Echocardiographic Criteria for the Definition of Ventricular Dysfunction Severity in Aortic Banded Rats

Vanessa Oliveira Moreira, Ana Valéria Barros de Castro, Marcelle Yumi Yaegaschi, Antonio Carlos Cicogna, Marina Politi Okoshi, Camila Alves Pereira, Flávio Ferrari Aragon, Mário Batista Bruno, Carlos Roberto Padovani, Katashi Okoshi

Faculdade de Medicina de Botucatu and Instituto de Biociências de Botucatu – Unesp - Botucatu, SP - Brazil

Objective

The purpose of this study was to identify echocardiographic parameters that allow distinguishing different levels of cardiac dysfunction in aortic banded rats.

Methods

Wistar male rats (90-100 g) were subjected to aortic banding (n=23) or a sham operation (n=12). The following echocardiographic parameters were evaluated and used to group rats into groups with similar characteristics using cluster analysis: absolute values and after normalization to body weight of left ventricular end-diastolic diameter (LVDD) and left atrial systolic diameter; left ventricular end-systolic diameter (LVSD); LV weight to body weight ratio (LVW/BW); three indexes of left ventricular shortening (endocardial fractional shortening, EFS; midwall FS, MFS; and posterior wall shortening velocity, (PWSV).

Results

The cluster analysis could group aortic banded rats into two groups: mild (n=13) and severe (n=9) stage of heart failure. There was no overlapping among the values of the 95% confidence interval of the following parameters between the two groups: LVDD, LVSD, EFS, MFS, LVW/BW, and PWSV.

Conclusion

It is feasible to distinguish two groups of aortic banded rats according to the level of cardiac dysfunction using those echocardiographic parameters. This allows to perform longitudinal studies in homogeneous groups of rats with aortic banding and cardiac dysfunction.

Key words

Echocardiogram, aortic stenosis, ventricular dysfunction, rats, cluster analysis.

Mailind Address: Katashi Okoshi • Travessa Cyro Targa, 115 - 18610-250 – Botucatu, SP - Brazil
E-mail: okoshi@cardiol.br

Received on 08/05/05 • Accepted on 11/11/05

In experimental models of heart injury, the difficulty in establishing an *in vivo* classification severity of ventricular dysfunction has restricted longitudinal studies aimed at verifying the effect of therapeutic interventions. Obtaining homogeneous groups of animals as regards the degree of heart dysfunction is of fundamental importance, since the beneficial or harmful effects of a treatment can be observed according to the prior impairment of the ventricular function.

The diagnosis of heart dysfunction in animals has traditionally been based on the measurement of left ventricle (LV) diastolic pressure, which requires ventricular catheterization with the risk of lesions to heart valves and the consequent alteration in heart performance[1]. Other criteria to diagnose heart failure (HF) are based on the presence of clinical and anatomical changes such as tachypnea, pleural effusion, ascitis, thrombus in the left atrium and right ventricle hypertrophy; most of these changes are identified after the animal is sacrificed[2-5].

The echocardiogram is an important ancillary exam for the clinical diagnosis of HF. It allows the evaluation of: 1) heart morphology and function; 2) progression of heart dysfunction caused by different types of injuries[6-8]; 3) effects of pharmacological interventions on the heart [4].

In small animals, the transthoracic echocardiogram is one of the most frequently used methods to assess the size of heart structures, and the contractile performance of the LV[4-10]. The identification of ventricular dysfunction is generally carried out by comparing the muscular shortening indexes with those obtained in the control group.

Sjaastad et al[1] have recently identified the most important variables for the echocardiographic diagnosis of HF in infarcted rats using the cluster analysis statistical method. This method was also used to determine the progression of heart function during the remodeling induced by the ligation of the coronary artery[2]. However, there are no reports in the literature about echocardiographic studies on experimental models of aortic stenosis (AS) focusing on degrees of ventricular dysfunction. The AS rat model is widely used because it induces gradual ventricular hypertrophy which progresses towards ventricular dysfunction. In this model, a clip is placed in the ascending aorta and stenosis develops in a proportionate fashion according to the growth of the animal's body.

