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# Long-term use of low-dose spironolactone in spontaneously hypertensive rats: Effects on left ventricular hypertrophy and stiffness

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**Abstract:**
The aim of the present study was to evaluate the effect of low-dose spironolactone initiated during the early stages of hypertension development and to assess the effects of chronic pressure overload on ventricular remodeling in rats. Male spontaneously hypertensive rats (SHRs) (4 weeks) were randomized to receive daily spironolactone (20 mg/kg) or vehicle (mineral oil) from 4 weeks to 8 months of age. Systolic blood pressure was measured non-invasively by tail-cuff pletysmography at baseline, 4 and 8 months. Hemodynamic assessment was performed at the end of treatment by arterial and ventricular catheterization. An *in situ* left ventricular pressure-volume curve was created to evaluate dilatation and wall stiffness. Systolic blood pressure at 1 month of age was higher in SHRs than in the Wistar group; it increased throughout the follow-up period and remained elevated with treatment (Wistar: 136 ± 2, SHR: 197 ± 6.8, SHR-Spiro: 207 ± 7.1 mmHg; $p < 0.05$). Spironolactone reduced cardiac hypertrophy (Wistar: 1.25 ± 0.03 SHR: 1.00 ± 0.03, SHR-Spiro: 0.86 ± 0.02 g; $p < 0.05$) and left ventricular mass normalized to body weight (Wistar: 2.51 ± 0.06, SHR: 2.70 ± 0.08, 2.53 ± 0.07 mg/g; $p < 0.05$). Moreover, the left ventricular wall stiffness that was higher in SHRs was partially reduced by spironolactone treatment (Wistar: 0.370 ± 0.032; SHR: 0.825 ± 0.058; SHR-Spiro: 0.650 ± 0.023 mmHg/ml; $p < 0.05$). Our results show that long-term spironolactone treatment initiated at the early stage of hypertension development reduces left ventricular hypertrophy and wall stiffness in SHRs.

**Key words:**
hypertension, spironolactone, ventricular hypertrophy, ventricular stiffness

Abbreviations: ACE – angiotensin converting-enzyme, ANOVA – analysis of variance, DBP – diastolic blood pressure, HF – heart failure, HR – heart rate, LV – left ventricle, LVEDP – left ventricular end-diastolic pressure, LVSP – left ventricular systolic pressure, RAAS – renin-angiotensin-aldosterone system, SBP – systolic blood pressure, SEM – standard error of mean, SHRs – spontaneously hypertensive rats

## Introduction

Hypertension is a common risk factor for heart failure (HF) and is associated with high mortality rates [18]. Activation of the renin-angiotensin-aldosterone system (RAAS) in hypertension directly affects cardiac re-

modeling, leading to interstitial and perivascular fibrosis, cardiomyocyte hypertrophy and apoptosis [17, 28]. Furthermore, hypertension increases plasmatic aldosterone levels [25], leading to left ventricular (LV) hypertrophy and myocardial fibrosis [15, 18]. Several studies have shown that aldosterone is produced not only in the adrenal cortex but also in the heart and blood vessels [27].

Experimental studies in rats have shown increased cardiac production of aldosterone and its receptor in different models of HF, such as myocardial infarction [21]. Similar findings have been observed in spontaneously hypertensive rats (SHRs), which develop HF after a compensated phase of cardiac function [12]. SHRs are used widely as models of essential hypertension due to their development of high blood pressure levels and cardiac and vascular remodeling [22], with cardiac hypertrophy [27] and increased collagen deposition [23].

Spironolactone, a mineralocorticoid receptor antagonist, has been used in humans for the treatment of hypertension and heart failure, with a resulting marked reduction in cardiovascular events [20]. Our group has shown that spironolactone reduces collagen deposition after coronary occlusion [15] and also reduces LV stiffness in adult hypertensive rats submitted to a high salt diet [2]. Mineralocorticoid receptor antagonism also ameliorates cardiac dysfunction in old SHRs [23]. Moreover, it has been shown that mineralocorticoid antagonists and renin-angiotensin system inhibitors given as a combination therapy can produce improvements in fibrosis and hypertrophy in hypertensive animals [3].

Studies of spironolactone use in hypertensive rats have been conducted mostly after short-term exposure to the drug, which would be considered an unusual scenario for clinical studies. Furthermore, studies with long-term treatment were initiated after hypertensive heart disease was already established. However, the early and long-term use of spironolactone to prevent cardiac remodeling in hypertension remains unknown. Therefore, the aim of this study was to evaluate the long-term effects on cardiac structure and function in SHRs treated with a low spironolactone dose initiated at early stages of hypertension development.

