

RESEARCH ARTICLE | Sex Differences in Cardiovascular and Cerebrovascular Physiology, Disease, and Signaling Mechanisms

Pressure-volume analysis reveals characteristic sex-related differences in cardiac function in a rat model of aortic banding-induced myocardial hypertrophy

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Ruppert M, Korkmaz-Icöz S, Loganathan S, Jiang W, Lehmann L, Oláh A, Sayour AA, Barta BA, Merkely B, Karck M, Radovits T, Szabó G. Pressure-volume analysis reveals characteristic sex-related differences in cardiac function in a rat model of aortic banding-induced myocardial hypertrophy. *Am J Physiol Heart Circ Physiol* 315: H502–H511, 2018. First published May 25, 2018; doi:10.1152/ajpheart.00202.2018.—Sex differences in pressure overload (PO)-induced left ventricular (LV) myocardial hypertrophy (LVH) have been intensely investigated. Nevertheless, sex-related disparities of LV hemodynamics in LVH were not examined in detail. Therefore, we aimed to provide a detailed characterization of distinct aspects of LV function in male and female rats during different stages of LVH. Banding of the abdominal aorta (AB) was performed to induce PO for 6 or 12 wk in male and female rats. Control animals underwent sham operation. The development of LVH was followed by serial echocardiography. Cardiac function was assessed by pressure-volume analysis. Cardiomyocyte hypertrophy and fibrosis were evaluated by histology. At *week 6*, increased LV mass index, heart weight-to-tibial length, cardiomyocyte diameter, concentric LV geometry, and moderate interstitial fibrosis were detected in both male and female AB rats, indicating the development of an early stage of LVH. Functionally, at this time, impaired active relaxation, increased contractility, and preserved ventricular-arterial coupling were observed in the AB groups in both sexes. In contrast, at *week 12*, progressive deterioration of LVH-associated structural and functional alterations occurred in male but not female animals with sustained PO. Accordingly, at this later stage, LVH was associated with eccentric remodeling, exacerbated fibrosis, and increased chamber stiffness in male AB rats. Furthermore, augmented contractility declined in male but not female AB animals, resulting in contractility-afterload mismatch. Maintained contractility augmentation, preserved ventricular-arterial coupling, and better myocardial compliance in female rats contribute to sex differences in LV function during the progression of PO-induced LVH.

NEW & NOTEWORTHY We investigated sex differences in pressure overload-induced left ventricular myocardial hypertrophy for the first time on the functional level by pressure-volume analysis. We

found that left ventricular hypertrophy was initially characterized by prolonged active relaxation, increased contractility, and maintained ventricular-arterial coupling in both sexes. However, at a later stage, augmented contractility declined in male but not female rats, resulting in contractility-afterload mismatch. Furthermore, in male rats, increased myocardial stiffness also contributed to hypertrophy-associated diastolic dysfunction.

cardiac function; contractility; left ventricular hypertrophy; pressure-volume analysis; sex differences; ventricular-arterial coupling

INTRODUCTION

In recent decades, sex differences have been reported to influence the development of many cardiovascular pathologies, including pressure overload (PO)-induced pathological left ventricular (LV) myocardial hypertrophy (LVH). In accordance, numerous aspects of LVH were taken under investigation to explore potential sex-related disparities. As a result, sexual dimorphisms in LV volumes, geometry, function, structural composition, and molecular background were also recognized. To summarize these key findings, in women with long-lasting hypertension or aortic stenosis as well in female rodents with aortic constriction, smaller LV dimensions (2, 4, 26), more concentric LV geometry (2, 4, 6), better systolic function (2, 4, 6, 9, 26), less interstitial fibrosis (10, 26), less apoptosis (7), and different regulation of genes encoding mitochondrial factors (28) were documented compared with their male counterparts with comparable mechanical overload.

Despite the above-mentioned extensive clinical and experimental research, potential sex-related differences with regards to LV hemodynamics have not yet been investigated. In recent years, the technique of LV pressure-volume (P-V) analysis became the “gold standard” method to assess numerous aspects of *vivo* cardiac function in animal models accurately (21). Particularly, in pathological conditions associated with sustained PO [aortic banding (AB), aortic stenosis, and arterial hypertension], the significance of P-V analysis is given by the fact that the robust increment in afterload makes the conventional load-dependent parameters inappropriate to characterize

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in vivo LV function. Calculation of preload- and afterload-independent parameters by P-V analysis, however, represents an adequate method to assess the contractile state of the myocardium even under extreme hemodynamic circumstances.

Thus, in the present study, we used the technique of P-V analysis to examine potential hypertrophy-associated sex differences in preload- and afterload-independent indices of in vivo LV function.

METHODS

Animals

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 86-23, Revised 1996). This investigation was approved by the ethics committee of the Land Baden-Württemberg for Animal Experimentation (G-198/16). Male ($n = 34$) and female ($n = 30$) Sprague-Dawley rats (160–180 g, Janvier) were housed in a room with a constant temperature of $22 \pm 2^\circ\text{C}$ with 12:12-h light-dark cycles. Animals were allowed access to a standard laboratory rat diet and water ad libitum during the whole experimental period.

Abdominal AB

After a 1-wk-long acclimatization period, rats underwent abdominal AB or sham operation, as previously described (22). In brief, anesthesia was induced by 5% isoflurane gas in a chamber and maintained by inhalation from a connected tube with 1.5–2% isoflurane in 100% O_2 . Animals were placed in a supine position on a controlled heating pad to maintain the body temperature at 37°C . The surgery was performed in midline laparotomy under sterile conditions. After the intestinal tract was taken aside, the peritoneal layer was dissected to visualize the abdominal aorta. A blunted, 22-gauge needle was used to constrict the aorta above the renal arteries. The abdominal muscle layer was sutured in a single interrupted fashion. Closure of the abdominal skin layer was ensured by subcutaneous continuous sutures. Sham-operated animals underwent the same procedure without the completion of the aortic constriction. Buprenorphine was administered subcutaneously (0.05 mg/kg) for postoperative analgesia.

Experimental Groups

Six weeks after AB or sham operation, rats were divided into the following eight experimental groups: male sham week 6 group (group 1; $n = 8$), male AB week 6 group (group 2; $n = 8$), female sham week 6 group (group 3; $n = 8$), female AB week 6 group (group 4; $n = 7$), male sham week 12 group (group 5; $n = 8$), male AB week 12 group (group 6; $n = 10$), female sham week 12 group (group 7; $n = 8$), and female AB week 12 group (group 8; $n = 7$).

