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# Sex differences in the response to angiotensin II receptor blockade in a rat model of eccentric cardiac hypertrophy

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Aim of study was to evaluate the development of cardiac hypertrophy (CH) in response to left ventricle (LV) volume overload (VO) caused by chronic aortic valve regurgitation (AR) in male and female rats treated or not with angiotensin II receptor blocker (ARB), valsartan. We studied 8 groups of Wistar rats: male or female, AR or sham-operated (sham) and treated or not with valsartan (30 mg/kg/day) for 9 weeks starting one week before AR surgical induction. As expected, VO from AR resulted for both male and female rats in significant LV dilation (39% vs. 40% increase of end-diastolic LV diameter, respectively; $p<0.0001$) and CH (53% vs. 64% increase of heart weight, respectively; $p<0.0001$) compared to sham. Sex differences were observed in the LV wall thickening in response to VO. In untreated AR males, relative LV wall thickness (a ratio of wall thickness to end-diastolic diameter) was reduced compared to sham, whereas this ratio in females remained unchanged. ARB treatment did not prevent LV dilation for both male and female animals but reversed LV wall thickening in females. Systolic and diastolic functions in AR animals were altered similarly for both sexes compared to sham. ARB treatment did not improve systolic function but help normalizing diastolic parameters in female AR rats. Increased LV expression of *Anp* and *Bnp* genes was normalized by ARB treatment in AR females but not in males. Other hypertrophy gene markers (*Fos, Trpc6, Klf15, Myh6* and *Myh7*) were not modulated by ARB treatment. The same was true for genes related to LV extracellular matrix remodeling (*Col1a1*, *Col3a1, Fn1, Mmp2, Timp1* and *Lox*). In summary, ARB treatment of rats with severe AR blocked the female-specific hypertrophic response characterized by LV chamber wall thickening. LV dilation, on the other hand, was not significantly decreased by ARB treatment.

# Sex differences in the response to angiotensin II receptor blockade in a rat model of eccentric cardiac hypertrophy.

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## ABSTRACT

Aim of study was to evaluate the development of cardiac hypertrophy (CH) in response to left ventricle (LV) volume overload (VO) caused by chronic aortic valve regurgitation (AR) in male and female rats treated or not with angiotensin II receptor blocker (ARB), valsartan. We studied 8 groups of Wistar rats: male or female, AR or sham-operated (sham) and treated or not with valsartan (30 mg/kg/day) for 9 weeks starting one week before AR surgical induction. As expected, VO from AR resulted for both male and female rats in significant LV dilation (39% vs. 40% end-diastolic LV diameter increase, respectively; $p<0.0001$) and CH (53% vs. 64% heart weight increase, respectively; $p<0.0001$) compared to sham. Sex differences were observed in LV wall thickening in response to VO. In untreated AR males, relative LV wall thickness (a ratio of wall thickness to end-diastolic diameter) was reduced compared to sham, whereas this ratio in females remained unchanged. ARB treatment did not prevent LV dilation in both male and female animals but reversed LV wall thickening in females. Systolic and diastolic functions in AR animals were altered similarly for both sexes. ARB treatment did not improve systolic function but help normalizing diastolic parameters such as left atrial mass and E wave slope in female AR rats. Increased LV gene expression of *Anp* and *Bnp* was normalized by ARB treatment in AR females but not in males. Other hypertrophy gene markers (*Fos*, *Trpc6*, *Klf15*, *Myh6* and *Myh7*) were not modulated by ARB treatment. The same was true for genes related to LV extracellular matrix remodeling (*Col1a1*, *Col3a1*, *Fn1*, *Mmp2*, *Timp1* and *Lox*). In summary, ARB treatment of rats with severe AR blocked the female-specific hypertrophic response characterized by LV chamber wall thickening. LV dilation, on the other hand, was not significantly decreased by ARB treatment.

## INTRODUCTION

Heart diseases are among the leading causes of mortality for both men and women. Roth et al. (2017) Cardiac hypertrophy (CH) is an adaptive response to overload (pressure (PO) or volume (VO)). Swynghedauw (1999) CH is an independent cause of morbidity and mortality from heart diseases. A sexual dimorphism is present in the hypertrophic response to an overload in both patients and in pre-clinical models. Blenck et al. (2016) It is believed that sex hormones can provide a protection against CH development and its main consequence, the evolution towards heart failure HF. For instance, it has been shown that evolution towards HF is quicker in animal models of PO in males than in females. Blenck et al. (2016)

We recently observed in a rat model of chronic (6 months) LV volume overload (VO) caused by severe aortic valve regurgitation (AR), that female animals developed as much if not more CH than males. Beaumont et al. (2017) However, male LVs showed more dilation and worse contractile function than those of females. Interestingly, LV remodeling in AR female rats is characterized by a more important increase LV wall thickness than in males. Beaumont et al. (2017) In another rat VO model (aorto-caval fistula), a faster progression toward HF was observed in males and resulted in poorer survival. Dent et al. (2010a) At the cellular and molecular levels, we also observed that male AR rat LVs showed an

important down-regulation of many fatty acid oxidation genes and an up-regulation of glucose metabolism genes, whereas this characteristic energy metabolism switch did not happen in females. Beaumont et al. (2017) Since sex steroids have a potent effect on differentiation, they could explain a large part of the sex dimorphism observed in CH. Leinwand (2003)