## Materials and Methods

### Animals and treatment

Animals were provided by our department colony at Federal University of Espírito Santo. During all treatment periods, the animals had free access to food and drinking water. All protocols were performed in accordance to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) and the ethical principles in animal experimentation of the Brazilian College of Animal Experimentation (COBEA), and this study was approved by the institutional Committee of Ethics on Animal Research (No. 012/2010).

One-month-old SHRs were randomly selected to receive 20 mg/kg per day of spironolactone (SHR-Spiro, 0.2 ml, *sc*/day, dissolved in mineral oil; n = 8) or only the vehicle (SHR; 0.2 ml, *sc*/day, n = 9). Aged-matched Wistar rats (n = 8) were used as normotensive controls. The injection site was often rotated to reduce the risk for a potential inflammatory reaction. The animals were weighed weekly to make any necessary adjustments to the drug dose.

### Non-invasive blood pressure assessment

Non-invasive measurement of tail-cuff pressure as an estimate of systolic arterial pressure was done at baseline (1 month) and at 4 and 8 months of age. Rats were warmed in a restraining chamber, and occluding cuffs and pneumatic pulse transducers were placed on the rat tail. A sphygmomanometer was inflated and deflated automatically, and the tail-cuff signals from the transducer were automatically collected using an IITC apparatus (IITC Inc., California, USA) connected to a computer. For each blood pressure measurement session, the mean of eight blood pressure readings was recorded for each rat.

### Hemodynamic measurements

At eight months of age (following seven months of treatment), animals were anesthetized with ketamine (Agener União, Brazil) and xylazine (Bayer, Brazil) (70/10 mg/kg, *ip*) to obtain hemodynamic data. The right common carotid artery was catheterized with a fluid-filled polyethylene catheter (P50) connected to a pressure transducer (TRI 21, Letica Scientific Instruments, Spain) and a digital system (Powerlab/4SP ML750, ADInstrument, Australia). The systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) were initially measured in the aorta. The catheter was then advanced into the left ventricular cavity to record systolic (LVSP) and end-diastolic (LVEDP) pressures. The maximum rate of pressure

rise and fall (d*P*/dt max and min, respectively) was obtained electronically, and all records were obtained at 2 kHz.

### *In situ* left ventricular pressure-volume relationship

After hemodynamic evaluation, the heart was arrested with 3 M KCl (0.2 ml, *iv*), and a double-lumen catheter (P50 inserted into P200) was inserted into the left ventricle through the aorta to obtain the *in situ* left ventricle diastolic pressure-volume relationship, as previously described [2]. In brief, the atrio-ventricular groove was occluded, and a small incision was made in the right ventricular free wall to avoid liquid accumulation inside this cavity and any compressor effect on the left ventricular chamber. Physiological saline (NaCl 0.9%) was pumped (BI 200, Insight Equipments, Brazil) into the left ventricular cavity at a constant rate (0.68 ml/min) through the P200 catheter up to a pressure of 30 mmHg, which was continuously monitored through the P50 catheter. Then, the left ventricle cavity was emptied, and another pressure-volume curve was obtained. Three curves were recorded in each heart over ten minutes.

The curves were separated into two parts to exclude possible interferences of dilatation on the stiffness index [2]. In the first segment, from 0 to 5 mmHg, the pressure curve followed a linear pattern during volume infusion, and the slope was proportional to the left ventricular dilatation. In the second segment, from 5 to 30 mmHg, the pressure increase during volume infusion followed a monoexponential pattern ($P = V_o \times e^{kv}$), where $V_o$ is the volume at 5 mmHg, k is the stiffness constant of the chamber and v is the volume infused. To determine the stiffness constant in the 5–30 mmHg interval without the interference of the first segment, the pressure scale was log-transformed. Therefore, the slope of the linear relationship between pressure and volume represents the stiffness constant of the left ventricular cavity.

After pressure-volume recording, the heart was removed, and the ventricles were separated, blotted and weighed. Hypertrophy was evaluated by the ventricle to body weight ratio.

### Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). One or two way analysis of variance (ANOVA) was used to compare the means of three groups, as appropriate, followed by the Fischer *post-hoc* test. The Pearson correlation was used to determine whether an association existed between two or more variables. Statistical significance was set at $p < 0.05$.