Echocardiography

Echocardiography was performed before AB/sham operation and 3, 6, 9, and 12 wk after AB/sham operation. For the sonographic measurements, the Vevo 2100 imaging system (FujiFilm VisualSonics, Toronto, ON, Canada) equipped with a 21-MHz linear probe was used. In brief, rats were anesthetized with 5% isoflurane in a chamber. After the induction of anesthesia, rats were placed on an automatic heating pad in a supine position with core temperature maintained at 37°C . The thorax of the animals was shaved to obtain an optimal acoustic window. Anesthesia was maintained by inhalation of 1–1.5% isoflurane gas in 100% O_2 . Images in two-dimensional parasternal long-axis and short-axis views as well as M-mode recordings at the midpapillary level were taken. The study was completed by analyzing

the digital images in a blinded fashion. On M-mode recorded images, LV internal diameters and anterior and posterior wall thicknesses in diastole and systole were measured. All values were calculated as the average from three consecutive cardiac cycles. From the measured parameters, ejection fraction (EF), LV mass index (LV mass/body weight), and relative wall thickness (RWT) were calculated.

LV P-V Analysis

LV P-V analysis was performed according to a previously described protocol (15) with minor modifications. At the end of the experimental period (at weeks 6 or 12, respectively), rats were anaesthetized by inhalation of 5% isoflurane gas in a chamber. After anesthesia was induced, rats were placed in a supine position on an automatic heating pad to keep the core temperature at 37°C . To maintain anesthesia during the whole hemodynamic measurement, rats were tracheotomized, intubated, and artificially ventilated with 1–1.5% isoflurane gas in 100% O_2 . Pancuronium in the dose of 2 mg/kg was administered intraperitoneally to induce muscle relaxation. A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2-Fr microtip pressure-conductance catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and subsequently advanced into the ascending aorta. After stabilization, arterial blood pressure was recorded. Then the catheter was guided to the LV under pressure control. With the use of a special P-V analysis program (PVAN, Millar Instruments), systolic arterial blood pressure (SBP), diastolic arterial blood pressure (DBP), mean arterial pressure (MAP), LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), heart rate (HR), $dP/dt_{\max}$, $dP/dt_{\min}$, LV end-diastolic volume, LV end-systolic volume, stroke volume (SV), cardiac output (CO), EF, arterial elastance [E_a ; calculated by the following equation: $E_a = \text{LVESP}/\text{SV}$ (24)], and the time constant of LV pressure decay τ ; according to the Glanz method (29)] were computed and calculated.

To detect load-independent contractility parameters, P-V loops were also registered at transiently decreasing preload, which was achieved by transient occlusion of the inferior caval vein. The slope of the end-systolic P-V relationship [ESPVR, according to the parabolic curvilinear model (12)] and preload recruitable stroke work (PRSW) were calculated as LV contractility indexes.

To characterize myocardial compliance, the slope of the end-diastolic P-V relationship (EDPVR) was derived from P-V analysis.

To assess LV mechanoenergetics, ventricular-arterial coupling (VAC) was determined as the ratio of E_a and ESPVR ($\text{VAC} = E_a/\text{ESPVR}$).

Parallel conductance was calculated, and volume calibration of the conductance system was performed as previously described (21). After completion of the hemodynamic measurements, animals were euthanized by exsanguination. After euthanasia, heart weights and tibial lengths were quickly measured. The ratio of heart weight-to-tibial length was calculated to assess the extent of LVH.

Histology

After organ weight measurements, the hearts were fixed in buffered paraformaldehyde solution (4%) and embedded in paraffin. Transverse, transmural, 5- μm thick slices of the ventricles were cut and placed on adhesive slides.

Hematoxylin and eosin staining was performed to measure cardiomyocyte diameter (CD) as a cellular marker of myocardial hypertrophy (22). In each sample, 100 longitudinally oriented cardiomyocytes from the LV were examined, and the diameters at transnuclear position were defined. The mean value of 100 measurements represented 1 sample.

The extent of myocardial fibrosis was assessed on picrosirius-stained sections (20). ImageJ software (NIH, Bethesda, MD) was used to identify the picrosirius red-positive area. Three transmural images (magnification: $\times 50$) were randomly taken from the free LV wall on each section. After

background subtraction, eye-controlled auto-threshold has been determined to detect positive areas. The fibrosis area (picrosirius red-positive area-to-total area ratio) was determined on each image, and the mean value of three images represents each animal.

LV mRNA Analysis

Quantitative real-time PCR was performed as previously described (14). In brief, LV myocardial tissue ($n = 7-8$) was homogenized in a lysis buffer (RLT buffer, Qiagen, Hilden, Germany) using the Tissue Lyzer LT (Qiagen) system. RNA was isolated with the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. The quality and concentration of the isolated RNA were assessed by the NanoDrop 2000 Spectrophotometer. Accordingly, optical density at 230, 260, and 280 nm was measured. The ratios of 230/260 and 230/280 nm were defined for quality control. The reverse transcription reaction (1 μ g total RNA in a reaction volume of 20 μ l) was completed using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed with the LightCycler 480 system using LightCycler 480 Probes Master and Universal Probe Library Probe 129 (Roche, Mannheim, Germany). Forward (5'-CCG-GTAGGTCTTCACATGG-3') and reverse (5'-GCTGACCTAGAG-GAAAACATTAAAGA-') primers for connective tissue growth factor (CTGF) were obtained from TIB Molbiol (Berlin, Germany). Gene expression data were normalized to GAPDH. All results are expressed as values normalized to a positive calibrator (a pool of cDNA from all samples of the corresponding sham group). The evaluation was performed with LightCycler 480 SW 1.5 software (Roche).

Statistics

All values are expressed as means $\pm$ SE. The distribution of the data sets was analyzed with the Shapiro-Wilk normality test. In case of non-normal distribution, data were transformed logarithmically. Two-way ANOVA with the factors "time" and "AB" was used to compare the four male (sham/AB week 6 and sham/AB week 12 groups) and four female (sham/AB week 6 and sham/AB week 12 groups) groups separately. To compare hypertrophy-associated alterations between the sexes directly, individual data of the AB groups were normalized to the mean value of the corresponding sham groups. An unpaired two-sided Student's *t*-test in the case of normal distribution or Mann-Whitney *U*-test in the case of non-normal distribution was used to compare hypertrophy-associated changes in each parameter.

In the case of echocardiographic measurements, two-way ANOVA with the factors "sex" and "AB" was performed to compare four groups (male/female sham and male/female AB groups) at five different time points (baseline, week 3, week 6, week 9, and week 12). After ANOVA, a Tukey post hoc test was selected in every case to examine intergroup differences.

The Spearman correlation test was performed to detect correlations between LV mass index and τ , between collagen area and LVEDP, and between collagen area and EDPVR as well.

A *P* value of <0.05 was used as the criterion of statistical significance.

