

# Myocardial Remodeling in Chronic Pressure or Volume Overload in the Rat Heart

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## OBJECTIVE

To compare cardiac structural changes in experimental pressure and volume overload models.

## METHODS

The study analysis included renovascular hypertensive rats (RVH, n=8), normotensive rats with volume overload caused by an aortocaval fistula (ACF, n=10) and control rats (CONT, n=8). After four weeks, tail cuff blood pressure (SBP) was recorded. Rats were killed, the hearts were excised and the right and left ventricles (RV&LV) were weighed (RVW&LVW). Using histological sections, myocyte cross sectional areas (MA). LV wall thickness (LVWT) LV cavity diameter (LVD), normalized LVWT (LVWT/LVD) and collagen volume fraction (CVF) were measured. The comparisons were made using the ANOVA and Tukey test for a significance level of 5%.

## RESULTS

Tail cuff blood pressure (mmHg) was higher in the RVH group (RVH =  $187 \pm 22$ ; CONT =  $125 \pm 10$ ; ACF =  $122 \pm 6$ ,  $p < 0.05$ ). LV hypertrophy was observed in the RVH and ACF groups. The ACF group presented a significant increase in size of LVD, compared to CONT and RVH. The absolute and normalized ventricular wall thickness were similar among the groups. The RVH group presented a significant increase in CVF compared to CONT group and ACF group.

## CONCLUSION

Cardiac remodeling patterns following volume or pressure overload are distinct, suggesting that their implications on ventricular dysfunction are not interchangeable.

## KEY WORDS

Renovascular hypertension, aortocaval fistula, collagen, ventricular hypertrophy

Myocardium is comprised of myocytes, vessels and collagen interstitial matrix. The equilibrium of these three compartments maintain cardiac form and function. Composition changes of these compartments reflect the process of myocardial remodeling that is closely related to cardiac dysfunction. The remodeling occurs in response to stimuli caused by mechanical or humoral agents on cardiac tissue<sup>1-3</sup>. In clinical practice, hypertrophy is more commonly seen in association with hemodynamic overload imposed by hypertension or volume overload. In the case of pressure overload, there is a synthesis of sarcomeres in parallel which increases the ventricle wall thickness resulting in concentric hypertrophy. In the case of volume overload, there is an increase of sarcomeres in series, associated with the slippage of myocyte bundles, resulting in eccentric hypertrophy. The presence of cardiac hypertrophy is an independent factor of higher morbidity and mortality caused by cardiovascular events<sup>4,5</sup>. However, this association has been studied extensively and is well established in chronic pressure overload, secondary to systemic hypertension<sup>6</sup>.

The description given for this condition is that the disproportional increase of the collagen interstitial matrix as well as the ventricular geometry change would reduce ventricular compliance leading to myocardial dysfunction<sup>7</sup>. Additionally, the slower growth of vessel density reduces the coronary reserve, potentially leading to myocyte oxygen and nutrient deficiencies causing further ventricular dysfunction. Therefore, the increased myocardial interstitial collagen in LV hypertrophy which may or may not be associated with relative ischemia, is the main cause of myocardial dysfunction, particularly diastolic dysfunction<sup>1,8</sup>. However, very few studies has been conducted regarding to the consequences of hypertrophy secondary to volume overload. It has been demonstrated that rats with arteriovenous fistula present unchanged interstitial collagen concentration<sup>9</sup>. In this experimental model, myocardial fibrosis is only described in conjunction with overt heart failure<sup>10</sup>.

Considering the distinct cardiac remodeling patterns resulting from different hemodynamic overloads, the objective of the study was to compare cardiac structural modifications in volume and pressure overload in rats.

## METHODS

The experimental protocol was submitted to the institution's Animal Research Ethics Committee for evaluation and was approved by protocol number 187/2001.

Male Wistar rats weighing 250 to 300 grams were used in the study. Randomly selected animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and submitted to a median laparotomy exposing the aorta and renal artery branches. The left renal artery was dissected to place a 7 x 1 mm silver clip with a 0.35 mm opening. The distal portion was closed ensuring that the

clip was fixed in the desired position in order to induce unilateral renal ischemia. Next, the abdominal cavity was closed in layers with continuous sutures. Eight rats were used for the experimental group with renovascular hypertension (RVH).