## Results

### Morphometric parameters

After seven months, Wistar rats were heavier than the rats in the SHR groups (Wistar: 497 ± 14 g, SHR: 361 ± 7 g, SHR-Spiro: 338 ± 5 g; $p < 0.05$). Table 1 depicts the morphological parameters found after seven months of treatment. Spironolactone reduced crude left ventricular weight in SHRs (Wistar: 1250 ± 30; SHR: 1000 ± 30; SHR-Spiro: 860 ± 20 g; $p < 0.05$) and left ventricular mass normalized to body weight (Wistar: 2.51 ± 0.06; SHR: 2.70 ± 0.08; SHR-Spiro: 2.53 ± 0.07 mg/g; $p < 0.05$). The other parameters, RV and lungs, were not significantly different between SHR groups.

### Hemodynamic evaluation

Using a non-invasive method, we observed that one-month-old SHRs (before treatment) showed higher systolic blood pressure than Wistar rats (Wistar: 115 ± 3, SHR: 130 ± 10, SHR-Spiro: 129 ± 6 mmHg; $p < 0.05$). The systolic blood pressure increased progressively during the observation period, without a sig-

**Tab. 1.** Morphological parameters after 7 months of follow up

| | Wistar (n = 8) | SHR (n = 9) | SHR-Spiro (n = 8) |
|---|---|---|---|
| LV (mg) | 1250 ± 30 | 1000 ± 30* | 860 ± 20*# |
| LV/BW (mg/g) | 2.51 ± 0.06 | 2.70 ± 0.08* | 2.53 ± 0.07*# |
| RV (mg) | 254 ± 12 | 202 ± 14* | 185 ± 13* |
| RV/BW (mg/g) | 0.50 ± 0.07 | 0.50 ± 0.03 | 0.54 ± 0.03 |
| Lungs (mg) | 3100 ± 90 | 2500 ± 170* | 2550 ± 100* |
| Lungs/BW (mg/g) | 6.2 ± 0.3 | 7.0 ± 0.6 | 7.2 ± 0.3 |

* $p < 0.05$ *vs.* Wistar; # $p < 0.05$ *vs.* SHR

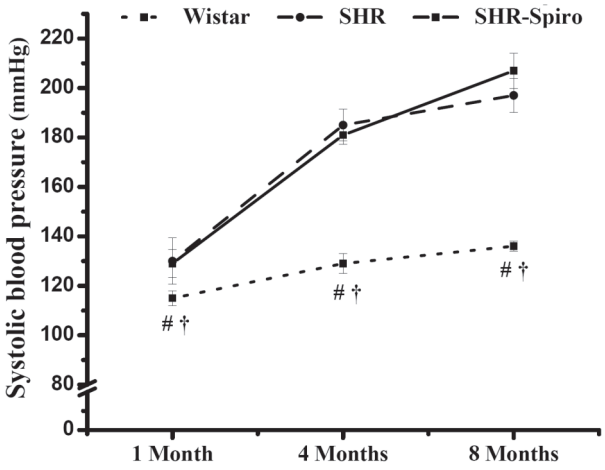


**Fig. 1.** Non-invasive measurement of systolic blood pressure by tail-cuff plethysmography. † $p < 0.05$ *vs.* SHR, # $p < 0.05$ *vs.* SHR-Spiro

nificant difference between the spironolactone-treated and the untreated SHR groups (Fig. 1). The hemodynamic parameters obtained in anesthetized animals are shown in Table 2. Additionally, spironolactone did not significantly affect blood pressure in the treated SHR group. As expected, the left ventricular systolic pressure was higher in SHRs than in Wistar rats. Moreover, LVEDP was also significantly higher in the SHR groups than in the Wistar rats. Left ventricular contractility and relaxation were assessed by +d*P*/dt max and –d*P*/dt min, respectively. The indexes of the systolic and diastolic performance of the left ventricle were slightly higher in SHRs (without statistical significance), and both were unaffected by spironolactone treatment.