We previously showed that blocking the renin-angiotensin-aldosterone system (RAAS) in male AR rats can reduce development of LV hypertrophy (LVH), improve myocardial function and survival Plante et al. (2009) Plante et al. (2004a) Arsenault et al. (2013). However, we did not investigate the benefits of inhibiting RAAS in female AR rats. Here, we wanted to compare the hypertrophic response to treatment of animals of both sexes with a severe LV volume overload. We studied the effects of an angiotensin II receptor antagonist, valsartan, on the hypertrophic response to severe LV volume overload from AR in rats of both sexes and over a relatively short duration of two months. We started treatment one week before AR induction instead as two weeks after as described in the chronic studies above. Our results suggest that angiotensin receptor blockade (ARB) with valsartan during the development of hypertrophy in female rats with severe AR abrogates LV wall thickening leading to a more eccentric remodeling similar to the one observed in males.

# METHODS

## Animals

Severe AR was induced by retrograde puncture one or two aortic valve leaflets under echocardiographic guidance as previously described. Arsenault et al. (2002) Only animals with 50% and more regurgitation were included in the study. The regurgitant fraction was estimated by the ratio of the forward systolic flow time–velocity integral (VTI) to the reversed diastolic flow VTI measured by pulsed Doppler in the thoracic descending aorta. Eleven male and ten female Wistar AR rats received daily valsartan (30 mg/kg/d) mixed in unsalted peanut butter (1:50;w:w). Untreated animals received equivalent amount of peanut butter (5-6 animals/gr.). Treatment was started one week before AR induction. We made sure that peanut butter was consumed by all animals, daily. In addition, 24 sham-operated male and female Wistar rats (Sham or Sh) were used as controls and received treatment following the same regimen as AR rats. A complete echo exam was performed before AR induction and at the end of the protocol as previously described. Plante et al. (2003)

## Echocardiography

An echocardiographic exam was performed two weeks after surgery to confirm AR severity and at the end of the protocol 26 weeks later as previously described. Arsenault et al. (2013) Arsenault et al. (2002) Plante et al. (2003) At the end of the protocol, the heart and the lungs were harvested and weighed. Heart chambers were dissected, weighted and the LV was then quickly frozen in liquid nitrogen and kept at -80 C until further use.

## Gene Expression Analysis by quantative RT-PCR

LV gene expression was quantified for 5-6 animals per group by quantitative RT-PCR as described elsewhere. Champetier et al. (2009) Pre-optimized primers were from QuantiTect (Qiagen) and IDT (Coralville, Iowa) (Table 1) and SsoAdvanced Universal SYBR Green Supermix (Bio Rad, Hercules, CA) was used.

## Statistical analysis

Results are presented as the mean and the standard error of the mean (SEM). Two-way ANOVA analysis was performed and Holm-Sidak's post-test was used for comparison between the groups (Graph Pad Prism 8.02, San Diego, CA). A Student's t-test was used when only two groups were compared. A p-value lower than 0.05 was considered significant.

# RESULTS

## Animal characteristics

Treatment with the angiotensin receptor blocker (ARB), valsartan, was initiated a week before surgery and lasted up until the end of the protocol 9 weeks later. Valsartan treatment had no significant effects on

**Table 1.** Name and symbol of all primer pairs used for gene expression analysis by quantitative RT-PCR. The table also includes catalogue numbers (from IDT or Qiagen) and the size of the amplicon.

| mRNA | Symbol | Catalog no. | Amplicon (bp) |
|---|---|---|---|
| procollagen-1 alpha-1 | Col1 | Rn.PT.58.7562513 | 134 |
| procollagen-3 alpha-1 | Col3 | Rn.PT.58.11138874 | 100 |
| fibronectin 1 | Fn1 | Rn.PT.58.18226984 | 114 |
| osteosarcoma viral oncogene homolog | Fos | QT01576330 | 73 |
| krüppel-like factor 15 | Klf15 | Rn.PT.58.12431283 | 129 |
| matrix metalloproteinase-2 | Mmp2 | Rn.PT.58.44737355 | 87 |
| myosin, heavy polypeptide 6, cardiac | Myh6 | Rn.PT.58.8646063 | 150 |
| myosin, heavy polypeptide 7, cardiac | Myh7 | Rn.PT.58.34623828 | 125 |
| natriuretic peptide precursor type A | Nppa, Anp | Rn.PT.58.5865224 | 79 |
| natriuretic peptide precursor type B | Nppb, Bnp | Rn.PT.58.5595685 | 108 |
| cyclophilin A | Ppia | Rn.PT.39a,22214830 | 140 |
| tissue inhibitor of metalloproteases 1 | Timp1 | Rn.PT.58.34442920 | 127 |
| transient receptor potential cation channelC6 | Trpc6 | Rn.PT.58.18089975 | 94 |

**Table 2.** Characteristics of sham-operated animals at the end of the protocol. BW: body weight. M: males, F: females and V: valsartan. Values are expressed as the mean +/- SEM. Group comparisons were made using Student's T-test. *: $p < 0.0001$ vs. the respective untreated group.