RESULTS

Effect of Sex on the Development of PO-Induced Pathological LVH

Week 6. At this early stage, LV mass index, heart weight/tibia length, CD, RWT, myocardial collagen area, and expression level of CTGF were enhanced in both male and female AB rats compared with their age- and sex-matched sham groups (Figs. 1 and 2). The extent of hypertrophy and fibrosis was comparable between the sexes at this early time point (Table 1).

Week 12. At this later stage, hypertrophy and fibrosis markers were also increased in male and female AB rats compared with their age- and sex-matched sham groups (Figs. 1 and 2).

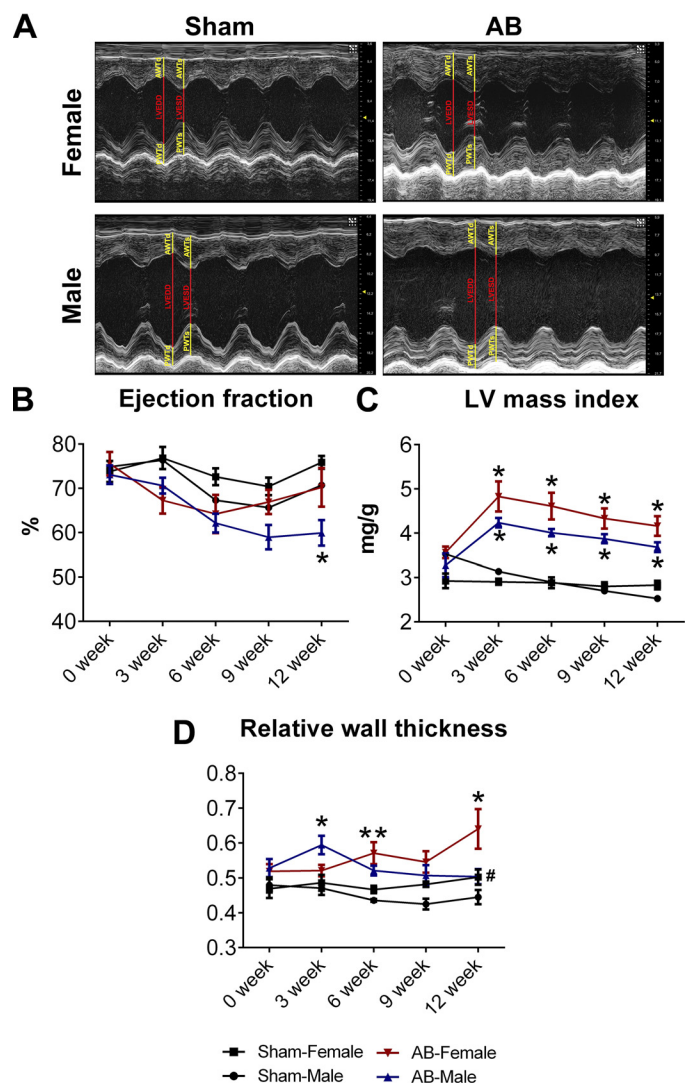


Fig. 1. Echocardiographic followup during the development of left ventricular (LV) myocardial hypertrophy in male and female rats. **A:** representative M-mode echocardiographic images at the midpapillary muscle level at week 12 are shown, indicating LV myocardial hypertrophy in male and female aortic banded (AB) rats. **B:** sustained pressure overload led to a reduced ejection fraction in male but not female AB rats. **C:** increased LV mass index confirmed the development of LV myocardial hypertrophy at weeks 3, 6, 9, and 12 in both sexes. **D:** relative wall thickness revealed concentric LV geometry at week 6 in both sexes. Although concentric remodeling was still observed after 12 wk of pressure overload in female rats, at this time in male rats eccentric LV geometry was detected. * $P < 0.05$ vs. age and sex-matched sham rats; # $P < 0.05$ vs. female AB rats. AWTd and AWTs, anterior wall thickness at diastole and systole, respectively; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; PWTd and PWTs, posterior wall thickness at diastole and systole, respectively.

However, the direct comparison of sexes revealed higher increments in heart weight/tibia length and CD in female compared with male rats (Table 1). In contrast, fibrosis markers showed a significant progression from week 6 to week 12 only in male AB rats (Fig. 2). Furthermore, the extent of the AB-associated increment in CTGF expression was also significantly higher in male compared with female rats (Table 1). In addition, increased RWT (concentric LV geometry) was only observed in female AB rats, whereas in male rats LVH was associated with eccentric remodeling (Fig. 1).

