thickness (RLVT), LA/AO, LVDD/BW, and LA/BW.

Left ventricle systolic function was assessed by using the shortening percentage (ΔD): [(LVDD – LVSD)/LVDD] X 100, and the diastolic function analyzed by the ratio between initial filling flow velocity (E wave) and of the atrial contraction (A wave) of the transmitral flow.

One day after the performance of echocardiography, corresponding to 21 weeks, the animals were sacrificed while under anesthesia with sodium pentobarbital. At this time, signs of heart failure were identified, according to Conrad et al [39] and Cicogna et al [11,18] as tachypnea, pleuropericardial effusion, ascites, and the presence of left atrial thrombus.

Regarding the statistical analysis, numerical data are expressed as mean ± standard deviation. Study of the morphometric variables and of the myocardial concentration of hydroxyproline was performed with analysis of variance (ANOVA) for the factorial scheme 2X4, for the entirely casual model, completed with the *Tukey* test of multiple comparisons. Results obtained in the echocardiographic study were analyzed with analysis of variance of the multivariate profiles for dependent groups (MANOVA). Significance level was considered 5%.

## Results

Corporal parameters are provided in table I. Body weight was similar between groups AoS and C in the period 2 weeks after surgery. Significant variations occurred between the groups: after 6 weeks body weight was greater in C; after 12 and 21 weeks, it was lower in the C groups in comparison with that in the AoS groups. Left ventricle weight was always greater in the AoS groups compared with that in the C groups. In the evolvement analysis, left ventricle weight increased significantly between 2 and 6 weeks in the 2 groups, C and AoS, and did not change in the other period of analysis. Right ventricle weight was greater in the AoS groups than in the C groups only in the 21-week period. In the evolvement analysis, RV weight had the same pattern as that of the left ventricle weight in the C groups and demonstrated the following results in the AoS groups: $AoS_2$<($AoS_6$=$AoS_{12}$)<$AoS_{21}$. LV/BW ratio was greater in AoS in the 4 periods assessed. In the C groups, the ratio was greater in $C_2$ than at other times and, in rats with AoS it had the following pattern: $AoS_2$>-$AoS_6$> ($AoS_{12}$=$AoS_{21}$). The RV/BW ratio was greater in the AoS group than in C only in the last period of evaluation. In the C groups, this ratio was greater in $C_2$ than in the other periods and, in AoS groups, it was similar in the 2- and 21-week periods, both greater than at 6 and 12 weeks.

Of the 12 animals in the AoS group, which evolved for 21 weeks, 3 had signs of heart failure, including tachypnea, pleural effusion, and ascites.

In the left ventricle histological evaluation, C groups had normal morphology. In the AoS groups, progressive

**Table I - Body parameters of rats**

| | | Experimental period (weeks) 2 (n=5) | 6 (n=7) | 12 (n=7) | 21 (n=7) |
|---|---|---|---|---|---|
| BW (g) | C | 155 ± 9[a] | 395 ± 33[b] | 383 ± 18[b] | 403 ± 40[b] |
| | | NS | * | * | * |
| | AoS | 161 ± 10[a] | 348 ± 42[b] | 418 ± 39[c] | 440 ± 48[c] |
| LV (g) | C | 0.43 ± 0.03[a] | 0.83 ± 0.07[b] | 0.80 ± 0.07[b] | 0.83 ± 0.08[b] |
| | | * | * | * | * |
| | AoS | 0.65 ± 0.08[a] | 1.26± 0.12[b] | 1.26 ± 0.16[b] | 1.41 ± 0.22[b] |
| RV (g) | C | 0.15 ± 0.02[a] | 0.24 ± 0.04[b] | 0.26 ± 0.04[b] | 0.25 ± 0.04[b] |
| | | NS | NS | NS | * |
| | AoS | 0.15 ± 0.02[a] | 0.25 ± 0.05[b] | 0.26 ± 0.04[b] | 0.40 ± 0.14[c] |
| LV/BW (mg/g) | C | 2.76± 0.14[b] | 2.10± 0.19[a] | 2.09± 0.12[a] | 1.92± 0.15 [a] |
| | | * | * | * | * |
| | AoS | 4.07± 0.44[c] | 3.64± 0.22[b] | 3.01± 0.19[a] | 3.20± 0.39[a] |
| RV/BW (mg/g) | C | 0.96± 0.11[b] | 0.61 ± 0.07[a] | 0.67 ± 0.09[a] | 0.57 ± 0.06[a] |
| | | NS | NS | NS | * |
| | AoS | 0.96 ± 0.15[b] | 0.72 ± 0.12[a] | 0.63 ± 0.11[a] | 0.92 ± 0.30[b] |