We hypothesized that cluster analysis will also allow us to identify criteria which can distinguish the different degrees of heart dysfunction as of the occurrence of tachypnea, in a model of pressure overload induced by the clamping of the root of the aorta that usually progresses towards LV hypertrophy and progressive HF[11,12].

The objective of this study was therefore to identify the echocardiographic variables that define the degrees of ventricular dysfunction from the compensated hypertrophy phase through the uncompensated phase in rats with AS. The identification of these variables will allow us to establish the stages of heart remodeling induced by pressure overload and to assess the effects of pharmacological interventions on the heart at different times of the progression of heart dysfunction.

Methods

Animals and experimental protocol - The procedures of the experiment were approved by the Ethics Committee for Research on Animals of the Medical School of Botucatu (Faculdade de Medicina de Botucatu, Unesp).

In this study, we used Wistar male rats, with body weight between 90 and 100 g, grown in the Central Bioterium – Unesp, Botucatu, Brazil. The animals were kept in individual cages at 23ºC room temperature, with 12-hour light/dark cycles. The animals were feed during all the experiment with Labina® chow and water *ad libitum*. The study included 35 animals. 12 were in the control group and 23 had AS.

Induction of heart failure through aortic stenosis - Aortic stenosis was induced according to the method previously described in our laboratory[5,13,14]. In summary, the animals were submitted to median thoracotomy and trichotomy after anesthesia with ketamine hydrochloride (50 mg/kg intraperitoneally) and xylazine hydrochloride (10 mg/kg intraperitoneally). Then the ascending aorta was dissected and a silver clip, with an internal diameter of 0.6 mm was placed at approximately 3 mm of the aorta's root. During the surgery, the rats were manually ventilated using positive pressure and given 1 ml of warm saline solution intraperitoneally. The control animals underwent the same surgery, but with no clip placed in the aorta.

Nineteen weeks after the induction of AS, the animals were observed daily to allow the detection of tachypnea, which is a sign of heart dysfunction.

Echocardiographic Study - The echocardiogram was performed after the detection of tachypnea, which is generally observed after 28 weeks of the AS.

The rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1 mg/kg), administered intraperitoneally. Following the trichotomy of the anterior part of the thorax, the animals were placed in the left lateral decubitus, and the exam was performed using a Hewlett-Packard Co. Sonos 2000 device, equipped with a 7.5 MHz electronic transducer. In order to measure the heart structures, we obtained monodimensional images (M-mode), guided by the bidimensional mode images, with the transducer in the parasternal short-axis position.

The LV assessment was performed with the M-mode cursor positioned just below the mitral valve plane at the level of the papillary muscles[15-17]. The image of the left atrium was obtained by positioning the M-mode cursor at

the level of the aortic valve plane. The images obtained in M-mode were recorded on a Sony Co. UP-890 printer.

Later heart structures were measured manually using a caliper. The following heart structures were measured: LV diastolic and systolic diameters (LVDD and LVSD respectively), LA diameter (LA) and the diastolic thickness of the interventricular septum (IST) and of the LV posterior wall (PWT).

LVDD and LA values were normalized for body weight (BW). LV systolic function was assessed based on the endocardial fractional shortening, EFS [(LVDD – LVSD) / LVDD X 100]; midwall fractional shortening, MFS, which is the variation between the diastolic and systolic values of the formula [(LV + ½ interventricular septum thickness + ½ posterior wall thickness) x 100]; and LV posterior wall shortening velocity, PWSV (maximum tangent of posterior wall systolic movement).

Left ventricle mass (LVM) was calculated using the following formula [7,15]: $[(LVDD + IST + PWT)^3 – LVDD^3] \times 1.04$ where the value 1.04 indicates the specific density of the myocardium. The LVM index (LVMI) was calculated by normalizing LVM for body weight.

Cluster analysis at stages of heart dysfunction - Considering the echocardiographic variables of 23 animals with As separately, we used the cluster analysis technique to establish similarity groups as to the severity of the disease. We used echocardiographic variables whose values increase progressively with the progression of the disease (LVDD, LA and LVMI), in addition to parameters indicative of the systolic function (EFS, MFS and PWSV) and LVSD, which is also related with the LV systolic function. The procedure employed to analyze the conglomerates considered the SAHN strategy for the formation of groups (SAHN involves sequential, agglomerative, hierarchical and non-overlapping clusters); the method of clustering was the closest neighbor technique (SLM: single linkage method), and the similarity coefficient was the median Euclidean distance[18]. The final representation of the cluster is described by the dendogram (fig.1).

