Relative Role of Left Ventricular Geometric Remodeling and of Morphological and Functional Myocardial Remodeling in the Transition from Compensated Hypertrophy to Heart Failure in Rats with Supravalvar Aortic Stenosis

Edson Antonio Bregagnollo, Marco Aurélio Mestrinel§, Katashi Okoshi*, Fábio Cardoso Carvalho*, Isamara Fernanda Bregagnollo#, Carlos Roberto Padovani+, Antonio Carlos Cicogna**

**Faculdade de Medicina de Botucatu-UNESP, §Pós-Graduando:Fisiopatologia em Clínica Médica- UNESP, +Instituto de Biociências-UNESP; # Faculdade de Medicina de Marília (FAMEMA) – Botucatu, Marilia - SP - Brazil*

Summary

Objectives: To evaluate the relative contribution of left ventricular (LV) geometric remodeling and of morphological and functional myocardial changes in rats with induced supravalvar aortic stenosis (SAS), in the transition from compensated hypertrophy to congestive heart failure (CHF).

Methods: Twenty one weeks after induction of SAS, the rats were classified as controls (CG, n=13), without congestive heart failure (SG, n=11), or with congestive heart failure (SG-HF, n=12). All groups were evaluated with echocardiographic, hemodynamic and morphological study of the myocardium.

Results: Twenty one weeks after SAS: mass index (SG-HF>SG>CG, p<0.05); systolic pressure (SG-HF= SG>CG, p<0.05); diastolic pressure (SG-HF>SG>CG, p<0.05); systolic and diastolic meridional stress (SG-HF>SG>CG, p<0.05); LV myocyte cross-sectional area (SG-HF>SG>CG, p<0.05) and hydroxyproline content (SG-HF>SG>CG, p<0.05). In the SG-HF group, LV geometric remodeling was characterized by a significant increase in dimensions and relative thickness of the normal wall (excentric remodeling), whereas the SG group presented a concentric remodeling. Indexes of LV performance in the SG-HF group were significantly lower than those of the SG group.

Conclusions: The SG-HF and SG groups differed primarily in the LV geometric remodeling and structural myocardial remodeling process, which established a chronically compensated state in the SG group and triggered CHF in the SG-HF group in the presence of equivalent degrees of impaired contractility.

Key words: ventricular remodeling, myocardial dysfuntion, pressure overload hypertrophy, congestive heart failure.

Introduction

Congestive heart failure is a syndrome resulting from several causes including ischemic coronary disease, inflammatory processes, heart valve diseases and high blood pressure[1,2]. In response to one of these aggressions, a process referred to as ventricular remodeling is triggered. Remodeling may be defined as the changes in size, geometry, shape, composition and heart function after aggression[1-4]. In pressure overloads, this mechanism initially preserves the global cardiac function. However, over time, the changes resulting from the chronic remodeling lead to a progressive left ventricular (LV) dysfunction that culminates in CHF or sudden death[1-3].

The factors responsible for LV failure include: adverse geometric LV remodeling[2,5,6], changes in myocardial composition[2-4,7], progressively decreased contractility[8-10], or a combination of these factors[2-4,11]. Several studies[2,5,6] show that adverse LV remodeling is the major factor contributing to the failure of the heart as a pump. This notion is supported by the observation that in post-infarction remodeling with global LV dysfunction, the contractile function of the remaining cardiomyocytes is normal or mildly impaired[10,12-14]. Another relevant aspect in pressure overloads is the abnormal collagen accumulation in the interstitium and around the coronary arterioles. Collagen is the main component of the extracellular matrix, and its accumulation, given its extremely high resistance, dramatically changes the physical characteristics of the myocardium and the ventricular function[7], and is pointed as a factor participating in the development of CHF[1,2,4,7]. With regard to myocardial contractility, experimental models of pressure overload – studies that evaluated animals with and without CHF evidenced discrepant results[2,5,11,15-19]. Brooks et al[19] reported that the contractile function of papillary muscles of spontaneously hypertensive rats was significantly different in animals with or without CHF, whereas Conrad et al[8], Bing et al[17], and Bing and Wigner[18] demonstrated that the active myocardial tension was not significantly different in animals

Mailing Address: Edson Antonio Bregagnollo •
Rua Capitão Andrade 567 - 18601-545 – Botucatu, SP - Brazil
E-mail: bregagnollogh@ig.com.br
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with or without CHF.