C- control; AoS- supravalvular aortic stenosis; BW- body weight; LV- left ventricle weight; RV- right ventricle weight. ANOVA and *Tukey*. [a, b, c] - groups that do not have the same letter had significant differences in the 4 evaluation periods (P<0.05); * P<0.05 vs respective control group; NS - nonsignificant.

hypertrophy of the myocytes was observed at 2-week follow-up. Intramural branches of the coronary artery had medium lower hypertrophy that was discreet after 2 and 6 weeks, becoming evident after 12 weeks (fig. 1). Myocardial fibrosis, interstitial fibrosis, or peripheral arteritis was not observed in all periods studied.

The cross sectional area of left ventricle myocytes was significantly greater in the AoS groups than in the C groups, in all periods studied. In both groups, a progressive increase of the myocyte cross sectional areas occurred during the evolvement process (fig. 2).

Myocardial concentration of hydroxyproline was greater in the AoS groups than in C groups, at 6 weeks of evolvement. In the C groups the concentration was similar in all periods of evaluation, and in AoS, a progressive increase in their values occurred with the evolvement of the hypertrophic process (fig. 3).

Mean and standard deviations of the left ventricle, left atrium, and aorta measures are presented in table II. LVDD was similar in the C groups and AoS in the 3 periods assessed and was greater in the 12- and 21-week periods, compared with that at 6 weeks, in both groups. When normalized for BW, the variable had similar patterns in the C and AoS groups, with a progressive reduction in their values during evolvement assessment ($P<0.05$). LVSD was smaller in $AoS_6$ than in $C_6$. In the C group, this parameter did not change in the experimental period, and in the AoS group, the 21-week period was significantly greater than that at 6 weeks. LVDT and LVDT/LVDD were greater in the AoS group in the 3 periods assessed and did not change with the age of the animal or the evolvement of the hypertrophic process. LA diameter was greater in the AoS group in the 3 periods, and its greatest value was in $AoS_{21}$ rather than in $AoS_6$ and $AoS_{12}$. AO diameter was greater in $AoS_{12}$ than in $C_{12}$. The LA/AO ratio was greater in group AoS in the 3 periods studied. In the 2 groups of animals, LA/AO did not change during evolvement, and LA/BW was greater in period 6 than in the 12- and 21-week periods.

Means and standard deviation of the heart rate (HR), and the systolic and diastolic function indexes are demonstrated in table III. Heart rate was greater in the AoS group in the 12- and 21-week periods, compared with the same period in group C. In group C, HR was lower in $C_{21}$ than in $C_6$, and in AoS this variable did not occur in the 3 periods analyzed. DD was greater in $AoS_6$ than in $C_6$. In group C, this index did not change with age, and in the AoS group a decrease occurred in its value in the 21-week period compared with that in the 6- and 12-week periods. E/A ratio was greater in $AoS_{12}$ and $AoS_{21}$ compared with that in groups C and $AoS_6$. In the control group, E/A did not change with age.

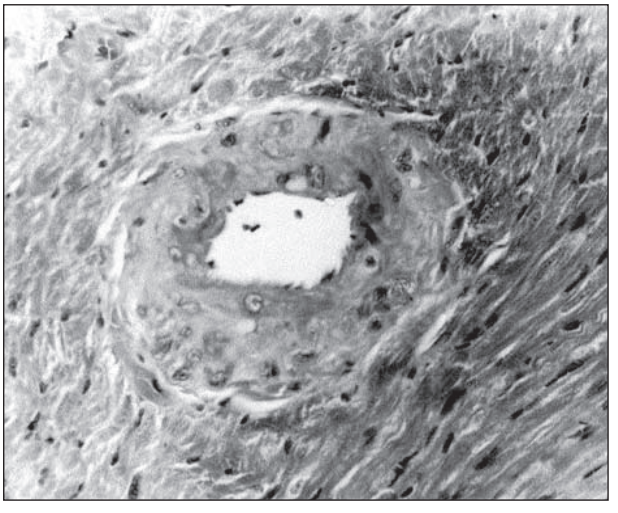

Fig. 1 - Medium layer hypertrophy of the intramural branch of the coronary artery in a rat with supravalvular aortic stenosis after 21 weeks. HE. 400X.