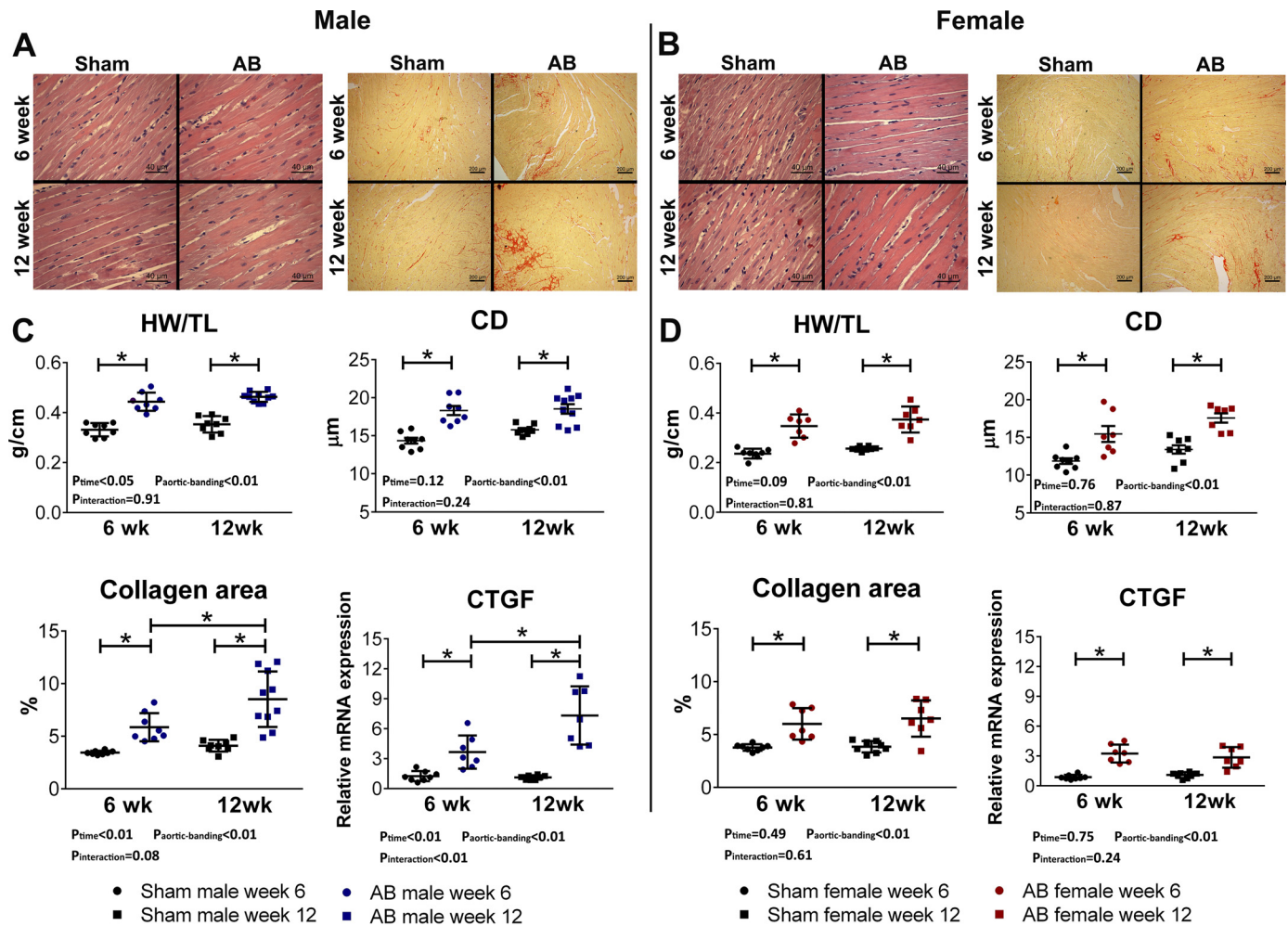


Fig. 2. Characterization of pathological left ventricular (LV) myocardial hypertrophy in male and female rats. *A* and *B*: representative photomicrographs of hematoxylin and eosin and picrosirius staining are shown, demonstrating enlargement of cardiomyocytes and intensified fibrosis in the aortic banded (AB) groups. *A*: male rats; *B*: female rats. *C* and *D*: in the AB groups in both sexes increased heart weight-to-tibial length ratio (HW/TL), cardiomyocyte diameter (CD), collagen area, and mRNA level of connective tissue growth factor (CTGF) were observed at weeks 6 and 12 as well, indicating the development of pathological LV myocardial hypertrophy. *C*: male rats; *D*: female rats. In male but not female rats, exacerbation of myocardial fibrosis (collagen area and CTGF expression) was also observed. * $P < 0.05$.

Characteristic Gender-Related Functional Differences in LVH

Hemodynamic results obtained from P-V analysis are summarized in Figs. 3 and 4 and Table 2.

Arterial Loading

At weeks 6 and 12, in both male and female AB rats increased SBP, DBP, and MAP confirmed the presence of increased afterload (Table 2). Furthermore, E_a showed significantly increased values in both sexes at week 12 (Fig. 4) compared with the corresponding sham groups.

Diastolic Function

Week 6. At this time, AB groups demonstrated prolonged τ in both sexes compared with sex-matched sham groups, indicating impaired ventricular relaxation. The degree of τ prolongation was similar between the sexes at week 6 (Tables 1 and 2).

Week 12. At this stage, prolongation of τ was also evident in both male and female AB rats. However, at this time, impairment of active relaxation occurred at a significantly greater extent in male AB rats compared with their female littermates (Tables 1 and 2). The increment of the LV mass index showed a strong correlation with prolongation of τ ($r = 0.53$, $P < 0.01$). Furthermore, LVEDP and EDPVR were also consistently elevated in male but not female AB animals at week 12, leading to substantial differences between the sexes (Tables 1 and 2). In addition, a significant correlation was detected between LVEDP and collagen area ($r = 0.30$, $P = 0.02$) and also between EDPVR and collagen area ($r = 0.28$, $P = 0.03$).

Load-Dependent Parameters of LV Systolic Function

Week 6. After 6 wk of sustained PO, the AB groups were associated with preserved EF in both sexes (Fig. 1 and Table 2). Furthermore, in female AB rats, SV and CO were slightly increased compared with the corresponding control group (Table 2).

Table 1. Comparison of pressure overload-induced alterations between the sexes

	6 Weeks			12 Weeks		
	Male rats	Female rats	P value	Male rats	Female rats	P value
Number of rats/group	8	7		10	7	
Δ LV mass index, %	49 $\pm$ 5	47 $\pm$ 12	0.90	46 $\pm$ 4	47 $\pm$ 8	0.88
Δ Relative wall thickness, %	27 $\pm$ 4	29 $\pm$ 11	0.84	13 $\pm$ 5	28 $\pm$ 11	0.21
Δ Heart weight/tibia length, %	34 $\pm$ 4	47 $\pm$ 8	0.13	31 $\pm$ 2	46 $\pm$ 8	<0.05*
Δ Cardiomyocyte diameter, %	28 $\pm$ 4	30 $\pm$ 9	0.78	17 $\pm$ 4	31 $\pm$ 5	0.04*
Δ Fibrosis area, %	70 $\pm$ 15	59 $\pm$ 15	0.60	108 $\pm$ 20	69 $\pm$ 17	0.19
Δ Connective tissue growth factor, %	193 $\pm$ 50	272 $\pm$ 39	0.24	557 $\pm$ 99	162 $\pm$ 36	<0.01*
Δ Systolic blood pressure, %	50 $\pm$ 3	62 $\pm$ 7	0.14	54 $\pm$ 3	60 $\pm$ 8	0.99
Δ Diastolic blood pressure, %	34 $\pm$ 3	49 $\pm$ 5	0.02*	38 $\pm$ 5	42 $\pm$ 6	0.65
Δ Mean arterial pressure, %	40 $\pm$ 3	54 $\pm$ 5	0.03*	44 $\pm$ 4	49 $\pm$ 7	0.54
Δ Heart rate, %	2 $\pm$ 3	3 $\pm$ 3	0.72	1 $\pm$ 2	2 $\pm$ 2	0.62
Δ dP/dt _{max} , %	39 $\pm$ 5	36 $\pm$ 10	0.80	30 $\pm$ 5	39 $\pm$ 6	0.22
Δ dP/dt _{min} , %	29 $\pm$ 4	18 $\pm$ 6	0.17	1 $\pm$ 3	23 $\pm$ 10	0.02*
Δ LV end-diastolic pressure, %	44 $\pm$ 16	31 $\pm$ 19	0.61	73 $\pm$ 23	5 $\pm$ 17	<0.05*
Δ LV end-systolic pressure, %	53 $\pm$ 5	46 $\pm$ 7	0.41	56 $\pm$ 2	50 $\pm$ 6	0.34
Δ LV end-diastolic volume, %	19 $\pm$ 2	22 $\pm$ 5	0.59	8 $\pm$ 6	1 $\pm$ 3	0.38
Δ LV end-systolic volume, %	33 $\pm$ 7	7 $\pm$ 12	0.07	38 $\pm$ 8	8 $\pm$ 4	0.01*
Δ Stroke volume, %	8 $\pm$ 5	35 $\pm$ 6	<0.01*	-15 $\pm$ 7	-5 $\pm$ 7	0.32
Δ Ejection fraction, %	-9 $\pm$ 4	7 $\pm$ 6	0.04*	-22 $\pm$ 4	-6 $\pm$ 5	0.02*
Δ Cardiac output, %	8 $\pm$ 5	42 $\pm$ 8	<0.01*	-16 $\pm$ 7	-2 $\pm$ 8	0.22
$\Delta\tau$, %	29 $\pm$ 4	26 $\pm$ 8	0.69	58 $\pm$ 6	33 $\pm$ 11	<0.05*
Δ End-diastolic pressure-volume relationship, %	10 $\pm$ 17	21 $\pm$ 24	0.71	76 $\pm$ 22	36 $\pm$ 21	0.23