| Parameters | ShM (n=6) | ShMV (n=6) | ShF (n=5) | ShFV (n=6) |
|---|---|---|---|---|
| Body weight, g | 586 +/- 9 | 637 +/- 31 | 318 +/- 6 | 328 +/- 3 |
| Tibial length, mm | 57 +/- 0.2 | 57 +/- 0.7 | 50 +/- 0.4 | 50 +/- 0.2 |
| Heart, mg | 1303 +/- 30 | 1322 +/- 68 | 836 +/- 22 | 812 +/- 26 |
| Heart/BW, mg/g | 2.2 +/- 0.06 | 2.1 +/- 0.06 | 2.6 +/- 0.04 | 2.5 +/- 0.07 |
| Left ventricle, mg | 1000 +/- 28 | 976 +/- 46 | 638 +/- 22 | 612 +/- 24 |
| Right ventricle, mg | 225 +/- 9 | 247 +/- 20 | 144 +/- 3 | 149 +/- 4 |
| Left atria, mg | 32 +/- 2 | 30 +/- 3 | 24 +/- 1 | 15 +/- 1* |

heart total weight in sham-operated animals, males and females (Table 2). The only significant difference was for the left atrial weight which was decreased by ARB treatment in females.

Comparing AR rats to sham ones, every parameters measured with the exception of body weight and tibial length were significantly increased as summarized in Tables 2 and 3. Moreover, heart total and indexed weights were significantly reduced in female animals treated with the ARB (Table 3). Only a trend for a decrease was present in males. This was also true for the left and right ventricles, which were smaller in female AR rats treated with valsartan. This was not observed in AR males.

In Figure 1, we illustrated variations in heart and heart chambers weights of AR animals compared to their respective sham-operated group. As expected, AR caused important cardiac hypertrophy in both male and female animals compared to sham (Figure 1). Valsartan had relatively little effects in blocking the development of cardiac hypertrophy in male animals. On the other hand, the hypertrophic response was slowed in female animals mainly for the left ventricle.

## Echocardiographic data

As for the animal and heart characteristics described above, most echocardiographic parameters were significantly changed by AR (Tables 4 and 5). ARB treatment with valsartan of sham-operated rats had relatively no effects on echocardiographic parameters measured in this study (Table 4). Calculated ejection fraction, although still in the normal range was lower in treated sham males. In AR animals, the effects of valsartan were observed on the LV wall thickness (intraventricular septal wall (SW) and posterior wall (PW), which were thinner in females compared to untreated AR rats. ARB had no effects in AR males (Table 5). Diastolic LV parameters were significantly altered in AR rats. Valsartan treatment had no effects on these parameters in males but significantly reduced E wave slope in females.

As illustrated in Figure 2, LV dilation caused by AR was similar in rats of both sexes. However, LV

**Table 3.** Characteristics of AR animals at the end of the protocol. BW: body weight. Values are expressed as the mean +/- SEM. Group comparisons were made using Student's T-test. *: $p < 0.0001$ vs. the respective untreated group.

| Parameters | ARM (n=6) | ARMV (n=11) | ARF (n=6) | ARFV (n=10) |
|---|---|---|---|---|
| Body weight, g | 599 +/- 31 | 619 +/- 12 | 337 +/- 12 | 332 +/- 9 |
| Tibial length, mm | 58 +/- 0.3 | 58 +/- 0.3 | 50 +/- 0.5 | 50 +/- 0.2 |
| Heart, mg | 1989 +/- 54 | 1889 +/- 87 | 1375 +/- 107 | 1115 +/- 59* |
| Heart/BW, mg/g | 3.3 +/- 0.08 | 3.1 +/- 0.11 | 4.1 +/- 0.31 | 3.4 +/- 0.14* |
| Left ventricle, mg | 1538 +/- 55 | 1426 +/- 58 | 1068 +/- 81 | 862 +/- 44* |
| Right ventricle, mg | 311 +/- 28 | 325 +/- 23 | 225 +/- 20 | 171 +/- 12* |
| Left atria, mg | 61 +/- 3 | 58 +/- 5 | 43 +/- 4 | 32 +/- 3* |

**Table 4.** Echocardiographic parameters of sham-operated animals at the end of the protocol. EDD: end-diastolic diameter, ESD: end-systolic diameter, SW: septum wall thickness, RWT: relative wall thickness, EF: ejection fraction, HR: heart rate, bpm: beats per minute, Values are expressed as the mean +/- SEM. Group comparisons were made using Student's T-test. *: $p < 0.05$ vs. the respective untreated group.