## Discussion

In this study, we assessed the cardiac function, morphology, and development of myocardial fibrosis in young rats undergoing induction of supravalvular aortic stenosis in different periods of follow-up.

AoS induction rapidly encourages left ventricle hypertrophy, which is evident in the early period of evaluation, with 2 weeks of follow-up. After 6 weeks, left ventricle weight or ventricular wall diastolic thickness did not increase, which could suggest that, after aortic stenosis induction, left ventricular hypertrophy occurs precociously and

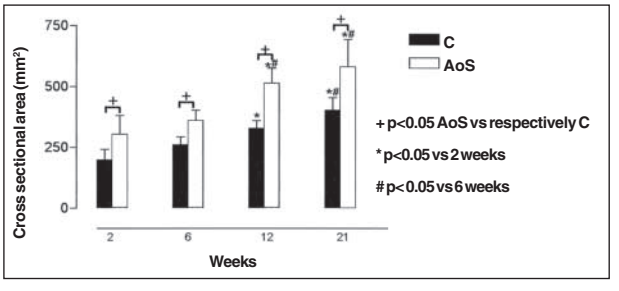


Fig. 2 - Myocyte cross sectional area of the left ventricle in the control groups (C) and supravalvular aortic stenosis (AoS) after 2, 6, 12, and 21 weeks of follow-up. Each column represents mean ± standard deviation +, *, # indicate significant differences between the groups according to the picture (ANOVA-two-way and *Tukey* test for $P<0.05$).

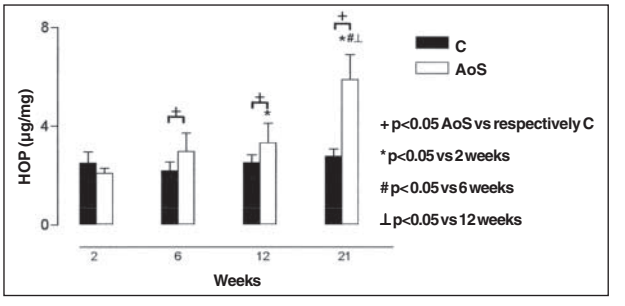


Fig. 3 - Myocardial concentration of hydroxyproline (HOP) of the control groups (C) and supravalvular aortic stenosis (AoS) after 2, 6, 12, and 21 weeks of follow-up. Each column represents mean ± standard deviation. +, *, #, - indicate significant differences between the groups according to the picture (ANOVA-two-way and Tukey test for $P<0.05$).