More recently, the supravalvar aortic stenosis (SAS) model has been used to promote gradual LV remodeling. In this model, the mechanisms responsible for the heart failure are complex and not fully understood. The objective of this study was to analyze the relative contribution of the geometric changes of the LV cavity, and of the morphological and contractile alterations of the myocardium in the transition from compensated hypertrophy to CHF in rats with pressure-overload induced left ventricular hypertrophy triggered by supravalvar aortic stenosis (SAS).

Methods

Animals and supravalvar aortic stenosis surgery - The experiment and procedures were approved by the Ethics Commission on Animal Research of *Faculdade de Medicina de Botucatu*. Male Wistar rats weighing between 70 and 80g were anesthetized with sodium pentobarbital (50mg/Kg – IP) and underwent SAS (SG; n=55). For this purpose, after median sternotomy, the ascending aorta was isolated and a stainless steel clip with a 0.6-mm inner diameter was placed in the ascending aorta as described in previous studies[21,22]. Matched rats underwent sham surgery (CG; n=23). Six weeks later, 10 rats undergoing sham surgery (CG_6 group) and 10 with SAS (SG_6 group) were randomized for simultaneous echocardiographic, hemodynamic and morphological assessments. The remaining rats were followed for 15 more weeks. Deaths occurring during this period were recorded. Twenty one weeks after SAS, the surviving rats were clinically assessed and underwent echocardiographic, hemodynamic, morphological and mechanical study of the papillary muscles isolated from the LV. The 21-week period was chosen because it encompasses the transition phase from compensated hypertrophy to heart failure[23] in this model.

Geometric alterations and global cardiac function were assessed using echocardiogram and hemodynamic study. Morphological alterations of the myocardium were assessed by determining the degree of LVH and quantification of myocardial collagen content. Contractility was studied in papillary muscle preparations that enable the assessment of the contractile status regardless of loading conditions, chamber geometry and neurohormonal disorders[20].

Echocardiographic study - After anesthesia with a combination of Ketamine (50mg/kg) plus xylazine (1mg/kg), IM, and chest shaving, the animals were placed in the left lateral position. A Sonos 2000 (Hewlett-Packard Medical Systems) echocardiograph equipped with a 7.5 MHz transducer was used to obtain two-dimensional short axis views of the LV, at the papillary muscles. M-mode records (sweep speed = 100 mm/s) were made right below the tip of the mitral valve leaflets[21,24]. The records were manually calibrated by the same observer according to the methodology proposed by the American Society of Echocardiography[25]. The variables analyzed were: heart rate (HR); LV systolic (LVSD) and diastolic diameters (LVDD); LV systolic (SPWT) and diastolic posterior wall thickness (DPWT), relative wall thickness (DPWT/LVDD); LV shortening fraction (ΔD%), and peak early to late transmitral flow velocity ratio (E/L ratio). The arithmetic mean of at least five consecutive cardiac cycles was considered to obtain the values of each variable.

Hemodynamic study - Twenty four hours after obtaining the echocardiograms, the animals underwent hemodynamic study. After anesthesia, they were placed in the supine position. A midline abdominal incision extending to the xyphoid process was performed. This would enable visualization of the heart through the diaphragm. Under direct visualization, a 25x9 hypodermic needle connected to a Stathan P23ID (0-300 mmHg) pressure transducer was introduced from the apex into the LV cavity. Pressure and the first time derivative of the LV pressure were then recorded. The horizontal plane of the thoracic midline was adopted as reference level zero. Values of hemodynamic variables were obtained considering the arithmetic mean of 10 consecutive cardiac cycles, and the sweep velocity was 100 mm/s. The association between echocardiographic and hemodynamic data enabled the calculation of the systolic (σms) and diastolic (σmd) meridional stress according to a methodology previously validated in the literature[26]. In summary, pressures, internal dimensions and LV (end-systolic and end-diastolic) posterior wall thickness were measured in the echocardiographic tracings (M mode), and the σms and σmd were estimated using the formula: $\sigma m = 0.334 \times LVP \times [LVID/(1 + PWT/LVID)]$[26] , where, LVP, LVID and PWT correspond to the values of left ventricular end-systolic and end-diastolic pressure, internal dimensions, and posterior wall thickness, respectively, for the calculation of σms and σmd values.