| Parameters | ShM (n=6) | ShMV (n=6) | ShF (n=5) | ShFV (n=6) |
|---|---|---|---|---|
| EDD, mm | 7.7 +/- 0.3 | 7.3 +/- 0.3 | 6.7 +/- 0.2 | 6.5 +/- 0.3 |
| ESD, mm | 3.3 +/- 0.1 | 3.6 +/- 0.2 | 2.9 +/- 0.2 | 2.8 +/- 0.3 |
| SW, mm | 1.2 +/- 0.06 | 1.1 +/- 0.04 | 1.1 +/- 0.04 | 0.9 +/- 0.02 |
| PW, mm | 1.9 +/- 0.02 | 2.1 +/- 0.08 | 1.7 +/- 0.13 | 1.6 +/- 0.02 |
| RWT | 0.40 +/- 0.032 | 0.44 +/- 0.022 | 0.42 +/- 0.018 | 0.39 +/- 0.018 |
| EF, % | 81 +/- 1 | 74 +/- 2* | 81 +/- 3 | 81 +/- 2 |
| HR, bpm | 393 +/- 13 | 374 +/- 10 | 413 +/- 17 | 421 +/- 14 |
| E wave, cm/s | 77 +/- 3 | 74 +/- 6 | 71 +/- 4 | 86 +/- 6 |
| A wave, cm/s | 38 +/- 3 | 45 +/- 6 | 40 +/- 2 | 43 +/- 3 |
| E wave slope | 2691 +/- 52 | 2391 +/- 115* | 2383 +/- 129 | 2699 +/- 127 |

septal and posterior walls thickening was more important in females. This was almost completely blocked by ARB treatment.

### Markers of LV hypertrophy and extracellular matrix remodeling

We measured LV gene expression for several hypertrophy markers. *Anp* and *Bnp* mRNA levels were increased in both male and female AR animals (Figure 3A). This increase was stronger for *Bnp* expression in females and was reversed by ARB treatment. Valsartan also reversed the increase in *Anp* expression in AR females. The expression of other hypertrophy markers was only changed in AR males but not in females (Figure 3B). Valsartan lowered *Fos* expression in both male and female animals but had little effects on the other two genes studied (*Trpc6* and *Klf15*), which were both up-regulated by LVH in males. As expected *Myh6* gene expression was reduced with AR whereas *Myh7* expression tended to increase. Valsartan treatment had no effect on the expression of both *Myh* genes. We did not observe important modulation of the extracellular markers studied. *Col1* was significantly increased in female AR rats and this was reversed by ARB treatment. *Timp1* was up-regulated in AR males and *Lox* in both males and females. The expression of the latter was reduced in ARB-treated males.

## DISCUSSION

In this study, we observed that angiotensin II receptor blockade using valsartan reduces the LV wall thickening taking place in AR female rats. We had previously shown that LV remodeling from AR in this model involves similar LV dilation in rats of both sexes but an excess of wall thickening in females. This results in a relatively maintained relative wall thickness (RWT) ratio, an index of LV remodeling. Beaumont et al. (2017)

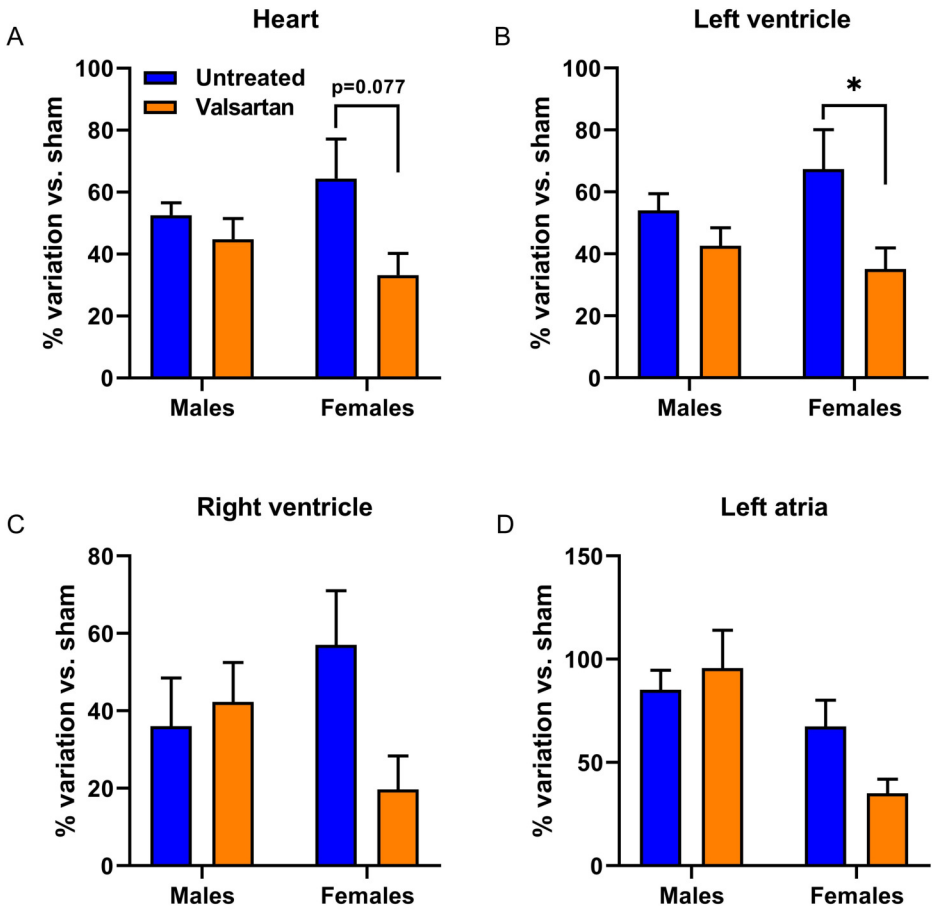


**Figure 1.** Effects of a 9-week treatment with valsartan on LV hypertrophy development caused by severe volume overload from AR. Results are expressed as the percentage of variation of the indicated parameter compared to the mean of the same parameter for the respective sham-operated group. A, Heart weight, B, Left ventricular weight, C, right ventricular weight and D, left atrial weight. Results are expressed as the mean +/- SEM. *: $p < 0.05$ between groups.