Values are means $\pm$ SE. The aortic banding-associated alterations normalized to the corresponding sham groups are shown. LV, left ventricular; τ , time constant of LV pressure decay according to the Glantz method. *Significant difference.

Week 12. At week 12, the echocardiographic measurement and invasive P-V analysis congruently confirmed reduced EF in the male AB group (Fig. 1 and Table 2). In contrast, values of EF did not differ from the corresponding sham group in female AB animals. The different alterations in EF resulted in a significant difference between the sexes (Table 1). Furthermore, the parameters of SV and CO showed also a tendency toward decreased values in male AB animals, whereas these tendencies could not be observed among female animals (Table 2).

Load-Independent Parameters of LV Contractility

Week 6. At week 6, P-V derived load-independent indexes of cardiac contractility (PRSW and ESPVR) were significantly increased in both male and female AB groups compared with their control groups (Figs. 3 and 4). Direct comparison of the sexes revealed no significant differences in the extent of contractility augmentation at this time (Figs. 3 and 4).

Week 12. At week 12, a significant reduction from the 6-wk state could be observed in PRSW and ESPVR among male AB rats. In contrast, in female animals, LV contractility remained increased and PRSW and ESPVR were significantly higher compared with the corresponding sham animals (Figs. 3 and 4). The differences in contractility changes between male and female rats were well reflected by direct sex comparison, which showed significantly greater increments in PRSW and ESPVR in female rats (Figs. 3 and 4).

Ventricular-Arterial Coupling

Week 6. At this early stage, augmentation in ESPVR exceeded the increment in E_a , resulting in a preserved VAC ratio in the AB groups in both sexes. Furthermore, the robust increment of ESPVR in female animals resulted in func-

tional overcompensation, as reflected by a reduced VAC ratio (Fig. 4).

Week 12. At this later stage, reduction of contractility in male AB rats led to an impairment in the VAC ratio, which showed significantly higher values in the AB week 12 group compared with the AB week 6 group. In contrast, the maintained contractility augmentation in female AB rats ensured a preserved VAC ratio even after 12 wk of PO. The sex differences in VAC appeared also during direct comparison at week 12 (Fig. 4).

DISCUSSION

To the best of our knowledge, this is the first study that has investigated the effect of sex on different aspects of LV hemodynamics in PO-induced LVH. The main findings of the present investigation are that 1) PO-induced LVH is initially associated with impaired active relaxation, increased LV contractility, and maintained VAC ratio in both sexes; 2) at a later stage of LVH development, augmented contractility declines in male AB rats, whereas it remains increased in female rats; 3) deterioration of the contractile capacity in male but not female rats leads to a contractility-afterload mismatch; 4) besides prolonged ventricular relaxation, increased myocardial stiffness also contributes to the diastolic dysfunction in male but not female AB rats.

Myocardial Hypertrophy and Fibrosis

In accordance with our previous experiences with this model (19, 20, 23), after 6 wk of PO we observed the development of marked LVH in the AB groups. Pathological remodeling of the LV was detected by echocardiography (increased LV mass index), by postmortem organ measurements (enhanced heart weight/tibia length), by histological analysis (increased CD and collagen area), and by increased CTGF gene expression in both sexes. At this time, the increment in myocardial mass and fibrosis occurred to a