**Table II - Echocardiographic analysis of the structural parameters of the heart**

| | | Experimental period (weeks) 6 (n=12) | 12 (n=12) | 21 (n=12) |
|---|---|---|---|---|
| LVDD (mm) | C | 7.51 ± 0.55[a] NS | 8.16 ± 0.64[b] NS | 8.39 ± 0.58[b] NS |
| | AoS | 7.38 ± 0.58[a] | 8.34 ± 0.96[b] | 8.50 ± 0.78[b] |
| LVDD/BW (mm/kg) | C | 23.8 ± 1.6[c] NS | 19.7 ± 2.2[b] NS | 16.9 ±1.2[a] NS |
| | AoS | 25.7 ± 3.3[c] | 21.5 ± 3.3[b] | 18.8 ± 3.1[a] |
| LVDS (mm) | C | 2.91 ± 0.47[a] * | 3.19 ± 0.73[a] NS | 3.35 ± 0.37[a] NS |
| | AoS | 2.24 ± 0.99[a] | 2.73 ± 1.52[ab] | 3.22 ± 1.36[b] |
| LVDT (mm) | C | 1.51 ± 0.16[a] * | 1.49 ± 0.13[a] * | 1.50 ± 0.09[a] * |
| | AoS | 1.76 ± 0.18[a] | 1.92 ± 0.22[a] | 1.95 ± 0.16[a] |
| LVDT/LVDD | C | 0.20 ± 0.03[a] * | 0.18 ± 0.02[a] * | 0.18 ± 0.01[a] * |
| | AoS | 0.24 ± 0.03[a] | 0.23 ± 0.03[a] | 0.23 ± 0.02[a] |
| LA (mm) | C | 5.03 ± 0.79[a] * | 5.24 ± 0.62[a] * | 5.47 ± 0.68[a] * |
| | AoS | 7.20 ± 1.80[a] | 7.35 ± 0.89[a] | 8.44 ± 1.02[b] |
| LA (mm) | C | 3.49 ± 0.24[a] NS | 3.53 ± 0.29[a] * | 3.79 ± 0.22[b] NS |
| | AoS | 3.47 ± 0.29[a] | 3.77 ± 0.25[b] | 3.96 ± 0.26[b] |
| LA/AO | C | 1.45 ± 0.24[a] * | 1.49 ± 0.21[a] * | 1.45 ± 0.16[a] * |
| | AoS | 2.08 ± 0.48[a] | 1.95 ± 0.25[a] | 2.14 ± 0.26[a] |
| LA/BW (mm/kg) | C | 15.9 ± 2.6[b] * | 12.5 ± 1.4[a] * | 11.0 ± 1.3[a] * |
| | AoS | 25.3 ± 7.7[b] | 18.5 ± 2.2[a] | 18.6 ± 2.8[a] |

C- control; AoS- supravalvular aortic stenosis; LVDD and LVDS- left ventricle (LV) diastolic and systolic diameters; BW- body weight; LVDT: LV wall diastolic thickness; LA- left atrium diameter; AO- aorta diameter. Variance analysis of the multivariate profiles for dependent groups (MANOVA). [a, b, c] - groups that do not have the same letters have statistically significant differences between the 3 evaluation periods (P<0.05); * P<0.05 vs respective control group; NS - nonsignificant.

then it becomes stable. However, morphometric evaluation demonstrated that the values of the cross sectional area of the myocytes increased progressively until 12 weeks, demonstrating the presence of additional hypertrophy during the evolvement of the process.

Echocardiographic study allowed the definition of left ventricular hypertrophy as concentric, characterized by increased wall thickness with normal or reduced dimensions of the ventricular cavity. According to several authors [7,26], animals start to develop heart failure in the 21st week after the induction of aortic stenosis. Thus, it would be expected that eccentric hypertrophy would be found during the last period of study. Because only 25% of our rats had heart failure after the 21 weeks of evolvement, it is probable that the final evaluation was performed when most animals were in the compensated phases of left ventricular hypertrophy and, therefore, still having concentric hypertrophy. However, the increase in right ventricle weight in the last assess-

**Table III - Left ventricle functional evaluation with echocardiography**

| | | Experimental period (weeks) 6 (n=12) | 12 (n=12) | 21 (n=12) |
|---|---|---|---|---|
| HR (bpm) | C | 281 ± 22[a] NS | 265 ± 26[ab] * | 245 ± 15[b] * |
| | AoS | 291 ± 21[a] | 291 ± 22[a] | 271 ± 29[a] |
| ΔD (%) | C | 61.3 ± 5.4[a] * | 61.2 ± 6.6[a] NS | 60.1 ± 2.9[a] NS |
| | AoS | 70.3 ± 10.8[b] | 68.4 ± 12.2[b] | 62.8 ±11.4[a] |
| E/A | C | 1.64 ± 0.27[a] NS | 1.61 ± 0.16[a] * | 1.72 ± 0.33[a] * |
| | AoS | 2.07 ± 1.55[a] | 4.20 ± 3.25[b] | 4.71 ± 2.08[b] |