**Tab. 2.** Hemodynamic parameters in anesthetized SHRs

| | Wistar (n = 8) | SHR (n = 9) | SHR-Spiro (n = 8) |
|---|---|---|---|
| HR (bpm) | 192 ± 8 | 235 ± 17* | 260 ± 22* |
| SBP (mmHg) | 109 ± 2 | 139 ± 3* | 130 ± 3* |
| DBP (mmHg) | 76 ± 3 | 93 ± 7* | 88 ± 4* |
| LVSP (mmHg) | 113 ± 2 | 150 ± 3* | 149 ± 4* |
| LVEDP (mmHg) | 4 ± 1 | 7.3 ± 1* | 7.7 ± 2* |
| +d*P*/dt max (mmHg/s) | 4854 ± 101 | 5225 ± 74 | 5145 ± 101 |
| -d*P*/dt max (mmHg/s) | –3698 ± 92 | –4036 ± 166 | –3972 ± 139 |

* $p < 0.05$ *vs.* Wistar

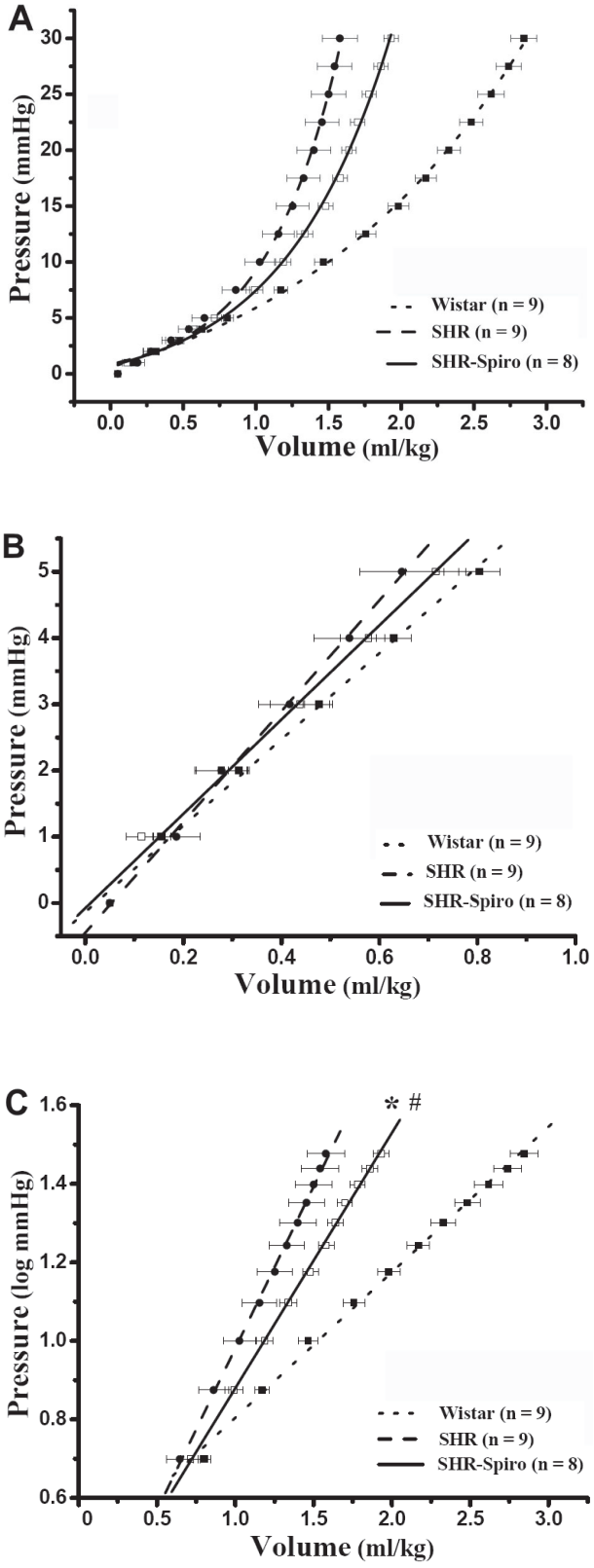


**Fig. 2.** Left ventricular *in situ* pressure-volume relationship. The entire curves (**A**) were divided into two segments to evaluate the left ventricular dilatation (**B**) and wall stiffness (**C**). * $p < 0.05$ *vs.* Wistar; # $p < 0.05$ *vs.* SHR