Results

The cluster analysis allowed us to place rats with AS into similar groups, according to the severity of the ventricular dysfunction. These groups were called mild dysfunction (n = 13) e severe dysfunction (n = 9) groups. In the statistical analysis, it was impossible to include one animal with AS into the groups mentioned above (fig.1).

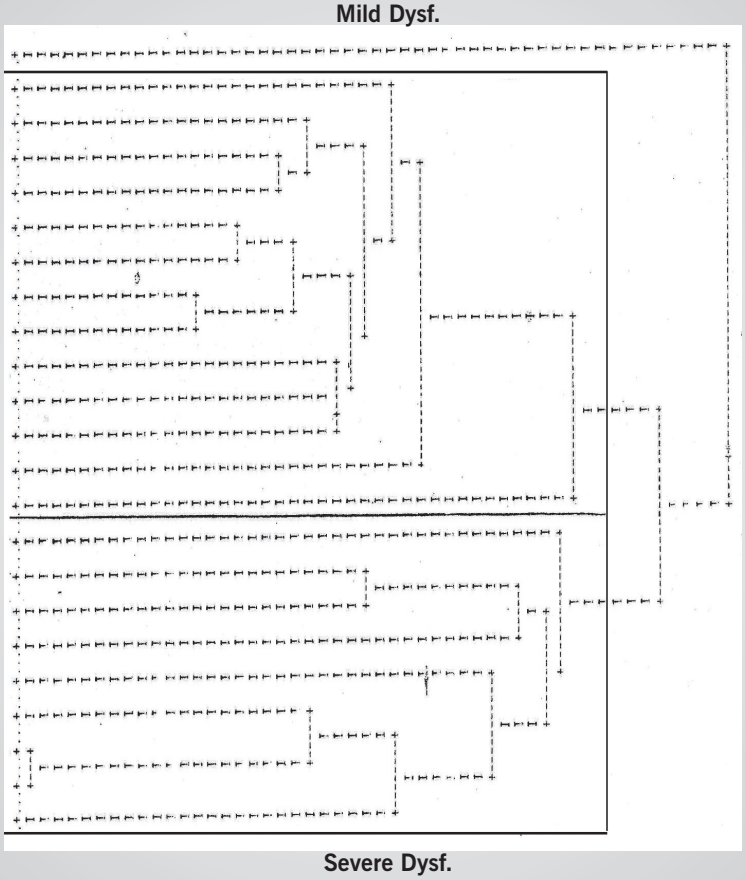

Fig. 1 *– Dendogram of cluster analysis according to the echocardiographic variables of rats with aortic stenosis and ventricular dysfunction.*

This animal had the lowest values for LV systolic function indexes and the highest values for LV and LA diameters among animals with AS. These values were outside the limits of the confidence intervals of the severe dysfunction group. Therefore, in our group of rats with AS, one animal presented more severe ventricular dysfunction than the group called severe dysfunction.

Table 1 shows the mean values ± standard deviation and the respective limits of the 95% confidence intervals for the echocardiographic variables analyzed considering the control and AS groups. The limits of the 95% confidence intervals are illustrated in figures 2 and 3. The echocardiographic variables which indicate LV and left atrium size and LV mass, normalized for body weight, presented higher values in the severe-AS group as compared with the mild-AS group. The variables indicative of left ventricle shortening (EFS, MFS and %Myoc.Short.) presented lower values in the severe-AS as compared with the mild-AS group. The confidence intervals of the variables LVDD, LVSD, % Endo.Short., % Myoc.Short., LVMI and PWSV did not present overlapping between the two groups.

The following variables obtained for rats without AS (control) showed overlapping with the mild-AS group: LVDD, LVDD/BW and PWSV. There was overlapping between the control groups and the severe-AS group for the variables LVSD, % Endo.Short. and % Myoc. Short. The control group presented no overlapping of confidence intervals with other groups for the values of LA/BW and LVMI.