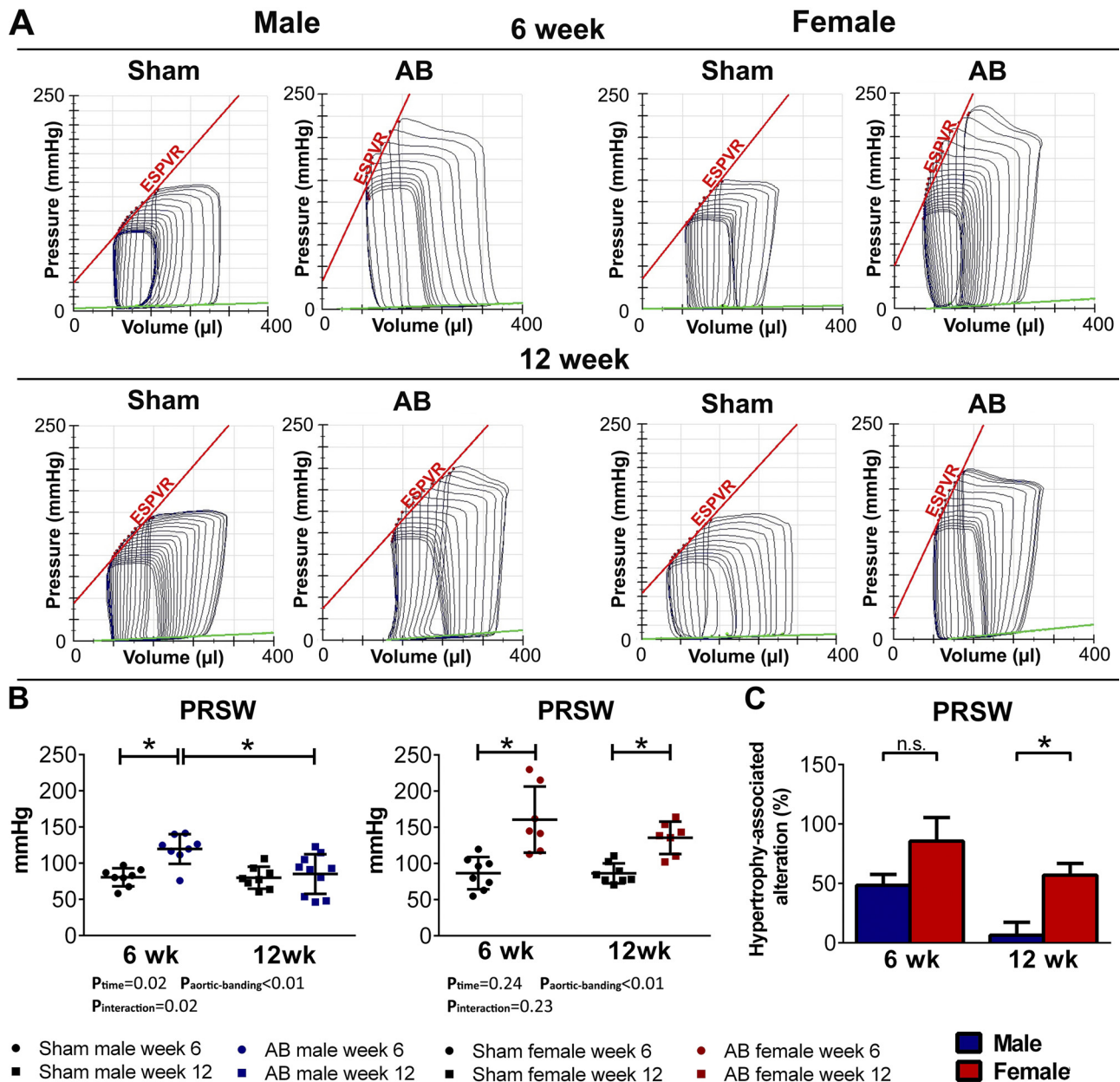


Fig. 3. Representative pressure-volume loops demonstrating sex differences in left ventricular (LV) myocardial hypertrophy. A: original recordings were obtained at different preloads during transient vena cava occlusion and showed a steeper slope of the end-systolic pressure-volume relationship (ESPVR) in male and female aortic banded (AB) rats at *week 6*, suggesting enhanced contractility. At *week 12*, in the AB groups, the slope of ESPVR decreased among male rats but remained increased among female rats, indicating sex differences. B: preload recruitable stroke work (PRSW). The linear relation between stroke work and end-diastolic volume was calculated as another parameter of cardiac contractility. In the AB groups at *week 6*, PRSW increased in both sexes. In contrast, at *week 12*, PRSW remained enhanced only among female rats, whereas it declined in male AB rats. C: direct comparison of hypertrophy-associated alterations in PRSW between the sexes revealed significantly better contractility in female AB rats at *week 12*. * $P < 0.05$.

comparable degree in male and female AB rats. Furthermore, assessment of LV geometry confirmed concentric remodeling (expressed as increased RWT) in both sexes, which is also in good agreement with prior findings (Figs. 1 and 2 and Table 1) (5).

In contrast to the adaptive 6-wk state, at *week 12*, late-stage characteristics of LVH were identified in male but not female AB rats. Accordingly, in the male AB group, LVH was associated with eccentric LV geometry (reduced RWT) and intensified extracellular matrix remodeling. In contrast, the hypertrophied hearts in the female group still showed concentric geometry and a similar extent of interstitial fibrosis as observed at *week 6*. Fur-

thermore, at this later time point, the extent of hypertrophy was greater in female rats, whereas CTGF gene expression occurred at a greater level in male rats (Figs. 1 and 2 and Table 1). Regarding the extent and geometry of LVH as well as fibrosis, our findings are in line with the results of Douglas et al. (5), who investigated sex-related myocardial differences in rats with 20 wk of AB.

Diastolic Function

The detailed analysis of P-V loops revealed prolonged active relaxation (τ) in the AB groups in both sexes. In line with