Myocardial structure study - After hemodynamic assessment, the animals were sacrificed, their hearts quickly removed, and the papillary muscles isolated for mechanical assessment. The atria were excised and the ventricles separated into LV muscle mass which included the septum and right ventricular free wall. The weights of these structures were normalized for the body weight of the respective animals. The cross-sectional area of the LV myocytes were measured using a software program according to the methodology standardized and described in a previous study[21,22]. Myocardial collagen content was estimated using the determination of hydroxyproline concentration in the LV muscle according to the Switzer technique[27] already standardized in our laboratory[21,22]. Lung and liver water contents were calculated from the moist and dry weights of fragments of these organs put into a stove at 100ºC for 72 hours to desiccate. At that moment the animals with (SG-HF, n = 12) and without CHF (SG, n = 11) were identified. The criteria used for the diagnosis of CHF were: tachydyspnea, ascitis, pleural or pericardial effusion, left atrial thrombus, right ventricular hypertrophy, and increased water content in the lungs and liver[5,8,15].

Mechanical study of the papillary muscles - After the hemodynamic study, a thoracotomy was performed; the hearts were quickly removed and placed into oxygenated Krebs-Henseleit solution at 28°C. LV papillary muscles were dissected, mounted between two steel clips and placed vertically in a contraction chamber containing Krebs-Henseleit solution (at 28ºC) added with a mixture of oxygen (95%) and carbon dioxide (5%). This solution was composed of: 118.5 mM NaCl, 4.69 mM KCl, 2.52 mM $CaCl_2$, 1.16 mM Mg $S0_4$, 1.18 mM KH_2PO_4, 5.50 mM glucose and 25. 88 mM NaHCO3, that kept

the pH between 7.40 and 7.45 and the PaO2 between 450 and 550 mmHg. The lower clip was connected to the Kyowa 120T – 20B force transducer by means of a steel wire passing through a mercury seal placed in the bottom of the contraction chamber. The upper clip was connected to a mechanical magnesium lever (lever arm ratio 4:1) to adjust the muscle length. The preparations were stimulated 12 times per minute with square wave pulses (5ms) using platinum electrodes at a voltage 10% higher than the threshold, so as to promote a maximal mechanical response. The muscles were kept in isotonic contraction for 60 minutes, and then left in isometric contraction and stretched progressively to the maximum value of contraction tension/length curve (Lmax), returning to isotonic contraction for 5 more minutes. After that, they were left in isometric contraction again and the Lmax was carefully determined. After 15 minutes, when the preparations were stable, the isometric contractions were recorded. The following parameters were determined: peak tension developed (TD, g/mm^2); resting tension (RT, g/mm^2), time to reach peak tension (TPT, ms), time for the tension developed to fall by 50% (TR50, ms); maximum rate of increase (+ dT/dt, g/mm^2/s) and reduction (-dT/dt, g/m^2/s) of TD. After the recordings were concluded, muscle length at Lmax was measured and the muscle was weighed. The cross-sectional area (SA, mm^2) was calculated assuming a cylindrical geometry and specific density of 1.0[20]. All variables of the mechanical study of the papillary muscles were normalized for SA.

Statistical analysis - All data are expressed as mean ± standard deviation (X ± sd). Student's t test for independent variables was used for the statistical comparisons between CG_6 and SG_6 variables. The analysis of variance for a model with one source of variation was used for the statistical comparison between CG, SG and SG-HF variables. When necessary, the Tukey test was additionally used for all possible pairs of means. The level of statistical significance used was 5% ($p<0.05$).

Results

The mortality rate observed in this experiment (48.9%) is similar to those reported in other studies[23,28] and in our laboratory[21]. In the period from the 6th to the 21st week, 22 (44.4%) animals out of the 45 with SAS died. Of the 23 survivors up to the end of the experiment, 12 (52.2%) showed signs of CHF, and tachydyspnea (n=12); left atrial thrombus (n=9), pleural effusion (n=8); ascitis (n=6), and pericardial effusion (n=5) were observed. In the CG group no deaths or CHF occurred.