Discussion

The emergence and development of the echocardiogram was a great advancement for *in vivo* assessment of the heart. In small animals used in experimental laboratories, this method enabled the follow-up of the effect of injuries and/or treatment on the heart, in an unlimited fashion as regards the number of times the exam is repeated. In rats with AS, the clinical signs that identify HF severity *in vivo* are limited. Additionally, the time elapsed until the first signs of HF appear is variable. In our laboratory, usually from 29 to 37 weeks elapse between the induction of AS and the appearance of tachypnea.

In the present study, the echocardiogram showed that the rats had different degrees of heart impairment when tachypnea was observed. Traditionally, in experimental studies, drugs are introduced at a predetermined time after the induction of AS[11,19]. Therefore, it may be that the animals were at different stages of heart impairment when the treatment was started. This heterogeneity is an important factor that may lead to discrepant results. In view of this, it is fundamental to seek echocardiographic criteria to classify the animals as regards the severity of ventricular dysfunction, thus allowing the use of animals with the most homogeneous degree of heart functional and structural impairment.

The cluster analysis technique is frequently used to group the object of study according to the similarity of its characteristics[1,20,21]. Sjaastad et al[1] established echocardiographic criteria for the detection of HF in rats, following myocardial infarction using the cluster analysis technique. In our study, using this statistical analysis, we aimed at classifying the heart impairment of animals with AS and tachypnea. With the number of rats used in this study, the cluster analysis allowed us to separate the rats with AS into two groups of animals which shared similar echocardiographic characteristics (mild-AS and severe-AS).

The variables LVDD, LVSD, % Endo.Short., % Myoc. Short., LVMI and PWSV did not present confidence interval values overlapping for the two groups. The variables LVDD/BW, LA and LA/BW presented overlapping between the mild-AS and severe-AS groups. Because LV and LA diameters are subject to the influence of body weight (BW) variation, their values have to be normalized for

Table 1 – Values of mean ± standard deviations and 95% confidence intervals of the echocardiographic variables studied

	Control (n=12)		Mild dysfunction (n=13)		Severe dysfunction (n=9)	
Variable	Mean ± SD	Confidence limit	Mean ± SD	Confidence limit	Mean ± SD	Confidence limit
LVDD	8.22±0.56	7.86-8.57	8.38±0.41	8.07-8.70	9.17±0.68	8.76-9.58
LVSD	3.96±0.57	3.60-4.32	2.60±0.67	2.08-3.11	4.56±0.76	4.09-5.02
LA	5.95±0.71	5.50-6.40	7.63±0.98	6.88-8.38	8.81±1.13	8.12-9.49
LVDD/BW	14.90±0.85	14.37-15.44	16.17±1.97	14.65-17.69	18.80±1.98	17.60-20.00
LA/BW	10.82±1.51	9.86-11.78	14.80±3.02	12.49-17.13	18.07±2.66	16.46-19.68
EMS	51.97±4.48	49.12-54.82	69.24±6.70	64.09-74.39	50.54±5.40	47.28-53.81
MFS	30.98±2.88	29.15-32.81	39.70±3.49	36.99-42.35	27.65±3.20	25.71-29.60
LVM index	1.89±0.28	1.71-2.07	2.77±0.54	2.35-3.18	3.69±0.74	3.25-4.14
PWSV	45.63±6.67	41.36-49.86	45.26±4.42	41.86-48.65	32.85±4.11	30.37-35.34

LVDD: left ventricle diastolic diameter in mm; LVSD: LV systolic diameter in mm; LA: left atrium diameter in mm; BW: body weight in kg; % Endo. Short.: percentage of endocardial shortening of LV; % Myoc.Short.: percentage of shortening of the central region of LV walls; LVM index: mass of LV/BW (g/kg); PWSV: LV posterior wall shortening velocity (mm/s)