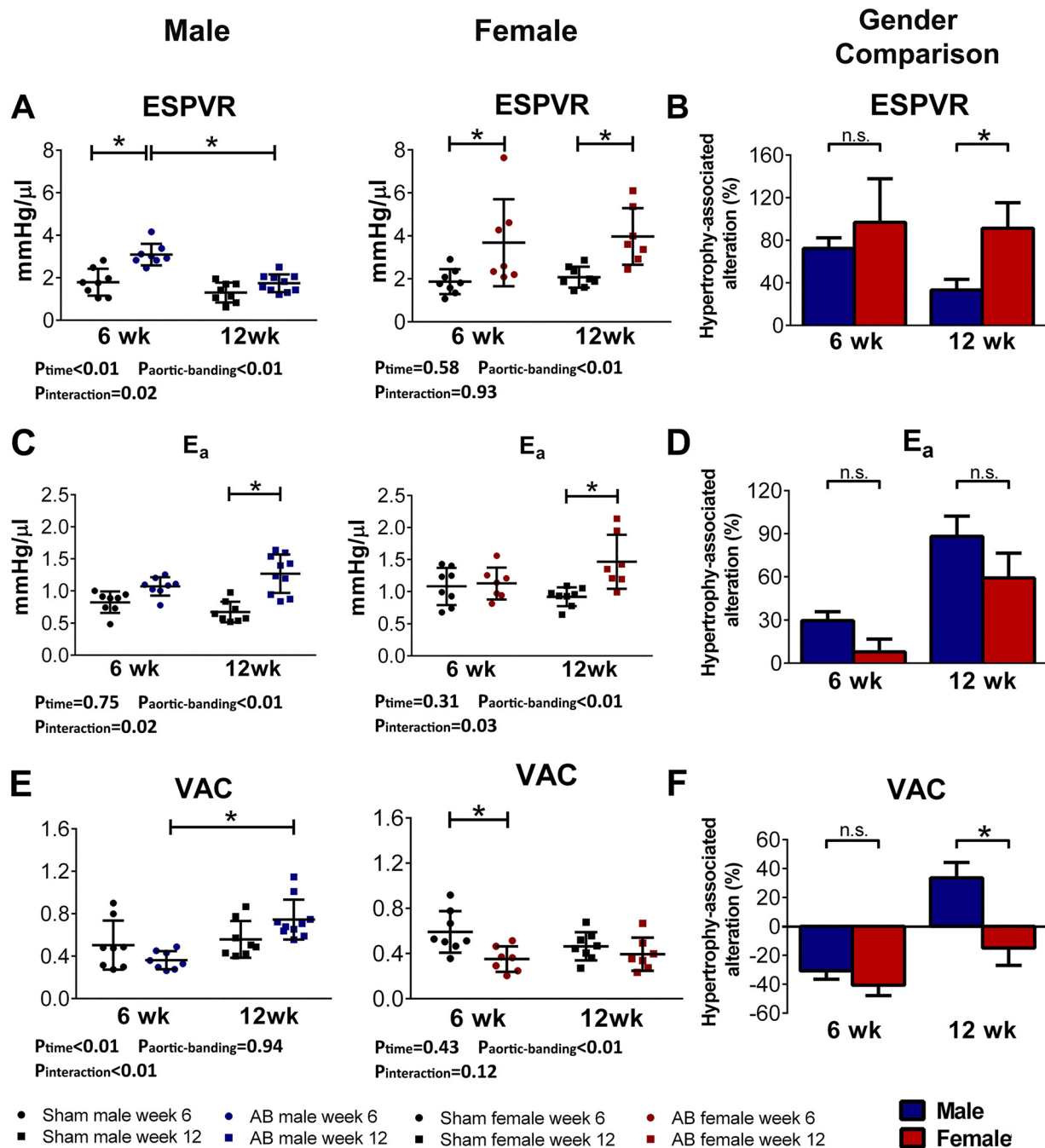


Fig. 4. Sex differences in left ventricular mechanoenergetics. **A**: the slope of end-systolic pressure-volume relationship (ESPVR) was significantly increased after 6 wk of aortic banding (AB), indicating augmented contractility in both sexes. In female AB rats, an increment in ESPVR was also observed at week 12, whereas cardiac contractility significantly decreased among male rats. **B**: direct comparison of hypertrophy-associated alterations in ESPVR between the sexes revealed significantly better contractility in female AB rats at week 12. **C**: arterial elastance (E_a) was significantly increased at week 12 in the AB groups in both sexes. **D**: no differences could be observed with regard to E_a between the sexes. **E**: the ventricular-arterial coupling (VAC) ratio was defined to assess mechanoenergetics. At week 6, in the AB groups, VAC decreased in both sexes; however, a functional overcompensation was only detected in female animals. At week 12, VAC increased in male but not female AB rats, indicating impaired mechanoenergetics. **F**: sex differences in VAC appeared also during direct comparison at week 12. * $P < 0.05$.

previous literature data (25), impairment of ventricular relaxation showed a strong correlation with the increment in LV mass index. The hypertrophy-related changes in τ among male rats were identical to those we observed in our previous consecutive studies with the abdominal AB rat model (19, 20, 23). However, the finding of impaired active relaxation in

female rats with sustained PO is novel. Furthermore, we found that the severity of active relaxation dysfunction was similar between the sexes at week 6. In contrast, at week 12, we detected significantly greater prolongation of τ in male AB rats compared with their female littermates. Despite the observed alterations in τ , we measured increased dP/dt_{min} values (an-

Table 2. Hemodynamic parameters in male and female rats 6 and 12 wk after AB or sham operation

	Male Rats				Female Rats			
	6 wk		12 wk		6 wk		12 wk	
	Sham	AB	Sham	AB	Sham	AB	Sham	AB
Number of rats/group	8	8	8	10	8	7	8	7
Systolic blood pressure, mmHg	144 ± 6	216 ± 5*	137 ± 5	211 ± 4*	144 ± 3	232 ± 10*	140 ± 5	224 ± 12*
Diastolic blood pressure, mmHg	112 ± 5	150 ± 3*	110 ± 4	152 ± 5*	112 ± 2	167 ± 6*	110 ± 5	156 ± 7*
Mean arterial pressure, mmHg	123 ± 5	172 ± 3*	119 ± 4	172 ± 4*	123 ± 2	188 ± 7*	120 ± 5	179 ± 8*
Heart rate, beats/min	355 ± 7	361 ± 9	360 ± 4	362 ± 8	362 ± 9	372 ± 10	374 ± 15	381 ± 7
dP/dt _{max} , mmHg/s	7,960 ± 310	11,080 ± 390*	8,230 ± 350	10,670 ± 410*	8,820 ± 410	12,040 ± 890*	9,500 ± 350	13,240 ± 560*
dP/dt _{min} , mmHg/s	-8,640 ± 240	-11,120 ± 350*	-9,590 ± 280	-9,630 ± 290†	-10,640 ± 590	-12,580 ± 650	-10,720 ± 420	-13,190 ± 1,060
LV end-diastolic pressure, mmHg	4.0 ± 0.7	5.7 ± 0.6	3.7 ± 0.4	6.4 ± 0.9*	3.7 ± 0.2	4.8 ± 0.7	3.2 ± 0.4	3.3 ± 0.5
LV end-systolic pressure, mmHg	132 ± 3	201 ± 6*	126 ± 4	196 ± 3*	139 ± 4	202 ± 10*	140 ± 3	210 ± 9*
LV end-diastolic volume, μ l	307 ± 17	364 ± 5	346 ± 21	373 ± 21	263 ± 25	320 ± 14	295 ± 5	297 ± 9
LV end-systolic volume, μ l	132 ± 13	175 ± 9	150 ± 13	207 ± 13*	127 ± 15	136 ± 15	136 ± 5	146 ± 6
Stroke volume, μ l	175 ± 18	189 ± 9	196 ± 12	166 ± 13	136 ± 11	184 ± 8*	159 ± 8	151 ± 11
Ejection fraction, %	57 ± 4	52 ± 2	57 ± 2	44 ± 2*	54 ± 3	58 ± 3	54 ± 2	51 ± 3
Cardiac output, ml/min	63.0 ± 7.1	68.0 ± 3.2	70.8 ± 4.7	59.8 ± 4.7	48.5 ± 3.0	68.4 ± 4.1*	59.1 ± 3.2	57.7 ± 4.7
τ , ms	13.8 ± 0.4	17.9 ± 0.6*	12.3 ± 0.6	19.4 ± 0.8*	12.3 ± 0.6	15.5 ± 1.0*	12.0 ± 0.4	15.9 ± 1.3*
End-diastolic pressure-volume relationship, mmHg/ μ l	0.035 ± 0.005	0.038 ± 0.006	0.028 ± 0.005	0.048 ± 0.006*	0.042 ± 0.006	0.051 ± 0.010	0.033 ± 0.004	0.045 ± 0.007

Values are means ± SE. AB, aortic banding; LV, left ventricular; τ , time constant of LV pressure decay according to the Glantz method. * $P < 0.05$ vs. the age-matched sham group. † $P < 0.05$ vs. the male AB week 6 group.

other conventional parameter of active relaxation) in the AB groups, findings that may appear controversial for the first time. However, it is important to note here that dP/dt_{min} is highly dependent on loading conditions, including changes in afterload (8). Therefore, the alterations in dP/dt_{min} reflected the robust increment in arterial blood pressure rather than an improved diastolic function in our AB model. Interestingly, we also found that PO-associated increment in dP/dt_{min} (as observed after week 6) declined in the male but not female AB group at week 12. Considering the fact that comparable arterial loading conditions were registered at weeks 6 and 12 among male AB rats, this alteration may indeed suggest a severe impairment of LV active relaxation (Tables 1 and 2). Our functional results might be underpinned by previous molecular experiments indicating sex-specific regulation of sarco(endo)plasmic reticulum Ca²⁺-ATPase during the development of LVH (27).