The values of the variables analyzed 6 weeks after SAS are shown in 1. The values of LVSP, LVEDP, LV/W, LVSA, SPWT, DPWT, DPWT/LVDD, and ΔD% in the SG_6 group were significantly higher, whereas the values of σms, σmd, +dP/dt, -dP/dt, LVSD, LVDD, E/L ratio, RV/W, LVHP, PWC, and LWC were similar to those of the CG_6 group ($p>0.05$). No animals of the CG_6 and SG_6 groups had signs of CHF.

The values of the echocardiographic, hemodynamic and morphological variables, as well as the mechanical studies of the papillary muscles obtained in the end of the experiment are shown in Tables 2 to 4. The animals with SAS presented significant increases of LV/W, A/W, LVSA, and LVHP, with SG-HF >SG>CG ($p < 0.05$), whereas the values of RV/W, PWC, and LWC of the SG-HF group were significantly higher than those of the CG and SG groups, which were similar (Table 2). The values of HR, LVSD, LVDD, E/L ratio, σms and σmd of the SG and SG-HF groups were SG-HF > SG > CG ($p < 0.05$), and the values of SPWT and DPWT were SG = SG–HF > CG; $p<0.05$). The values of DPWT/LVDD and ΔD% were CG=SG>SG-HF; $p<0.05$). In the SG and SG-HF groups there were significant and similar increases of LVSP (SG=SG-HF>CG; $p<0.05$), increase of LVEDP (SG-HF>SG>CG; $p< 0.05$) and a significant reduction of +dP/dt and – dP/dt (CG>SG>SG-HF; $p< 0.05$) as shown in Table 3. Surprisingly, the analyses of the mechanical behavior of the papillary muscles during isometric contractions evidenced the same degree of myocardial contractile dysfunction. The values of

Variables	CG_6(n=10)	SG_6(n=10)
W (g)	382 ± 10	391 ±11
LV/W (mg/g)	2.38 ± 0.10	3.25 ± 0.24*
RV/W (mg/g)	0.53 ± 0.06	0.55 ± 0.10
LVSA (μm2)	287 ± 13	389 ± 27*
LVHP (mg/g)	3.58 ± 0.53	3.97 ± 0.60
LVSP(mmHg)	105 ± 8	195 ± 14*
LVEDP (mmHg)	4 ± 1	8 ± 2*
+dP/dt (mmHg/s)	6.326 ± 208	6.476 ± 261
– dP/dt (mmHg/s)	2.985 ± 222	3.107 ± 355
LVSD (mm)	4.80 ± 0.08	4.10 ± 0.10
LVDD (mm)	8.20 ± 0.20	7.83 ± 0.20
SPWT (mm)	2.91 ± 0.10	3.81 ± 0.11*
DPWT (mm)	1.60 ± 0.12	2.32 ± 0.12*
DPWT/LVDD	0.39 ± 0.02	0.60 ± 0.03*
E/L	1.64 ± 0.27	2.05 ± 1.05
σms (Kdyn/cm^2)	40.1 ± 3.6	36.8 ± 3.40
σmd (Kdyn/cm^2)	7.10 ± 0.50	7.8 ± 0.40
ΔD (%)	49 ± 5	59 ± 5*
PWC (%)	81 ± 0.90	80 ± 1.1
LWC (%)	68 ± 0.90	67 ± 0.90

*W:body weight; LV and RV:left and right ventricles; LVSA: cross-sectional area of the myocytes; LVHP: LV muscle hydroxyproline content; LVSP and LVEDP: LV systolic and end-diastolic pressures; +dP/dt and – dP/dt: first positive and negative time derivative of LV pressure; LVSD and LVDD: LV end-systolic and diastolic diameters; SPWT and DPWT: LV systolic and diastolic posterior wall thickness; 2xDPWT/LVDD : systolic and diastolic posterior wall thickness; E/L: peak early to late transmitral flow velocity ratio; σms and σmd: LV systolic and diastolic meridional stress; ΔD (%): LV shortening percentage; PWC and LWC: lung and liver water content; g: grams; mg: milligrams; μm^2:square micra; mm: millimeters; mmHg: millimeters of mercury; Kdyn/cm^2:Kilodyna /square centimeter; %: percentage; *p < 0.05*

Table 1 - Means ± standard deviation of morphological, echocardiographic and hemodynamic variables obtained in the control group (CG6) and stenosis group (SG6) six weeks after supravalvar aortic stenosis induction

TD, + dT/dt, -dT/dt were CG > SG = SG-HF; $p<0.05$, and the values of RT, TPT and TR_{50} were SG = SG-HF > CG; $p<0.05$, as shown in Table 4.