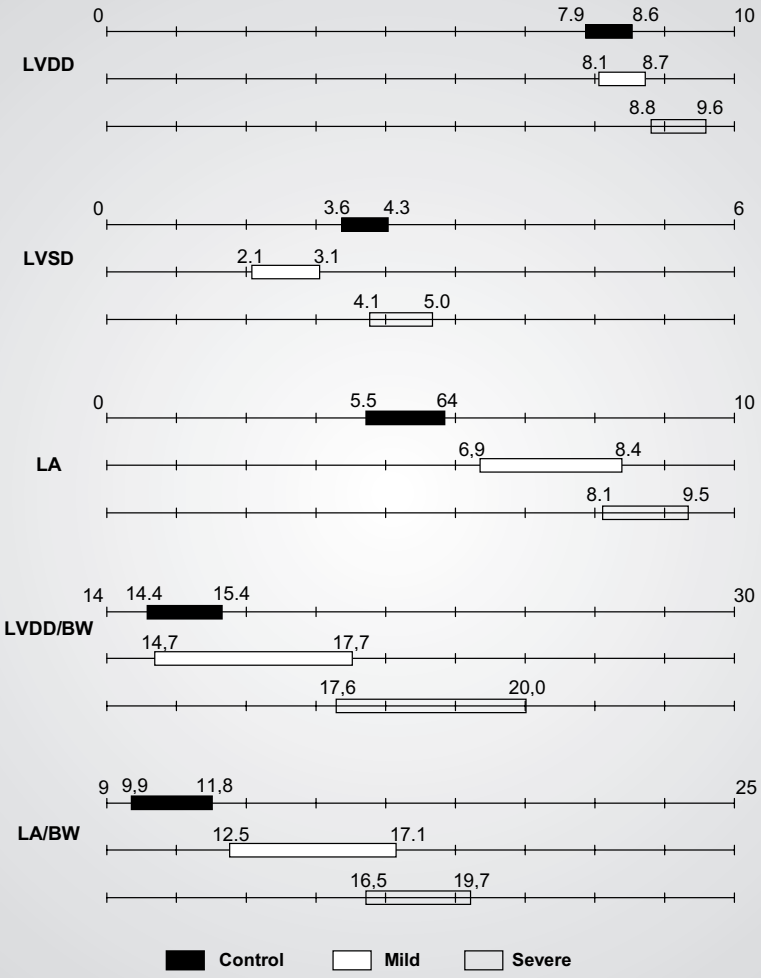

***Fig. 2** – Confidence interval of echocardiographic variables of rats with aortic stenosis and ventricular dysfunction, grouped according to the cluster analysis technique. LVDD: left ventricle diastolic diameter; LVSD: LV systolic diameter; LA: left atrium diameter; LVDD/BW: LVDD normalized for body weight; LA/BW: LA normalized for body weight*

BW. However, this procedure can also cause errors in the interpretation of the results since the LV and LA diameters are not exactly proportional to BW. Therefore, the joint use of variables which did not present overlapping, with the exception of LVDD and LVSD, will allow an utmost safe distribution of rats with aortic stenosis into two different degrees of ventricular dysfunction. The absolute values of LVDD and LVSD are to be used with caution because these parameters usually require normalization in accordance to the animals' body weight.

As described in the results section, some variables presented overlapping between the control and AS groups. This overlapping is understandable, if we consider the ventricular remodeling that takes place after the placement of the clip in the aorta[12]. In this model of pressure overload, as the animal grows, progressive stenosis develops in the LV anterograde flow with the consequent development of myocardial hypertrophy. This increase in myocardial thickness occurs concurrently with a progressive increase in ventricular shortening index values while LVDD values (concentric hypertrophy) remain stable and LVSD decreases.

Even before reaching the peak of systolic shortening values, the animal begins to present tachypnea due to LV diastolic dysfunction resulting from impaired relaxation and ventricular compliance. When the mechanism of hypertrophy becomes unable to compensate the overload, the enlargement of the ventricle begins, and the indexes of ventricular shortening start to decrease as systolic dysfunction develops with a full blown manifestation of heart failure, although it is difficult to characterize AS severity based on tachypnea, which is the sign commonly observed at this stage of AS. Therefore, even with manifest systolic dysfunction, the values of the ventricular shortening indexes will be elevated as compared with the control animals. With the progression of systolic