### Left ventricular pressure-volume relationship

The pressure-volume curve (Fig. 2A) was divided into two segments for analysis. The first segment (0–5 mmHg) was fitted to a linear regression (Fig. 2B), and the inclination gives a dilatation index of the left ventricular cavity. The second segment (5–30 mmHg) was adjusted to a monoexponential model (pressure = $V_0 \times e^{kv}$), with k giving the left ventricular stiffness index (Fig. 2C). According to this model, the left ventricular dilatation index was similar for all groups (Wistar = 6.5 ± 1.2; SHR = 8.2 ± 1.5; SHR-Spiro = 7.2 ± 1.1 mmHg/ ml, p = 0.09; Fig. 2B), despite a trend toward higher values in SHRs. The ventricular stiffness, however, was significantly higher in SHRs compared to Wistar rats, and spironolactone treatment partially prevented ventricle stiffening in hypertensive animals (Wistar: 0.370 ± 0.032; SHR: 0.825 ± 0.058; SHR-Spiro: 0.650 ± 0.023 mmHg/ml; $p < 0.05$; Fig. 2C). Figure 3 shows a significant association between left ventricular hypertrophy (assessed by left ventricle weight to body weight ratio) and stiffness index ($r = 0.606$; $p < 0.05$).

## Discussion

Hypertension produces adaptive changes in the cardiovascular system due to pressure overload, which commonly leads to heart failure. The development of left ventricular hypertrophy and the stiffening of the left ventricular chamber results in diastolic dysfunction that facilitates heart failure progression and an increased risk of death.

The structural rearrangement that occurs in the left ventricular wall due to chronic pressure overload determines the degree of ventricular stiffening, thus affecting the systolic and diastolic function of the heart chambers [26]. It is already known that RAAS induces left ventricular remodeling [30]. Moreover, both angiotensin-converting enzyme (ACE) inhibitors and/or $AT_1$ receptor blockers are able to prevent the development of cardiac hypertrophy in hypertensive rats [22] as well as reduce cardiac hypertrophy in humans [1]. Studies have shown that aldosterone participates directly in several processes of ventricular remodeling. Okoshi et al. [17] have shown that aldosterone produces cardiomyocyte hypertrophy in *in vitro* conditions. Furthermore, in some clinical trials, aldosterone receptor blockers have been shown to reverse cardiac hypertrophy in several cardiovascular diseases [9, 19]. Unfortunately, these findings have not yet been translated to clinical practice [14].

Aldosterone antagonists exert beneficial effects in hypertensive rats. For example, Baumann et al. [3] treated SHRs with spironolactone or the angiotensin II receptor blocker losartan in the prehypertensive phase of 4 weeks, after which the drugs were discontinued. Following a drug wash-out period, it was observed that cardiac fibrosis was reduced by both treatments. However, cardiac hypertrophy was reduced only by losartan. The spironolactone dose used by Baumann et al. [3] was lower (1 mg/kg) than the dose used in our study (20 mg/kg). Thus, we found less ventricular hypertrophy than expected in spironolactone-treated SHRs. Furthermore, this short, 4-week treatment period of SHRs only during the prehypertensive stage followed by drug discontinuation was recently studied by our group. We showed that ACE inhibition with captopril was effective in reducing ventricular and arterial remodeling during the treatment period, but not after the drug was withdrawn [22]. In the present study, spironolactone was administered long-term, and the same dose was maintained throughout the treatment period. This strategy avoids a possible loss of drug effect over time.

Conversely, Kambara et al. [11] showed that in a rat model of spontaneous hypertension and heart failure, daily spironolactone (20 mg/kg) was ineffective in reducing cardiac hypertrophy and fibrosis, unlike the results seen after treatment with captopril. In our study, the treatment was initiated in an early phase of blood pressure increase and cardiac hypertrophy development. Thus, our results regarding cardiac hypertrophy seem not to be through a regressive effect but potentially through slowness in the hypertrophic signaling. Our results also suggest that the beneficial effects in ventricular hypertrophy reduction are independent of blood pressure changes.

It is already known that SHRs present hyperactivation of the sympathetic nervous system from the prehypertensive phase [6, 7], as is observed with the increased heart rate found in SHRs in our study. It has been reported that aldosterone antagonists prevent the degeneration of left ventricular function, collagen deposition and hypertrophy induced by chronic β-adrenergic activation in SHRs [4, 29]. This pathway might partially explain our results regarding LV hypertrophy.