The classic view of cardiac remodeling induced by a hemodynamic overload is that pressure overload (hypertension or aortic stenosis; afterload) is associated with initial concentric LV hypertrophy (wall thickening and equal or smaller chamber volume). On the other hand, volume overload situations (valve regurgitation; preload) induce chamber dilatation with no or little increase in wall thickness or eccentric remodeling. Katz and Rolett (2016) This view is probably more accurate for male animal models than for females as evidenced in the present study. Here in the AR rat model, we observed that LV dilation was similar and relatively unaffected by angiotensin receptor blockade by valsartan both in males and females. Blood regurgitation from the aorta to the LV during diastole is a somewhat stable determinant of the disease and cannot be modulated significantly by ARB. On the other hand, LV wall thickening was more important in female AR rats compared to males but was reversed by treatment leaving a LV morphology relatively similar between the sexes.

Arteriovenous (AV) shunt is a form of global volume overload where sex differences have been studied with some details in the past in rats. Although this form of VO is less relevant on a clinical standpoint but remains, the most popular model in the literature. In 2002, Gardner and collaborators first reported that female rats with AV shunt developed less hypertrophy, evolved less towards heart failure and had better survival than males. Gardner et al. (2002) Ovariectomy removed this advantage over males.

**Table 5.** Echocardiographic parameters of AR animals at the end of the protocol. EDD: end-diastolic diameter, ESD: end-systolic diameter, SW: septum wall thickness, RWT: relative wall thickness, EF: ejection fraction, HR: heart rate, bpm: beats per minute, Values are expressed as the mean +/- SEM. Group comparisons were made using Student's T-test. *: $p<0.05$ and **: $p<0.01$ vs. the respective untreated group.

| Parameters | ARM (n=6) | ARMV (n=11) | ARF (n=6) | ARFV (n=10) |
|---|---|---|---|---|
| AR severity, % | 65 +/- 4 | 64 +/- 4 | 68 +/- 6 | 66 +/- 3 |
| EDD, mm | 10.7 +/- 0.2 | 10.3 +/- 0.3 | 9.4 +/- 0.2 | 8.7 +/- 0.3 |
| ESD, mm | 6.2 +/- 0.1 | 6.0 +/- 0.3 | 5.3 +/- 0.2 | 4.8 +/- 0.3 |
| SW, mm | 1.3 +/- 0.04 | 1.1 +/- 0.07 | 1.3 +/- 0.03 | 0.9 +/- 0.05** |
| PW, mm | 2.3 +/- 0.02 | 2.4 +/- 0.10 | 2.7 +/- 0.15 | 2.1 +/- 0.07** |
| RWT | 0.34 +/- 0.013 | 0.35 +/- 0.015 | 0.43 +/- 0.013 | 0.38 +/- 0.015* |
| EF, % | 66 +/- 2 | 66 +/- 2 | 68 +/- 3 | 70 +/- 2 |
| HR, bpm | 365 +/- 12 | 377 +/- 11 | 386 +/- 15 | 380 +/- 9 |
| E wave, cm/s | 101 +/- 9 | 99 +/- 7 | 102 +/- 6 | 94 +/- 4 |
| A wave, cm/s | 57 +/- 6 | 63 +/- 5 | 65 +/- 6 | 68 +/- 4 |
| E wave slope | 3547 +/- 407 | 3578 +/- 180 | 4094 +/- 251 | 3275 +/- 190* |

Brower et al. (2003) A few years later, Dent and collaborators then characterized this model further by echocardiography and at the molecular level. Both groups also showed that estrogen could reverse the adverse effects of ovariectomy in females. Dent et al. (2010b) In the AR rat model, we did not observe majors effects related to the loss of estrogens in female rats. Drolet et al. (2006)

Sex differences are caused by the presence or absence of sex hormones but also by sex chromosomes and epigenetics. Kessler et al. (2019) The Y chromosome has been shown to influence susceptibility to hypertension and development of adverse cardiac remodeling processes. Incomplete X chromosomes inactivation in females can influence disease progression after a myocardial infarction. Moreover, our comprehension of the role of epigenetics especially in female cardiovascular disorders is still in its infancy.