Discussion

In this study, we assessed the relative contribution of changes in ventricular geometry, myocardial morphology and contractile status in the transition phase from compensated remodeling to CHF in rats with SAS. The assessments performed 6 weeks after SAS evidenced that the animals in the SG_6 group presented concentric left ventricular hypertrophy (LVH), LV performance indexes, myocardial contractility and relaxation indexes, LV σms and σmd, and LVHP similar to those observed in the CG_6 group (Table 1). In previous studies we showed that, in this experimental model, six weeks after SAS induction, the left ventricular function is hyperdynamic and the myocardial contractility is normal[22,24]. During this period, the improvement in LV performance may be attributed to the LV concentric remodeling, since we verified a significant correlation (0.74, $p<0.05$) between ΔD% and relative left ventricular wall thickness[24]. The present study corroborates previous results by demonstrating that six weeks after SAS induction the increase in the LV wall thickness completely normalizes the systolic and diastolic parietal stress. We also demonstrated that LVHP, E/L ratio, +dP/dt, -dP/dt, RV/W, PWC, and LWC were similar in the CG_6 and SG_6 groups ($p<0.05$) and that LV/W, LVSA, DPWT, DPWT/LVDD, LVSP, LVEDP values in the SG_6 group were significantly higher than those of the CG_6 group ($p<0.05$). All together, these results allow us to conclude that concentric LVH normalized the parietal stress, thus enabling a hyperdynamic ventricular function even in the presence of a significant pressure overload.

At the end of the experiment, in contrast to the concentric hypertrophy observed in the SG group, rats from the SG-HF group presented eccentric geometric remodeling characterized by increased LVSD, LVDD and reduced SPWT/LVDD. In animals with SAS, the σms and σmd and ΔD% were significantly higher (SG-HF>SG>CG; $p<0.05$) and lower (CG>SG>SG-HF; $p<0.05$) than those of the group CG, respectively. Morphological changes of the myocardium were more significant than in animals with CHF, with values of LV/W, A/W, RV/W, LVHP and LVSA in the SG-HF group significantly higher when compared to those of the SG group. However, mechanical assessments performed in isolated muscle preparations evidenced the same degree of impairment of the myocardial contractile function in the SG and SG-HF groups. Thus, we can conclude that the SG and SG-HF groups differed primarily in the LV geometric remodeling process and myocardial morphological remodeling that led to a chronically compensated state in the SG group and to CHF in the SG-HF group.

A previous study conducted in our laboratory demonstrated that rats without CHF presented persistent and stable pressure overload in the period between 6 and 21 weeks after SAS induction[21,24]. The results of this study corroborate the previous findings and demonstrate that the same behavior is extensive to animals with CHF. The high rate of animals with CHF is similar to that previously found in our laboratory[21,22], being consistent with several other studies[5,8,19,29].

Although cardiac remodeling is an important mechanism to compensate chronic pressure overloads, it is usually associated with CHF and death over time[1-3]. Cardiac remodeling involves a series of structural, biochemical, molecular and geometric LV changes, such as reexpression of fetal genes that codify isoforms of myosin; enzymes that regulate the intracellular calcium cycle; imbalance between supply and demand of myocardial oxygen; induction of myocytes to necrosis and/or apotosis and to the abnormal collagen accumulation that occur as a consequence of the direct mechanical stimulation and/or of the activation of several neurohormonal factors, notably angiotensin II, endothelin, norepinephrine and aldosterone[1-4,6,7,30,31]. Together, these mechanisms progressively affect the global cardiac function, culminating in CHF or sudden death[1-6,30-33].

In chronic pressure overloads the mechanisms triggering CHF include geometric alterations of the LV cavity[5,6,32], morphological alterations of the myocardium[1-4,7,33], impairment of the myocardial contractile function[1,2,8,10,11], or a combination of these factors[2-4,11,33].