C- control rats; AoS- rats undergoing supravalvular aortic stenosis; HR- heart rate; ΔD- shortening percentage; E/A- ratio between the initial filling flow velocity (E wave) and the atrial contraction (A wave) of the transmitral flow. Variance analysis of the multivariate profiles for dependent groups (MANOVA). [a, b] - groups that do not have the same letters have statistically significant differences between the 3 evaluation periods (P<0.05); * P<0.05 vs respective control group; NS- nonsignificant.

ment period demonstrates that the change from compensated left ventricular hypertrophy to heart failure may have already occurred. Histological analysis of the myocardium confirmed the findings of other authors [7,26], who did not find relevant tissue lesions, such as myocyte necrosis, extensive areas of myocardial fibrosis, or peripheral arteritis, after 21 weeks of aortic stenosis induction. In our study, in addition to myocyte cell hypertrophy, we observed the presence of vascular remodeling characterized by medium-layer hypertrophy in the intramyocardial arteries, which was discreet in the first assessment periods and became evident after 12 weeks of follow-up. Thickening of the medium layer of the arteries is usually found in human beings with systemic hypertension [40]. This finding may be due to hypertrophy or hyperplasia of the smooth muscle, or both; to an increase in water and/or collagen content of the vascular wall; and thickening of the basal membrane [40].

Although this model is not associated with severe fibrosis [7], myocardial concentration of hydroxyproline was greater in the AoS groups at 6 weeks, and it increased progressively during the evolvement of the hypertrophic process. In the present study, we did not perform morphometric quantification of the collagen content and, therefore, we were not able to state whether the increase was due to the interstitial collagen or perivascular. Weinberg et al [26], using *picrosirius* red dye, verified the presence of a moderate increase in interstitial and perivascular collagen 21 weeks after the induction of aortic stenosis.

The analysis of left ventricle systolic function demonstrated that in the initial phases of the hypertrophic process the shortening is increased. A decrease in contractile function of the chamber occurred over time, evidenced by the progressive increase in LVSD and by the reduction of ΔD. These indexes were significantly different between the periods of 6 and 21 weeks after aortic stenosis. It is possible to detect the 3 evolvement phases of systolic function during the hypertrophic process in this model: initially, the presence of supranormal performance, which is followed by unchanged cardiac performance, and, finally, by depression of the contractile capacity of the heart. Similar results were observed by other authors who also assessed cardiac function sequentially with echocardiography [7,22].

Diastolic dysfunction may be detected in the 12th week of evolvement by the E/A ratio, which was significantly greater in the AoS groups than in the respective C groups. After this period, additional variation was not observed in the values of this ratio. However, the increase in LA diameter, verified in the 6th week, demonstrates that diastolic dysfunction may have occurred earlier. Similar results were observed during the serial study of left ventricle function with echocardiography [22]. E/A increase reflects the restrictive pattern of ventricular filling alteration. Currently, this filling model has been attributed, in great part, to the increase in left atrial pressure and also to the increase in passive stiffness of the ventricular chamber, which may be due to the increase in wall thickness or interstitial fibrosis, or both of these [22].

In conclusion, induction of supravalvular aortic stenosis in young rats promotes early development of concentric left ventricular hypertrophy with cardiac function initially supranormal, followed by diastolic dysfunction in the 12th week of evolvement. Reduction of systolic ventricular performance occurred only in the late period after 21 weeks of AoS induction. Cardiac function alterations are followed by myocardial fibrosis without expressive histological lesions. These characteristics demonstrate that the model is suitable and potentially useful for studies on physiopathology and treatment of cardiac hypertrophy due to chronic pressure overload both in the compensated stage and during transition to heart failure.

## Acknowledgments

For the valuable technical support of Vitor Marcos de Souza, José Carlos Georgette, and Mário Augusto Dallaqua and to the support of CNPQ/PIBIC.