The sex-and-age matched rats were also randomly selected and anesthetized as described above and submitted to a median laparotomy to create an infra-renal aortocaval fistula (ACF group). The procedure described by Garcia & Diebold<sup>11</sup> was used and consisted of the dissection of the abdominal aorta and inferior vena cava from the renal artery to the origin of the iliac arteries. Next, the vessels were obstructed using a vascular clip placed just below the renal artery and the anterior wall of the aorta was punctured with a 1.5 mm external diameter, 16 gauge needle. The needle was advanced approximately 1 cm into the aorta and, passing through the median vessel wall into the vena cava, creating a aortocaval fistula. After withdrawal of the needle, the puncture orifice was sealed with a drop of cyanoacrylate glue. Next, the vascular clip was removed to reestablish blood flow, taking care to verify fistula patency through visualization, by transparency, of the blood flow into the vena cava. The abdominal cavity was closed in layers using continuous sutures.

Finally eight Wistar rats of the same sex and age as described before were kept-without surgery procedures and were used as controls (CONT). The animals were housed in cages in groups of two or three and placed in a vivarium with a twelve hour light cycle, temperature and humidity controls. The rats received a standard diet and water *ad libitum*. The experimental groups and the controls were studied after four weeks of evolution. Tail cuff blood pressure (SBP) was taken using an automatic sphygmomanometer (Narco BioSystem, Austin, Texas, USA). Next, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and submitted to a median thoracotomy. The heart was removed and the ventricles were separated and weighed.

Morphological Study – Coronal sections, approximately 3 mm thick, taken 5 mm from the tip of the ventricles were fixed in a 10% tamponed formaldehyde solution for 48 hours followed by immersion in a 70% alcohol solution for 48 hours. After fixation, the tissues were embedded in paraffin. Four micron thick histological sections were stained with Hematoxylin-Eosin (HE) or Masson, and 6 micron thick histological sections were stained with Picro Sirius red, specific for collagen. The myocyte cross sectional areas (MA) were measured in the sections stained by HE or Masson, using a LEICA DM LS microscope connected to a video camera and IBM compatible computer, equipped with the image analysis program Image Pro-plus (Media Cybernetics, Silver Spring, Maryland, USA). Fifty to seventy cells were analyzed per slide. The selected cells presented a circular shape and were located in the subendocardium layer of the

ventricles walls. This care was taken in order to have the best uniformity in the myocyte shape among the groups. The average cross sectional areas obtained for each group were used as an indicator of cell hypertrophy.

The slides stained with Picro Sirius red were used to quantify the interstitial collagen volume fraction using video densitometry. Images of the cardiac tissue were taken and analyzed using the system described above. The elements of the cardiac tissue were identified according to color level. Therefore, the collagen fibers were visualized in red and the myocytes in yellow. The collagen volume fraction (CVF) was calculated automatically and corresponded to the sum of the collagen areas divided by the sum of the collagen tissue and myocyte areas. On average, thirty fields were analyzed using a lens with magnification of 40X. Perivascular collagen was excluded from the analysis.

Remodeling of the ventricular chamber was evaluated using the slides stained with HE, measuring the left ventricular internal diameter (LVS) and the myocardial wall thickness (LVWT) and calculating the ratio wall thickness and ventricular diameter (LVWT/LVD).

Statistical analysis – The variables were presented as mean  $\pm$  standard deviation (SD) and comparison between the groups were made by analysis of variance (ANOVA), followed by multiple comparison Tukey test. The analyses were made using the statistical package SigmaStat for Windows, version 2.03 (SPSS, San Raphael, CA, USA), and a 5% significance level was accepted.

## RESULTS

Tail cuff blood pressure was significantly higher in the RVH group compared to CONT and ACF groups (fig. 1). The morphometric data are presented in table 1. After four weeks left ventricular weight, LVW corrected to body weight and myocyte cross sectional area were similar in RVH and ACF groups, indicating similar degree

of LV hypertrophy. In the volume overload model (ACF) there was an increase in the right ventricle weight, RVW corrected by body weight and right ventricle myocyte cross sectional area compared to the CONT and RVH groups.

RVH group showed an increased collagen volume fraction when compared to CONT and ACF groups (fig. 2). LV wall thickness was statistically similar among the experimental groups. LV cavity diameter was significantly larger in the ACF group in relation to the other two groups. Left ventricular wall thickness and normalized wall thickness cavity diameter (LVWT/LVD) were comparable among the groups.