Geometric alterations leading to a dilated and more

Variables	CG (n = 13)	SG(n = 11)	SG-HF(n = 12)
W (g)	460 ± 57	463 ± 32	483 ± 80
LV/W (mg/g)	1.8 ± 0.1	2.8 ± 0.3*	3.7 ± 0.2*+
RV/W (mg/g)	0.50 ± 0.01	0.55 ± 0.01	1.11 ± 0.1*+
A/W (mg/g)	0.02 ± 0.01	0.04 ± 0.01*+	0.08 ± 0.01 *+
LVSA (μm^2)	338 ± 25	451 ± 32*	508 ± 36*+
LVHP (μg/mg)	3.65 ± 0.67	6.64 ± 0.63*	9.21 ± 1.38*+
PWC (%)	77 ± 1	79 ± 2*	84 ± 3*+
LWC (%)	65 ± 4	66 ± 2	71 ± 6*+

*CG: Control Group; SG: Stenosis Group; SG-HF: Stenosis Group + Heart Failure; W: body weight; LV/W, RV/W, A/W: left and right ventricular weight and atrial weight corrected for W; LVSA: cross-sectional area of the myocytes; LVHP: left ventricular hydroxyproline content; PWC and LWC: lung and liver water content, respectively; *p < 0.05 vs CG; +p < 0.05 vs SG*

Table 2 - Means ± standard deviation of the morphological variables obtained at the end of the experiment

Variable	CG (n=13)	SG (n=11)	SG-HF (n=12)
HR (bpm)	282 ± 12	228 ± 20	338 ± 10*+
LVSD (mm)	4.90 ± 0.20	6.81 ± 0.32*	9.42 ± 0.41*+
LVDD (mm)	8.42 ± 0.22	8.9 ± 0.38	14.31 ± 0.42*+
SPWT (mm)	3.01 ± 0.11	3.92 ± 0.21*	3.90 ± 0.24*
DPWT (mm)	1.72 ± 0.11	2.74 ± 0.23*	2.65 ± 0.31*
DPWT/LVDD	0.40 ± 0.02	0.58 ± 0.03*	0.44 ± 0.02+
E/L	1.72 ± 0.33	6.71 ± 2.10*	11.85 ± 1.10*+
ΔD (%)	47 ± 4	44 ± 3	31 ± 4+
σms (Kdyn/cm²)	36.6 ± 3.10	78.6 ± 4.8*	104.6 ± 7.8*+
σmd (Kdyn/cm²)	6.9 ± 0.4	20.1 ± 1.1*	43.2 ± 3.2*+
LVSP (mmHg)	108 ± 5	199 ± 13*	191 ± 12*+
LVEDP (mmHg)	4 ± 1	10 ± 3*	21 ± 3*+
+dP/dt (mmHg/s)	7846 ± 300	5236 ± 253*	3127 ± 238*+

*HR: heart rate; LVSD and LVDD: LV end-systolic and diastolic diameters; SPWT and DPWT: LV systolic and diastolic posterior wall thickness; DPWT/LVDD: relative LV posterior wall thickness; E/L: early (E) and late (L) peak velocity ratio of the transmitral flow; E/L: peak early to late transmitral flow velocity ratio; ΔD%: LV shortening percentage; σms and σms: LV wall systolic and diastolic meridional stress; LVSP and LVEDP: LV systolic and end-diastolic pressures; +dP/dt and -dP/dt: positive and negative time derivative of LV pressure. * p<0.05 vs CG; +p<0.05 vs SG*

Table 3 - Echocardiographic and hemodynamic values in the control group (CG), aortic stenosis group (SG), and aortic stenosis + congestive heart failure (SG-HF)

Variables	CG (n=13)	SG (n=11)	SG-HF (n=12)
TD (g/mm²)	6.4 ± 0.90	4.95 ± 1.07*	4.48 ± 0.83*
RT (g/mm²)	0.77 ± 0.35	1.27 ± 0.20*	1.36 ± 0.33*
TPT(ms)	162 ± 15	185 ± 19*	194 ± 25*
TR50 (ms)	165 ± 18	182 ± 33*	184 ± 25*
+ dT/dt (g/mm²/s)	62 ± 11	42 ± 11*	37 ± 8*
– dT/dt (g/mm²/s)	45 ± 6	20 ± 7*	21 ± 4*
SA (mm²)	1.22 ± 0.19	1.28 ± 0.36*	1.36 ± 0.21*