## References

1. Cicogna AC, Okoshi MP, Okoshi K. História natural da remodelação miocárdica: da agressão aos sintomas. Rev Soc Cardiol Estado de São Paulo 2000; 10:8-16.
2. Olivetti G, Cigola E, Maestri R, Lagrasta C, Corradi D, Quaini F. Recent advances in cardiac hypertrophy. Cardiovasc Res 2000; 45:68-75.
3. Kaplan NM. Systemic hypertension: mechanisms and diagnosis. In: Braunwald E, Zipes DP, Libby P, eds. Heart Disease. A Textbook of Cardiovascular Medicine. Philadelphia: W.B. Saunders Company, 2001:941-71.
4. Okoshi MP, Matsubara LS, Franco M, Cicogna AC, Matsubara BB. Myocyte necrosis is the basis for fibrosis in renovascular hypertensive rats. Braz J Med Biol Res 1997; 30:1135-44.
5. Rossi MA, Peres LC. Effect of captopril on the prevention and regression of myocardial cell hypertrophy and interstitial fibrosis in pressure overload cardiac hypertrophy. Am Heart J 1992; 124:700-9.
6. Rodrigues MAM, Bregagnollo EA, Montenegro MR, Tucci PJF. Coronary vascular and myocardial lesions due to experimental constriction of the abdominal aorta. Int J Cardiol 1992; 35:253-7.
7. Weinberg EO, Schoen FJ, George D, et al. Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 1994; 90:1410-22.
8. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J 1963; 27:282-93.
9. Bing OHL, Brooks WW, Robinson KG, et al. The spontaneously hypertensive rat as a model of the transition from compensated left ventricular hypertrophy to failure. J Mol Cell Cardiol 1995; 27:383-96.
10. Brooks WW, Bing OHL, Robinson KG, Slawsky MT, Chaletsky DM, Conrad CH. Effect of angiotensin-converting enzyme inhibition on myocardial fibrosis and function in hypertrophied and failing myocardium from the spontaneously hypertensive rat. Circulation 1997; 96:4002-10.
11. Cicogna AC, Robinson KG, Conrad CH, et al. Direct effects of colchicine on myocardial function. Studies in hypertrophied and failing spontaneously hypertensive rats. Hypertension 1999; 33:60-5.