Besides active ventricular relaxation, the other aspect of diastole, namely, passive filling, was also impaired in the male AB group at week 12, as evidenced by increased LVEDP and EDPVR (Table 1). Because myocardial compliance (the passive expansion capability of the LV) is primarily determined by the structural composition of the ventricular wall and we found that interstitial fibrosis also occurred at the greatest level in the male AB group at week 12, our functional and histological findings were in line with each other. Accordingly, a significant correlation was discovered between fibrosis and stiffness parameters.

Load-Dependent Parameters of LV Systolic Function

The conventional parameters (EF, SV, and CO) confirmed preserved systolic function in both sexes at week 6. Furthermore, in female AB animals, the excessive functional adaptation to sustained PO led to increased systolic performance

(reflected by higher SV and CO values) at this early stage of LVH (Table 2). Therefore, although systolic function was maintained in male and female AB rats as well, the lack of functional overcompensation in male rats resulted in sex-related differences already at this early time (as seen in the percent change of EF; Table 1). The finding that sex-dependent disparities in systolic function begins to manifest early after the onset of LVH is supported by a previous study by Weinberg et al. (27). In their isolated heart experiment, they observed a better-preserved LV contractile reserve capacity in female rats compared with male rats after 6 wk of AB.

At week 12, we detected deteriorated systolic function in male AB rats. In small animal models of PO-induced LVH, the development of systolic dysfunction is mainly dependent on the severity of the applied aortic constriction. In line with our previous experience with this model (23) as well as with others' findings (16), functional signs of an early transition to systolic heart failure is expected to manifest at this time in male rats. Accordingly, a reduction of EF was observed by echocardiography and by invasive hemodynamic measurement at week 12 in the male AB group (Fig. 1 and Table 2). In female AB rats, EF still proved to be preserved after 12 wk of PO, resulting in substantial differences between the sexes (Table 1). Therefore, in terms of conventional systolic parameters (EF, SV, and CO), our results corresponded to prior clinical (2, 4, 6, 26) and experimental observations (5).

Load-Independent Parameters of LV Contractility

To assess LV systolic performance independently from loading conditions, two parameters of LV contractility (ESPVR and PRSW) were calculated. Traditionally, ESPVR has been introduced as a fairly load-independent index of contractility. Although ESPVR indeed proved to be a valuable index to characterize LV contractility, it has also been reported that it is

not completely independent of chamber geometry (11). Given the fact that in our model characteristic differences were found regarding LV geometry between male (eccentric remodeling) and female (concentric remodeling) AB animals, another contractility parameter was also calculated. PRSW has been described as a parameter independent of LV size, therefore representing a valuable tool to assess cardiac contractility (11). In our model, robust increments in both parameters (ESPVR and PRSW) were observed after 6 wk of PO in male and female animals as well, confirming augmented LV contractility. Similarly, in female AB rats, increments in ESPVR and PRSW could be also detected after 12 wk of PO. In contrast, the maladaptive remodeling in male AB rats was accompanied with reduced values of ESPVR and PRSW (Figs. 3 and 4), leading to substantial differences in cardiac contractility between the sexes at this particular time point.

Therefore, the P-V analysis-derived load-independent parameters provided evidence that substantial functional compensation (increment in LV contractility) occurred also in the male AB *week 6* group and in the female AB *week 12* group as well. However, the conventional load-dependent systolic parameters (EF, SV, and CO) failed to demonstrate this fine functional adaptation. Our observation draws attention to the fact that only preload- and afterload-independent parameters can assess LV function in pathological conditions with extreme hemodynamic circumstances (such as AB, aortic stenosis, and arterial hypertension).

Ventricular-Arterial Coupling

In our investigation, VAC was defined to explore potential differences in LV mechanoenergetics between the sexes. VAC expresses the interaction between the LV and connecting arterial system, and it provides an accurate estimation of LV net performance (13). Impaired VAC has been reported during end-stage heart failure as a result of increased E_a and decreased contractile capacity (1). In our model, at *week 6*, the increment in LV contractility (ESPVR) was sufficient to compensate the enhanced afterload (E_a) in both sexes, resulting in a preserved VAC ratio at this particular time point. In addition, in female AB rats, the functional overcompensation to PO (as also observed in the increased conventional systolic parameters) was also associated with reduced VAC (Fig. 4). Similar to other functional parameters, sexual differences in the VAC ratio became even more pronounced after 12 wk of AB. At this time, in male rats, reduction of previously increased contractility resulted in a mismatch between the ventricle and the elevated arterial loading condition. In contrast, the maintained contractility augmentation in female rats led to a preserved VAC ratio despite the enhanced E_a (Fig. 4).

Limitations

In the present investigation, aortic constriction to a standard size was used to induce PO in rats. However, male animals grew to a greater body weight, which raised the possibility that in male rats a relatively more severe aortic constriction developed. Therefore, to exclude the possibility of different degrees of PO in male and female rats, numerous parameters of afterload were calculated (SBP, DBP, MAP, and E_a), which showed no differences between the sexes. Accordingly, it could be hypothesized that indeed genetic and hormonal fac-

tors have mainly contributed to the observed sex-dependent differences in our model.

Another limitation of the study might be that the gradual rise of arterial blood pressure (proximal to the aortic constriction) has not been registered in our investigation. However, literature data clearly indicate that in the abdominal AB rat model, a substantial increment of arterial blood pressure occurs already in the first 2 wk after the surgical application of the constriction (18). Therefore, it can be confidently stated that the severe increment in afterload had been long persisted before the selected time points (*weeks 6* and *12*) in the present study.

Furthermore, it has to be also noted here that the AB rat model involves numerous mechanistic components that ultimately evoke the pathological remodeling of the LV. Accordingly, previous investigations have indicated that constricting the abdominal aorta at the suprarenal level not only induces PO in the proximal part of the aorta but also leads to the hypoperfusion of the kidneys with subsequent activation of the renin-angiotensin-aldosterone system (3, 17). Therefore, it cannot be ruled out that in our study, neurohumoral factors also contributed to the development of LVH.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.R., S.K.-I., B.M., M.K., T.R., and G.S. conceived and designed research; M.R., S.K.-I., S.L., and W.J. performed experiments; M.R., S.K.-I., S.L., W.J., L.L., A.O., A.A.S., and B.A.B. analyzed data; M.R. interpreted results of experiments; M.R. prepared figures; M.R., S.K.-I., T.R., and G.S. drafted manuscript; S.K.-I., B.M., M.K., T.R., and G.S. edited and revised manuscript; M.R., S.K.-I., S.L., W.J., L.L., A.O., A.A.S., B.A.B., B.M., M.K., T.R., and G.S. approved final version of manuscript.

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