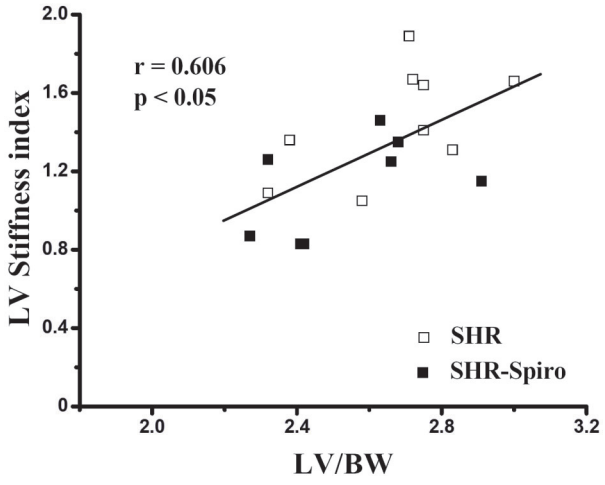


**Fig. 3.** Correlation between left ventricular hypertrophy (evaluated by LV/BW) and the chamber stiffness index

Changes in the extracellular myocardial matrix can lead to ventricular stiffening. For example, in patients with mild dilated cardiomyopathy, spironolactone reduced left ventricular stiffness and fibrosis [10]. Some studies have shown the ability of aldosterone antagonists to decrease collagen deposition in the myocardium of hypertensive rats [3, 23]. Moreover, in a model of pressure overload by aortic constriction, eplerenone, a new selective mineralocorticoid receptor blocker, reduced ventricular and perivascular fibrosis and hypertrophy [13]. Additionally, it was reported that eplerenone administration to Dahl salt-sensitive rats in the compensatory hypertrophic phase reduced cardiac hypertrophy and ventricular stiffness [16]. Our group recently reported that adult hypertensive rats present with an enhanced left ventricular stiffness phenotype and that high dose spironolactone abrogates this effect [2]. Our results showed that SHRs (8 months of age) had higher left ventricular stiffness compared to normotensive controls. Furthermore, we have also found that spironolactone reduces left ventricular stiffness in SHRs. Correlation analysis showed a significant association between left ventricular hypertrophy and stiffness. This fact reinforces the idea that left ventricular hypertrophy also contributes to an increase in chamber stiffness in SHRs. Therefore, the antihypertrophic effect of long term spironolactone use in these animals may also contribute to stiffness reduction.

However, another parameter related to ventricular relaxation, –d*P*/dt max, remained unchanged in our study. It is important to point out that hemodynamic variables are influenced by anesthesia. In fact, anesthesia exerts greater effects in SHRs than Wistar rats because of the high sympathetic hyperactivity found in SHRs, which can be inferred in our data by the lower heart rate observed in these animals. A higher sympathetic drive to the heart tends to accelerate calcium reuptake in the sarcoplasmic reticulum and, thus, increases –d*P*/dt max. Therefore, the reporting of hemodynamic variables under anesthesia represents a limitation of our study.

In a model of hypertension associated with high salt intake and a continuous infusion of aldosterone, spironolactone (20 mg/kg) reduced interstitial and perivascular fibrosis [5]. At the same dose, spironolactone reduced reactive fibrosis in the infarcted myocardium [15]. However, these effects related to aldosterone receptor blockers were not confirmed in other studies. For example, eplerenone did not reduce left ventricular hypertrophy and myocardial fibrosis in SHRs beyond the observed improvement in coronary perfusion [24]. Collagen assessment is another limitation to our data analysis. Collagen proliferation can be observed in SHRs, mainly in adult SHRs, and could be an important determinant of chamber stiffness. Furthermore, mineralocorticoid receptor antagonists can interfere with fibrotic proliferation. However, it has been shown that the low dose used in our work was unable to reduce fibrosis in SHRs [11]. Moreover, the association of hypertrophy with chamber stiffness shown in our study reinforces the view that hypertrophic regression may provide an important opportunity to treat left ventricular stiffness.

The long-term use of spironolactone is often limited in patients due to the undesired adverse effects, such as breast tenderness and gynecomastia, which affects about 10% of treated men [8]. Eplerenone has been used to treat several conditions, including hypertension and heart failure, and has shown similar efficacy as spironolactone without the same adverse effects and toxicities [14, 19]. Therefore, experimental and clinical studies suggest that an aldosterone antagonist may represent a useful strategy to prevent matrix remodeling in chronic diseases such as hypertension, preventing heart failure.

In summary, our data show that low-dose spironolactone given to SHRs for a long-term period and initiated at an early stage of hypertension development, when blood pressure is beginning to increase, results in decreased left ventricular hypertrophy and higher

left ventricular compliance, independently of alterations in blood pressure levels. These results support the beneficial effects of aldosterone antagonists and support their use as standard therapy.

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