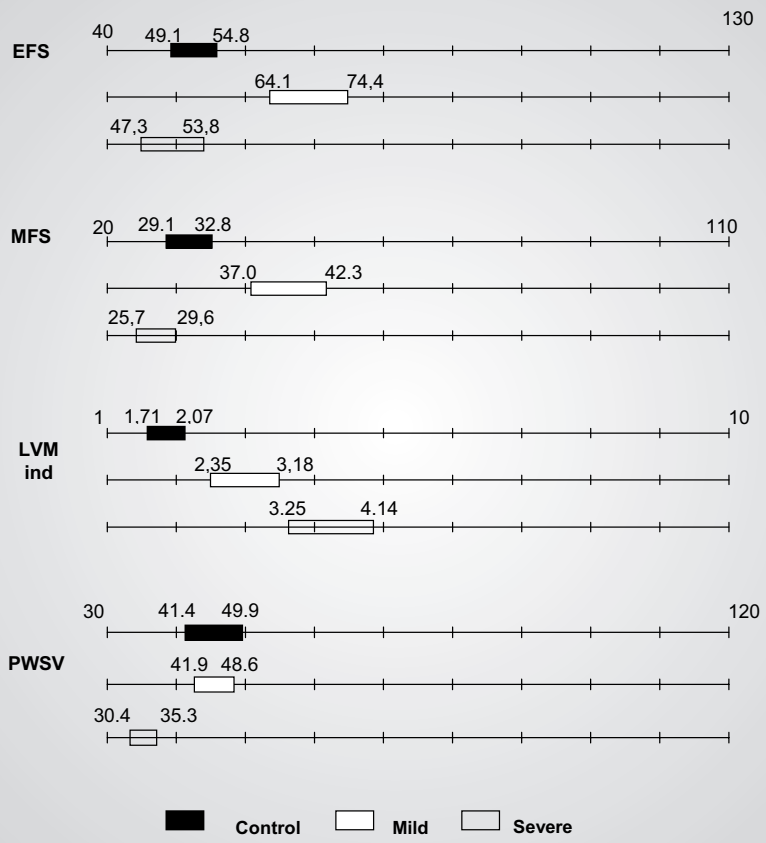

***Fig. 3** – Confidence interval of echocardiographic variables of rats with aortic stenosis and ventricular dysfunction, grouped according to the cluster analysis technique. EFS: endocardial fractional shortening; MFS: midwall fractional shortening; LVMI: left ventricle mass index; PWSV: LV posterior wall shortening velocity*

dysfunction, those indexes will converge to the controls and will eventually become lower. Because of the reasons stated above, the values of muscle shortening indexes did not present overlapping between the control and mild-AS groups and presented overlapping between the control and severe-AS groups.

In an attempt to minimize the effect of myocardial hypertrophy on the increase of the percentage of shortening between the endocardium of the interventricular septum and the LV posterior wall (% Endo.Short.), we also calculated the percentage of shortening between the central points of these walls (% Myoc.Short.)[9,22]. However, the conclusions were not different from the % Endo. Short. index, probably because of the high degree of ventricular hypertrophy. The LV mass index was the only variable which did not present overlapping between the three groups. In our study, the difference between the control and AS groups was also distinguished by using the measure of LV wall thickness (data not presented).

In conclusion, the cluster analysis allows the classification of rats with AS in groups with different degrees of heart impairment. The quantification of ventricular dysfunction and consequently the use of groups of animals with homogeneous heart impairment will play an important role in experimental studies on physiopathology and treatment of ventricular hypertrophy and heart failure.

Acknowledgement

The authors are thankful to Fundunesp (process n.00758/04) and to Novo Nordisk® for the partial funding of this study. The authors are also thankful to Mirella Oliveira Silva for her support in the carrying out of this study.

Potencial Conflict of Interest

No potential conflict of interest relevant to this article was reported.