12. Cicogna AC, Padovani CR, Okoshi K, Aragon FF, Okoshi MP. Myocardial function during chronic food restriction in isolated hypertrophied cardiac muscle. Am J Med Sci 2000; 320:244-8.
13. Hirsch AT, Talsnec CE, Schunkert H, Paul M, Dzau VT. Tissue specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ Res 1991; 69:475-82.
14. Kabour A, Henegar JR, Devineni VR, Janicki JS. Prevention of angiotensin II induced myocyte necrosis and coronary vascular damage by lisinopril and losartan in the rat. Cardiovasc Res 1995; 29:543-8.
15. Ratajska A, Campbell SE, Sun Y, Weber KT. Angiotensin II-associated cardiac myocyte necrosis: role of adrenal catecholamines. Cardiovasc Res 1994; 28:684-90.
16. Trippodo NC, Frohlich ED. Similarities of genetic (spontaneous) hypertension: man and rat. Circ Res 1981; 48:309-19.
17. Cicogna AC, Padovani CR, Georgette JC, Aragon FF, Okoshi MP. Efeito da restrição protéico-calórica sobre a função mecânica dos músculos cardíacos hipertrofiados. Arq Bras Cardiol 1999; 72:431-5.
18. Cicogna AC, Robinson KG, Conrad CH, Squire R, Okoshi MP, Bing OHL. Participação do estado contrátil e do relaxamento miocárdico na disfunção ventricular durante a transição hipertrofia-falência cardíaca. Arq Bras Cardiol 1997; 69:381-4.
19. Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. J Clin Invest 1990; 86:1913-20.
20. Feldman AM, Weinberg EO, Ray PE, Lorell BH. Selective changes in cardiac gene expression during compensated hypertrophy and the transition to cardiac decompensation in rats with chronic aortic banding. Circ Res 1993; 73:184-92.
21. Bruckschlegel G, Holmer SR, Jandeleit K, et al. Blockade of the renin-angiotensin system in cardiac pressure-overload hypertrophy in rats. Hypertension 1995; 25:250-9.
22. Litwin SE, Katz SE, Weinberg EO, Lorell BH, Aurigemma GP, Douglas PS. Serial echocardiographic-doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy: chronic angiotensin-converting enzyme inhibition attenuates the transition to heart failure. Circulation 1995; 91:2642-54.
23. Turcani M, Rupp H. Development of pressure overload induced cardiac hypertrophy is unaffected by long-term treatment with losartan. Mol Cell Biochem 1998; 188:225-33.
24. Condorelli G, Morisco C, Stassi G, et al. Increased cardiomyocyte apoptosis and changes in proapoptotic and antiapoptotic genes bax and bcl-2 during left ventricular adaptations to chronic pressure overload in the rat. Circulation 1999; 99:3071-8.
25. Schunkert H, Orzechowski HD, Bocker W, Meier R, Riegger GAJ, Paul M. The cardiac endothelin system in established pressure overload left ventricular hypertrophy. J Mol Med 1999; 77:623-30.
26. Weinberg EO, Lee MA, Weigner M, et al. Angiotensin AT1 receptor inhibition: effects on hypertrophic remodeling and ACE expression in rats with pressure-overload hypertrophy due to ascending aortic stenosis. Circulation 1997; 95:1592-600.
27. Gonçalves G, Okoshi K, Okoshi MP, et al. Efeito da inibição da enzima de conversão da angiotensina sobre a hipertrofia ventricular esquerda induzida por sobrecarga pressórica. Rev Soc Cardiol Estado de São Palulo 1998; 8:2.
28. Bregagnollo EA, Okoshi K, Okoshi MP, Padovani CR, Cicogna AC. Efeitos do lisinopril sobre as características da hipertrofia ventricular esquerda na vigência de sobrecarga pressórica persistente. Rev Soc Cardiol Estado de São Paulo 2001; 11:32.
29. Zierhut W, Zimmer H-G, Gerdes AM. Effect of angiotensin converting enzyme inhibition on pressure-induced left ventricular hypertrophy in rats. Circ Res 1991; 69:609-17.
30. Böcker W, Hupf H, Grimm D, Kurzidim K, Schunkert H. Effects of indapamide in rats with pressure overload left ventricular hypertrophy. J Cardiovasc Pharmacol 2000; 36:481-6.
31. Grimm D, Huber M, Jabusch HC, et al. Extracellular matrix proteins in cardiac fibroblasts derived from rat hearts with chronic pressure overload: effects of beta-receptor blockade. J Mol Cell Cardiol 2001; 33:487-501.
32. Switzer BR. Determination of hydroxyproline in tissue. J Nutr Biochem 1991; 2:229-321.
33. Matsubara LS, Matsubara BB, Okoshi MP, Franco M, Cicogna AC. Myocardial fibrosis rather than hypertrophy induces diastolic dysfunction in renovascular hypertensive rats. Can J Physiol Pharmacol 1997; 75:1328-34.
34. Matsubara LS, Bojikian BB, Okoshi MP, Cicogna AC, Janicki JS. Alterations in myocardial collagen content affect rat papillary muscle function. Am J Physiol 2000; 279:1534-9.
35. Okoshi MP, Okoshi K, Dal Pai V, Dal Pai-Silva M, Matsubara LS, Cicogna AC. Mechanical, biochemical, and morphological changes in the heart from chronic food restricted rats. Can J Physiol Pharmacol 2001; 79:754-60.
36. Okoshi K. Estrutura e Função do Coração de Ratos Normotensos e Hipertensos Submetidos à Restrição da Ingestão Alimentar: Estudo In Vivo Pelo Ecocardiograma e In Vitro do Coração Isolado. Botucatu: UNESP, 2000:106p.
37. Simone G, Devereux R, Camargo M, et al. In vivo left ventricular anatomy in rats with two-kidney, one clip and one-kidney, one clip renovascular hypertension. J Hypertens 1992; 10:725-32.
38. Douglas PS, Katz SE, Weinberg EO, Chen MH, Bishop SP, Lorell BH. Hypertrophic remodeling: gender differences in the early response to left ventricular pressure overload. J Am Coll Cardiol 1998; 32:1118-25.
39. Conrad CH, Brooks WW, Robinson KG, Bing OHL. Impaired myocardial function in spontaneously hypertensive rats with heart failure. Am J Physiol 1991; 260:H136-45.
40. Schwartzkopff B, Motz W, Frenzel H, Vogt M, Knauer S, Strauer BE. Structural and functional alterations of the intramyocardial coronary arterioles in patients with arterial hypertension. Circulation 1993; 88:993-1003.