Pharmacological interventions for cardiovascular diseases and heart failure are often less prescribed in women. Their absorption, distribution , metabolism and clearance is often different. It is not excluded that the differences we observed here may have been related to sex-specific handling of ARB by females compared to males. Unfortunately, since the sex differences in the response to treatment in preclinical models of cardiovascular diseases have received only little attention. In the pressure overload SHR (spontaneously hypertensive rats) model of LV hypertrophy, head-to-head comparison of treatment in animals of both sexes have seldom been performed. In 1982, Pfeffer and collaborators showed similar effects between male and female SHR of two anti-hypertensive agents hydralazine and guanethidine on LV hypertrophy. Pfeffer et al. (1982a) Captopril (ACE inhibitor) has been shown to be effective to block LV hypertrophy in both males and females but was not compared in the same study. Pfeffer et al. (1982b) Pfeffer et al. (1983) More recently, the effects of vasopeptidase inhibitor omapatrilat and the ARB irbesartan in combination with a diuretic were studied in SHR/stroke prone male and female animals. Graham et al. (2004) Both regimen were efficient to lower LV hypertrophy development and this was similar for males and females. Romero and collaborators observed a better response of male SHR to atrial natriuretic treatment than for females although benefits were present for all animals. Romero et al. (2015)

Sex dimorphism in the response to treatment has not been studied before in VO rodent models. The present study design is also different from previous studies we made on male animals using this model. Arsenault et al. (2013) Plante et al. (2004b) Zendaoui et al. (2011) Plante et al. (2009) Plante et al. (2008) Here, we started treatment before the surgical induction of valve regurgitation instead of two weeks after and thus, when cardiac hypertrophy is already present. This study was relatively short (2 months) instead of being more chronic (6 months and more). We consider that at the end of the protocol the animals were still in the compensated phase of the disease although both systolic and diastolic functions indicators were already significantly but not severely altered. We showed in previous chronic studies that renin-angiotensin-aldosterone system (RAAS) inhibition using either angiotensin converting enzyme inhibitors such as captopril or angiotensin II receptor antagonists such as losartan can reduce the extent in LV hypertrophy, dilation and improve survival. Plante et al. (2009) Arsenault et al. (2013) By comparing

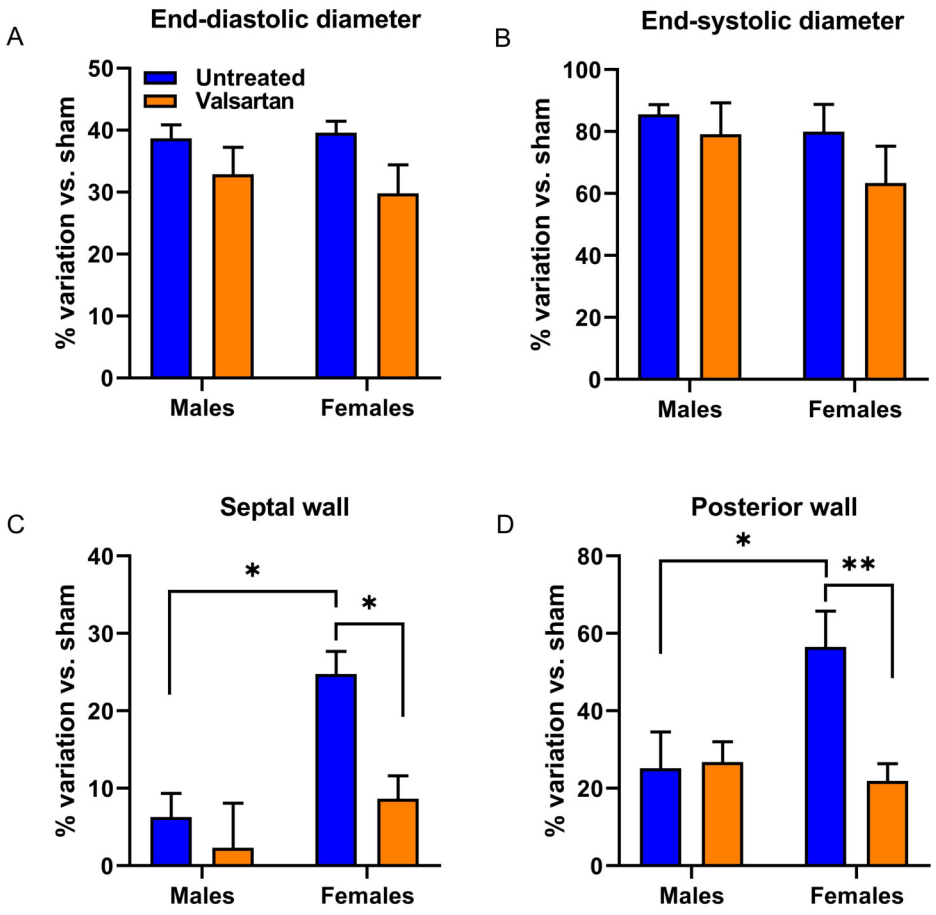


**Figure 2.** Effects of a 9-week treatment with valsartan on LV hypertrophy development caused by AR on echocardiographic parameters. Results are expressed as the percentage of variation of the indicated parameter compared to the mean of the same parameter of the respective untreated sham-operated group. A, End-diastolic diameter, B, End-systolic diameter, C, Inter-ventricular septal wall thickness and D, posterior wall thickness. Results are expressed as the mean +/- SEM. *: $p < 0.05$ and **: $p < 0.01$ between groups.

these studies performed in males to the present one, we notice that the RAAS implication during the early stages of LV remodeling after AR induction does not seem to be as important as the one of the mTORC signaling pathway weobserved in a previous study. Drolet et al. (2015) Rapamycin inhibition of mTOR signaling was able to reduced the extent of LV dilation in AR males, which was not the case here. It is possible that a higher dosage of valsartan may have provide a better inhibition. On the other hand, the dosage of valsartan we used in this study was similar to other studies performed in the past in rats ranging from 10 to 30 mg/kg/daily. Li et al. (2002) Der Sarkissian et al. (2003) Tachikawa et al. (2003)

The present study also enlightened that development of LV hypertrophy in this model first involves rapid LV dilation to accommodate the increased blood volume during diastole in males. This is accompanied with a mild raise in systolic blood pressure as previously reported. Plante et al. (2009) Then, later in the disease, LV dilation and mass continue to increase and this can be blocked by inhibiting the RAAS. Plante et al. (2004a) Arsenault et al. (2013) In females however, RAAS activation leads to LV wall thickening early in the disease. This helps maintain a relatively normal LV morphology (or relative wall thickness) although gain of mass is relatively as important as for males.