*TD and RT: tension developed and resting tension; TPT: time to reach peak tension; TR50: time for the TD to fall by 50% of its peak value; + dT/dt and - dT/dt: rate of increase and reduction of TD; SA: cross-sectional of the papillary muscles. * p< 0.05 vs GC.*

Table 4 - Means ± standard deviation of the papillary muscle variables in the control (CG), aortic stenosis (SG) and aortic stenosis + congestive heart failure (SG-HF) groups

spherical LV cavity result in a series of functional disadvantages, among which we can point out: increased parietal stress, afterload maladjustment, increased oxygen consumption, low subendocardial perfusion, sustained hemodynamic overload and maintenance of activation of mechanical and neurohormonal stimuli participating in the cardiac remodeling process[2-6,33-36]. Varying degrees of mitral regurgitation occur as a result of the left ventricular dilatation, which add volumetric overload to the LV, which, in turn, predisposes to additional ventricular dilatation[2,6,33], thus creating a vicious cycle.

These aspects were demonstrated in the present study. Considering that LVSD, LVDD, E/L, σms, σmd, LVEDP, LVPH, LVSA values were SG-HF > SG > CG; p<0.05; ΔD% and DPWT/LVDD values were CG> SG> SG-HF; p<0.05); and LVSP was SG=SG-HF>CG; p<0.05, we can assume that the preload reserve depletion and afterload maladjustment effectively contributed to the development of CHF. The echocardiographic and hemodynamic results clearly showed that these mechanisms were present in the animals with SAS, and were more significant in the SG-HF group. Therefore, we can assume that this is the mechanism that most probably triggered CHF in the animals of the SG-HF group (Table 2).

Morphological myocardial remodeling was significantly more evident in the SG-HF group than in the SG group (Table 1). The percentage increases of morphological variables of the SG and SG-HF groups compared to those of the CG group are shown in Figure 1. Hypertrophic and failing hearts frequently show a significant increase in interstitial fibrosis and myocyte

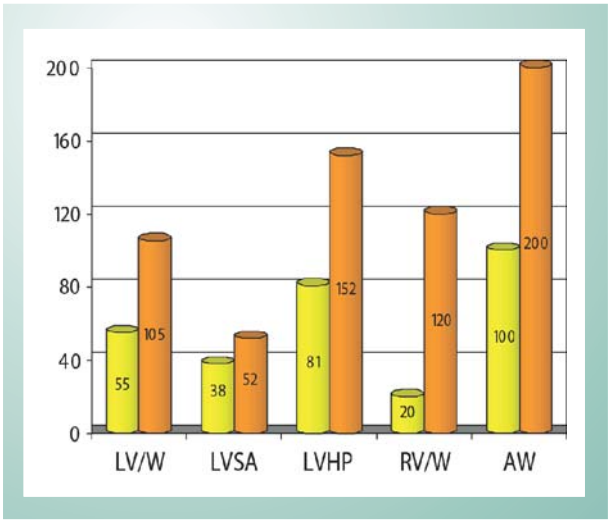

Fig. 1 - Percentage variations of morphological parameters in the stenosis (SG) and stenosis plus heart failure (SG-HF) groups in relation to the control group (CG).

hypertrophy that harden the LV and negatively affect initially the diastolic function, and later[1,2,7,33] the systolic function. Several studies support the idea that the morphological myocardial remodeling, more significant in the SG-HF group, also may have contributed to the onset of CHF[2-5,7,32], in addition to the adverse geometric alterations of the LV cavity. In this study, we demonstrated that the LVPH, LV/W, LVSA, RV/W, and A/W values of the SG-HP group were significantly higher than those of the SG group ($p<0.05$), showing that the degree of morphological myocardial remodeling in the SG-HF group was more aggressive than that of the SG group (Table 2). The more significant increase of these morphological variables that occur in response to mechanic overloads has an adverse effect on cardiac adaptation to pressure overloads, since they increase muscle rigidity and affect the LV diastolic and systolic functions, thus collaborating to the development of CHF[1,3,7,33].