## DISCUSSION

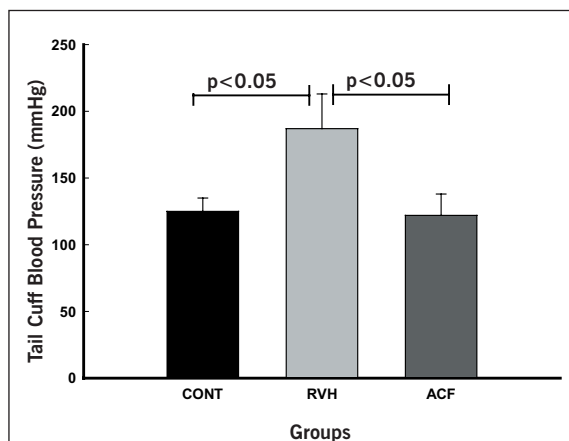
The objective of the present study was to compare cardiac remodeling secondary to either chronic volume or pressure overload.

In the pressure overload model, all indices showed left ventricular hypertrophy. The LV weight and myocyte sectional area grew significantly in relation to the control group. The maintenance of normalized wall thickness suggests that the hypertrophy was still developing and had not yet modified the ventricular chamber geometry to the concentric pattern. Therefore, it is reasonable to assume that in this period, the increased afterload in the hypertensive animals was not totally compensated by the developing hypertrophy. Nevertheless, the absence of right ventricle hypertrophy, in this experimental group, suggests that the myocyte cellular growth is more dependent on mechanical stimulus than on the trophic effects of neurohormonal activation. This results is agreement with other authors that evaluated similar models<sup>12</sup>. It is known that unilateral renal ischemia (Goldblatt - 2 kidney, 1 clip model) is the experimental model of renovascular hypertension where the activation of the rennin-angiotensin-aldosterone system is the leading factor in the genesis of hypertension<sup>13</sup>, hypertrophy and myocardial

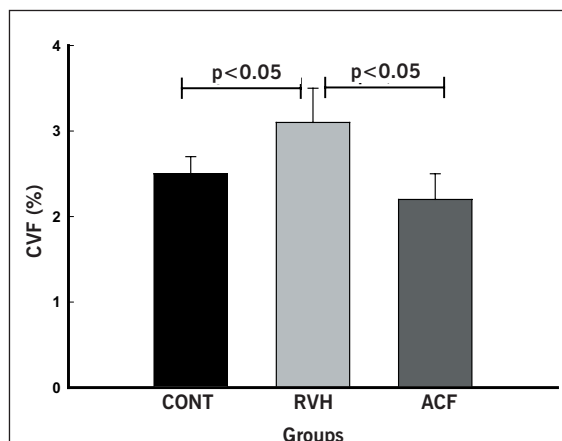
Table 1 – Mean and standard deviations of the variables for the renovascular hypertension (RVH), aortocaval fistula (ACF) and control (CONT) rat groups

Variable	CONT (n=8)	RVH (n=8)	ACF (n=10)
BW (g)	319 $\pm$ 22	305 $\pm$ 22	331 $\pm$ 29
LVW (g)	0.63 $\pm$ 0.04	0.77 $\pm$ 0.06*	0.84 $\pm$ 0.11*
RVW (g)	0.21 $\pm$ 0.03	0.21 $\pm$ 0.01	0.34 $\pm$ 0.05*#
LVW/BW (mg/g)	1.97 $\pm$ 0.2	2.53 $\pm$ 0.17*	2.54 $\pm$ 0.31*
RVW/BW (mg/g)	0.66 $\pm$ 0.1	0.69 $\pm$ 0.04	1.02 $\pm$ 0.15*#
MA-LV ( $\mu^2$ )	157 $\pm$ 5	191 $\pm$ 4*	208 $\pm$ 23*
MA-RV ( $\mu^2$ )	153 $\pm$ 12	161 $\pm$ 10	200 $\pm$ 17*#
LVWT (mm)	2.86 $\pm$ 0.91	3.17 $\pm$ 0.72	3.28 $\pm$ 0.95
LVD (mm)	7.49 $\pm$ 0.49	7.68 $\pm$ 0.59	8.94 $\pm$ 0.70*#
LVWT/LVD	0.38 $\pm$ 0.11	0.41 $\pm$ 0.08	0.37 $\pm$ 0.10