It is not clear if the effects of angiotensin receptor blockade we observed in AR females provide

benefits in the context of a volume overload. On one side, valsartan recreates in AR females an eccentric LV morphology similar to males, which have a worse outcome than untreated AR females after 6 months. Arsenault et al. (2013) On the other, diastolic function seems to be improved by ARB as evidenced by less left atrial hypertrophy and better echocardiographic diastolic parameters.

Expression of various LV genes associated with hypertrophy or extracellular matrix remodeling was then assessed. In the case of *Anp* and *Bnp* genes, expression was more elevated in AR rats and valsartan normalized their expression only in females suggesting that LV wall tension may have been improved by treatment. For three other markers, namely *Fos*, *Trpc6* and *Klf15*, gene expression was only altered in untreated AR males but not in females as previously reported.Interestingly, *fos* expression was lowered by ARB both in males and females. As for genes related to extracellular matrix remodeling, very few differences between the sexes or treatment were registered. Volume overload is not associated with important myocardial fibrosis at least in the early steps of the disease. Ryan et al. (2007) This is also true in the AR rat model where collagen total myocardial content, at least in males, is still normal up to 9 months. Lachance et al. (2009)

We chose to not directly address the influence of sex hormones by castration or ovariectomy in this study. This would have added a level of complexity. We recently observed that loss of sex hormones reduced LV hypertrophy in AR males but not in females. On the other hand, loss of estrogens resulted in less LV cardiac output increase and better diastolic function (unpublished observations).

Additional studies are needed to better understand sex differences in the response to treatment in both pressure overload and volume overload pre-clinical models of LV hypertrophy. It is not clear how we can translate the observations made here and future ones to the human situation. On the other hand, our state of knowledge about heart diseases in women and how to treat them is still lagging. Regitz-Zagrosek et al. (2010) Blenck et al. (2016) We need even more basic knowledge to address this gap. We want to point out several limitations in this study. One is the relatively short duration of the protocol and as mentioned, the fact that we did not study the effects of sex hormones on the response to treatment. Aortic regurgitation is a relative rare disease in the Western world and is more prevalent in poorer countries. On the other hand, this disease is still lacking proven pharmaceutical options that could delay valve replacement, we consider that an effort on this is important.

In conclusion, we showed that female AR rats have a stronger early response to treatment with an angiotensin receptor antagonist, valsartan than males. This response is mainly concentrated on a female-specific feature of the LV remodeling in response to volume overload, LV wall thickening.

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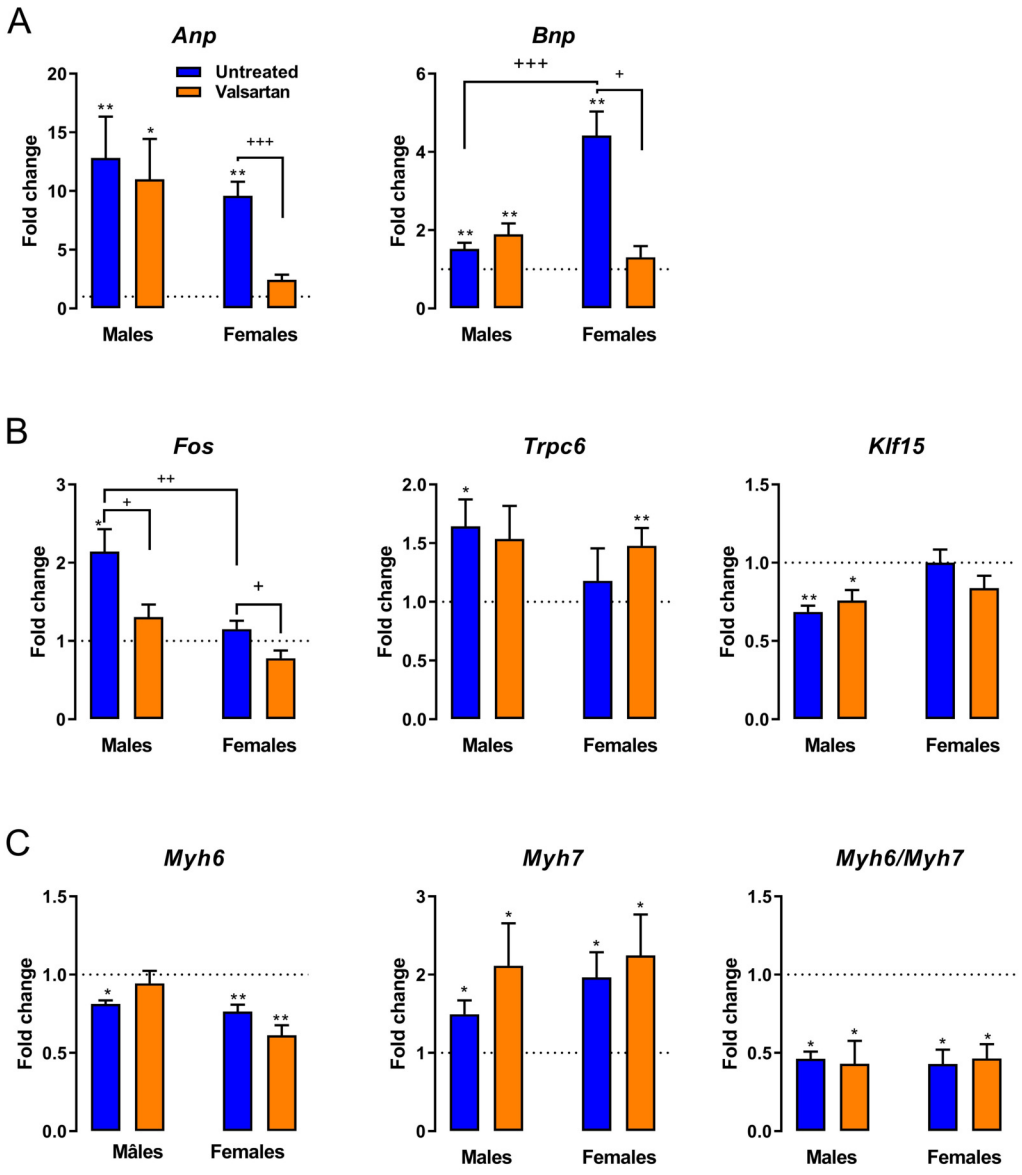