Stimuli to cardiac remodeling include mechanical and biochemical factors that act on receptors, ion channels and integrins present in the sarcolemma[1-4,10,33,35,37] , resulting in increased protein synthesis, fetal gene reexpression, cardiomyocyte necrosis and/or apoptosis, disproportion between muscular and interstitial myocardial compartments, fibroblast proliferation and collagen accumulation.

In contrast to the differences observed in the global myocardial performance, myocardial morphology and LV cavity geometry indexes, the degree of impairment of myocardial contractility and relaxation observed in the isolated papillary muscle preparations was similar in both the SG and SG-HF groups (Table 4). These preparations reliably show the intrinsic myocardial contractile properties without the interference of geometric alterations of the LV cavity, load conditions, imbalance between oxygen supply and demand or fluctuations in the neurohormonal systems activity. Additionally, they allow the normalization of values of mechanical variables for the cross-sectional area of the muscles, so that muscles of different sizes can be adequately compared, which could hardly be achieved in isolated heart preparations or in situ[20]. Our results are consistent with those reported by Norton et al[5] and inconsistent with those of other studies in rats with and without CHF, in which the myocardial contractile dysfunction was significantly more increased in rats with CHF[8,12,19,38]. Norton et al[5] concluded that the transition from compensated hypertrophy to heart failure in rats undergoing abdominal aortic banding resulted from an adverse LV geometric remodeling. Conrad et al[8] showed that the tension developed and papillary muscle shortening velocity of spontaneously hypertensive rats with CHF were depressed when compared to those obtained in rats without CHF. Similar conclusions were reported with papillary muscles obtained from Dahl rats with CHF[38]. Morii et al[12] suggested that the transition from compensated hypertrophy to heart failure in Dahl rats was due to impairment of myocardial contractility and abnormal LV geometric remodeling. These studies used pressure/LV end-systolic volume ratios as an index of myocardial contractility. This contractility index, like the +dP/dt, inherently reflects the characteristics of the mechanical behavior of the ventricular chamber and not specifically the intrinsic myocardial contractility[39,40]. A previous study conducted in our laboratory demonstrated that the pressure/LV diameter ratios, as well as the pressure/volume ratios are significantly different during sudden and sustained elevations of blood pressure[40]. Isolated papillary muscle preparations used in this study enable an efficient control of factors determining contractility, and the normalization of indexes for muscles of different dimensions; also, they do not suffer interferences from neurohormone alterations and from geometric characteristics of the LV cavity[20,39-41]. Thus, the presence of distinct patterns of geometric remodeling and loading conditions demonstrated in the SG and SG-HF groups makes complex the characterization of the real degree of impairment of the myocardial contractility when these indexes are used in intact heart preparations or in situ. In this study, we observed that the degree of impairment of the myocardial contractile function of the SG and SG-HF groups were similar, whereas the indexes of LV performance of the SG-HF group were significantly worse when compared to the SG group. Aurigemma et al[41] reported similar findings. However, we should note that the differences in experimental models used to assess the transition phase to CHF in pressure overloads may, at least in part, justify the discrepancies reported. For instance, myocardial dysfunction in spontaneously hypertensive rats may be due to combined effects of hypertension and age[8,15,17,19]. In abdominal aortic banding, hypertension starts immediately after the surgical procedure[5,42-44], whereas in the SAS model pressure overload is supposed to start gradually[23,28,29], being mild at the moment of the surgical procedure and progressively increasing as the animals grow up. In our study, the rats are young, unlike those which assessed spontaneously hypertensive rats. In the model used by Norton et al[5] and in this experiment, death rates were high and similar, indicating a more aggressive pressure overload state in young animals. Values significantly higher of the LV/W, LVSA, LVHP, RV/W and A/W variables demonstrate that the cardiac remodeling process is more active and also more adverse in animals that developed CHF. In summary, our results indicate that in the transition phase from compensated hypertrophy to CHF, in the SAS experimental model, the alterations of cavity geometry,

myocardial morphology, and LV loading conditions are more important than the degree of impairment of myocardial contractility for the onset of CHF.

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Potential Conflict of Interest

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

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