References

1. Sjaastad I, Sejersted OM, Ilebekk A, Bjornerheim R. Echocardiographic criteria for detection of postinfarction congestive heart failure in rats. J Appl Physiol. 2000; 89: 1445-54.
2. Francis J, Weiss RM, Wei SG, Johnson AK, Felder RB. Progression of heart failure after myocardial infarction in the rat. Am J Physiol Regul Integr Comp Physiol. 2001; 281: R1734-45.
3. Collins KA, Korcarz CE, Lang RM. Use of echocardiography for the phenotypic assessment of genetically altered mice. Physiol Genomics. 2003; 13: 227-39.
4. Satoh S, Ueda Y, Suematsu N, et al. Beneficial effects of angiotensin-converting enzyme inhibition on sarcoplasmic reticulum function in the failing heart of the Dahl rat. Circ J. 2003; 67: 705-11.
5. Bregagnollo EA, Okoshi K, Bregagnollo IF, Padovani CR, Okoshi MP, Cicogna AC. Efeitos da inibição prolongada da enzima de conversão da angiotensina sobre as características morfológicas e funcionais da hipertrofia ventricular esquerda em ratos com sobrecarga pressórica persistente. Arq Bras Cardiol. 2005; 84: 225-32
6. Okoshi K, Matsubara LS, Okoshi MP, et al. Food restriction-induced myocardial dysfunction demonstrated by the combination of *in vivo* and *in vitro* studies. Nutr Res. 2002; 22: 1353-64.
7. Paiva SAR, Zornoff LAM, Okoshi MP, et al. Ventricular remodeling induced by retinoic acid supplementation in adult rats. Am J Physiol Heart Circ Physiol. 2003; 284: H2242-6.
8. Paiva SAR, Zornoff LAM, Okoshi MP, Okoshi K, Cicogna AC, Campana AO. Comportamento de variáveis cardíacas em animais expostos à fumaça de cigarro. Arq Bras Cardiol. 2003; 81: 221-4.
9. Reffelmann T, Kloner RA. Transthoracic echocardiography in rats. Evaluation of commonly used indices of left ventricular dimensions, contractile performance, and hypertrophy in a genetic model of hypertrophic heart failure (SHHF-Mcc-facp-Rats) in comparison with Wistar rats during aging. Basic Res Cardiol. 2003; 98: 275-84.
10. Litwin S, Katz S, Morgan J, Douglas P. Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat. Circulation. 1994; 89: 345-54.
11. Momken I, Kahapip J, Bahi L, et al. Does angiotensin-converting enzyme inhibition improve the energetic status of cardiac and skeletal muscles in heart failure induced by aortic stenosis in rats? J Mol Cell Cardiol. 2003; 35: 399-407.
12. Ribeiro HB, Okoshi K, Cicogna AC, et al. Estudo evolutivo da morfologia e função cardíaca em ratos submetidos a estenose aórtica supravalvar. Arq Bras Cardiol. 2003; 81: 562-8.
13. Gonçalves G, Zornoff LAM, Ribeiro HB, et al. O Bloqueio do sistema renina-angiotensina atenua a remodelação cardíaca de ratos submetidos a estenose aórtica. Arq Bras Cardiol. 2005; 84: 304-8.
14. Carvalho RF, Cicogna AC, Campos GER, et al. Myosin heavy chain expression and atrophy in rat skeletal muscle during transition from cardiac hypertrophy to heart failure. Int J Exp Path. 2003; 84: 201-6.
15. Litwin SE, Katz SE, Weinberg EO, Lorell BH, Aurigemma GP, Douglas OS. 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.
16. Simone G, Devereux RB, Camargo MJ, 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.
17. 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.
18. Padovani CR. Análise de Agrupamento ("Cluster Analysis"). Publicação do Depto. Matemática e Estatística – ESALQ/USP, Piracicaba – SP, 1982, 16p.
19. Butz S, Driamov S, Remondino A, et al. Losartan but not enalaprilat acutely reduces reperfusion ventricular tachyarrhythmias in hypertrophied rat hearts after low-flow ischaemia. J Pharm Pharmacol. 2004; 56: 521-8.
20. Hansell AL, Lam KA, Richardson S, Visick G, Soriano JB. Medical event profiling of COPD patients. Pharmacoepidemiol Drug Saf. 2004; 13: 547-55.
21. Ebrahim S, Montaner D, Lawlor DA. Clustering of risk factores and social class in childhood and adulthood in British women's heart and health study: cross sectional analysis. BMJ. 2004; 328: 861-5.
22. Ballo P, Mondillo S, Guerrini F, Barbati R, Picchi A, Focardi M. Midwall mechanics in physiologic and hipertensive concentric hypertrophy. J Am Soc Echocardiogr. 2004; 17: 418-27.