**Figure 3.** Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of genes encoding for several hypertrophy markers. The results are reported in arbitrary units (AU) as the mean +/- SEM (n=5-6/gr.). Messenger RNA levels of the respective sham group were normalized to 1 and is represented by the dotted line. *: $p < 0.05$ and **: $p < 0.01$ vs. respective untreated sham group. +: $p < 0.05$, ++: $p < 0.01$ and +++: $p < 0.001$ between the indicated groups.

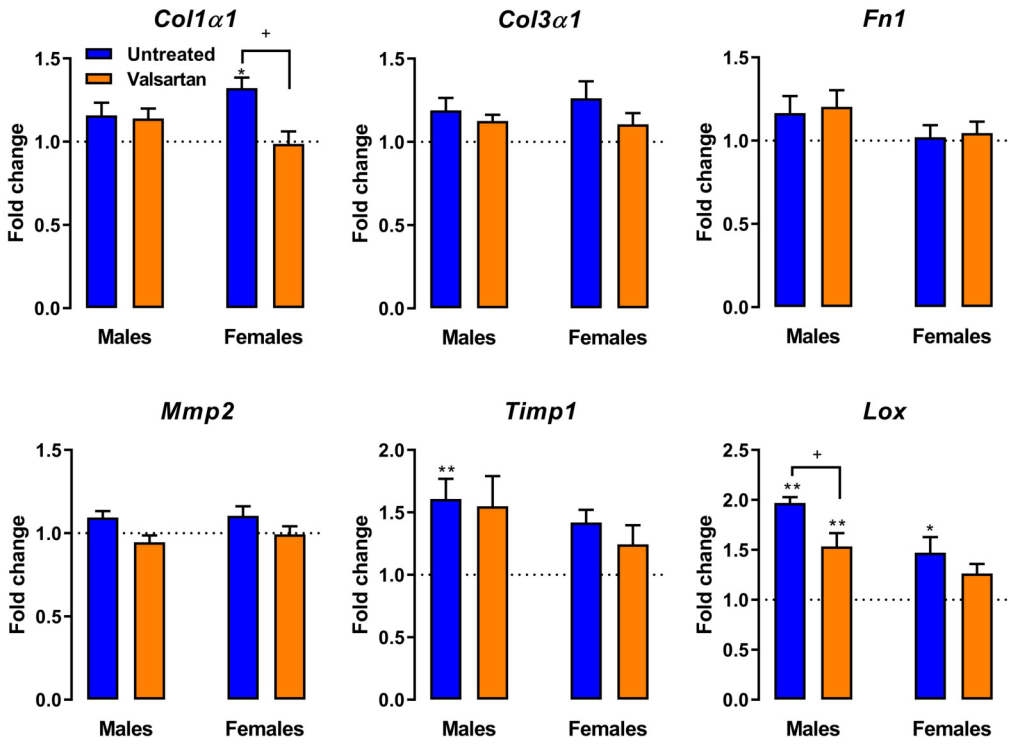


**Figure 4.** Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of genes encoding for several extracellular matrix markers. The results are reported in arbitrary units (AU) as the mean +/- SEM (n=5-6/gr.). Messenger RNA levels of the respective sham group were normalized to 1 and is represented by the dotted line. *: $p < 0.05$ and **: $p < 0.01$ vs. respective untreated sham group. +: $p < 0.05$ between the indicated groups.