BW: body weight; LVW: left ventricle weight; RVW: right ventricle weight; LVW/BW & RVW/BW: ventricle weight normalized by body weight; MA: myocyte cellular area; LVWT/LVD: left ventricle wall thickness and left ventricle cavity diameter ratio; \*p<0.05 x CONT; #p<0.05 x RVH (ANOVA followed by the Tukey test)



**Fig. 1** – Graphical representation of the mean and standard deviations of tail cuff blood pressure for the renovascular hypertension (RVH), aortocaval fistula (ACF) and control (CONT) rats groups. Analyzed using ANOVA, followed by the Tukey test



**Fig. 2** – Graphical representation of the mean and standard deviations of the interstitial collagen fraction (CVF) for the renovascular hypertension (RVH), aortocaval fistula (ACF) and control (CONT) rat groups. Analyzed using ANOVA, followed by the Tukey test

interstitial fibrosis<sup>14</sup>, as well as vascular remodeling<sup>15</sup>. Sharifi et al<sup>16</sup> observed increased angiotensin-converting enzyme activity both in the plasma and cardiac tissue of rats with renovascular hypertension, with two and twelve weeks of evolution, and concluded that the increased angiotensin-converting enzyme tissue activity has an important role in the alterations of the target organ in this experimental model. Therefore, both the mechanical effects of pressure overload and the humoral effects of the activation of the local and systemic renin-angiotensin system are relevant in the modulation of the ventricular remodeling.

Myocardial interstitial fibrosis was observed in the RVH group in accordance with previous results from our laboratory<sup>8</sup> and other researchers<sup>17,18</sup>. This component of cardiac remodeling would be the result of both humoral fibroblast stimulation leading increased collagen synthesis<sup>17,19,20</sup> and cellular necrosis<sup>21</sup>.

The rats submitted to the aortocaval fistula presented a distinct hypertrophy pattern in comparison with hypertensive rats. The myocyte cellular area, ventricular weight and right ventricular weight normalized to body weight were higher than in CONT and RVH groups indicating combined right and left ventricular hypertrophy. Diastolic myocyte distension would be the main mechanical stimulus for right ventricle hypertrophy. However, the role of pulmonary hypertension caused by increased volume should not be discharged<sup>22</sup>. Therefore, it must be considered that the stimulus for right ventricular hypertrophy in aortocaval fistula could be multifactorial, that is volume and pressure overload on pulmonary circulation<sup>22</sup>. Liu et al<sup>22</sup> studying a similar model, showed that the myocyte increases both in width and length which helps maintain the muscular form. During the compensated phase, this growth contributes to the maintenance of the chamber geometry without ventricular dilation. Afterwards, when the capacity of the muscle to be hypertrophied is exhausted, the cavity radius increases

disproportionally and leads to heart failure<sup>23</sup>.

In the present study the LVWT/LVD ratio was preserved, which suggests that in the four week of the experiment, eccentric hypertrophy was still compensated. This stage of the remodeling allows the maintenance of wall tension and preservation of cardiac performance despite the hemodynamic overload.

The collagen interstitial matrix is also a factor to be studied in the remodeling process of volume overload secondary to aortocaval fistula. It is known that the quantity of interstitial collagen is dependent on the interaction between the synthesis process and protein degradation. Pressure overload and the angiotensin II humoral stimulus are the main stimulating agents for collagen synthesis while muscular distension caused by volume overload contributes to its degradation<sup>24</sup>, in a process that probably involves collagenase activation due to mastocyte degranulation<sup>25</sup>.

After performing the aortocaval fistula the metalloproteases are rapidly activated and remain significantly elevated during the following five days, returning to basal conditions in fourteen days<sup>25</sup>. Concurrent with the increase of metalloprotease activity, there is a significant reduction in the collagen interstitial matrix<sup>24</sup>. In this study, it was observed that the ACF group maintained the amount of interstitial collagen, suggesting an equilibrium between degradation and interstitial matrix synthesis after four week. The most relevant implication of these observations in that the results of medical literature that show a direct relationship between myocardial hypertrophy and cardiovascular morbidity and mortality do not necessarily apply to the hypertrophy pattern caused by volume overload.

Our results show that pressure or volume overloads cause distinct patterns of cardiac remodeling suggesting that their implications on ventricular dysfunction are not interchangeable.

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