Myofilament Ca\(^{2+}\) sensitivity correlates with left ventricular contractility during the progression of pressure overload-induced left ventricular myocardial hypertrophy in rats

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Aim: Here we aimed at investigating the relation between left ventricular (LV) contractility and myofilament function during the development and progression of pressure overload (PO)-induced LV myocardial hypertrophy (LVH). Methods: Abdominal aortic banding (AB) was performed to induce PO in rats for 6, 12 and 18 weeks. Sham operated animals served as controls. Structural and molecular alterations were investigated by serial echocardiography, histology, quantitative real-time PCR and western blot. LV function was assessed by pressure-volume analysis. Force measurement was carried out in permeabilized cardiomyocytes. Results: AB resulted in the development of pathological LVH as indicated by increased heart weight-to-tibial length ratio, LV mass index, cardiomyocyte diameter and fetal gene expression. These alterations were already present at early stage of LVH (AB-week6). Furthermore, at more advanced stages (AB-week12, AB-week18), myocardial fibrosis and chamber dilatation were also observed. From a hemodynamic point of view, the AB-wk6 group was associated with increased LV contractility, maintained ventriculo-arterial coupling (VAC) and preserved systolic function. In the same experimental group, increased myofilament Ca\(^{2+}\) sensitivity (pCa\(^{50}\)) and hyperphosphorylation of cardiac troponin-I (cTnl) at Threonine-144 was detected. In contrast, in the AB-wk12 and AB-wk18 groups, the initial augmentation of LV contractility, as well as the increased myofilament Ca\(^{2+}\) sensitivity and cTnl (Threonine-144) hyperphosphorylation diminished, leading to impaired VAC and reduced systolic performance. Strong correlation was found between LV contractility parameters and myofilament Ca\(^{2+}\) sensitivity among the study groups. Conclusion: Changes in myofilament Ca\(^{2+}\) sensitivity might underlie the alterations in LV contractility during the development and progression of PO-induced LVH.

Keywords:
Myocardial hypertrophy
Myofilament function
Ca\(^{2+}\) sensitivity
Contractility

Abbreviations: AB, aortic banding; ANP, atrial natriuretic peptide; AWT\(_d\), anterior wall thickness in diastole; β/α-MHC, beta/alpha myosin heavy chain ratio; CD, cardiomyocyte diameter; cMyBP-C, cardiac myosin binding protein-C; CO, cardiac output; cTnl, cardiac troponin-I; dp/dmax, maximal slope of systolic pressure increment; dP/dmax, maximal slope of diastolic pressure decrement; dP/dmax-EDV, dp/dmax-end-diastolic volume relationship; E\(_a\), arterial elastance; EDPVR, end-diastolic pressure-volume relationship; ESPVR, end-systolic pressure-volume relationship; F\(_{\text{max}}\), Ca\(^{2+}\)-saturated maximal force; F\(_{\text{passive}}\), Ca\(^{2+}\)-independent passive tension; HW/TL, heart weight to tibial length ratio; LVEDD, left ventricular end-diastolic diameter; MAP, mean arterial pressure; pCa\(^{50}\), myofilament Ca\(^{2+}\) sensitivity; PO, pressure overload; PRSW, preload recruitable stroke work; P–V, pressure-volume; PWT\(_p\), posterior wall thickness in diastole; SV, stroke volume; SW, stroke work; Tau, time constant of left ventricular pressure decay; VAC, ventriculo-arterial coupling

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2.2 Abdominal aortic banding

After a one-week-long acclimatization period, abdominal aortic banding (AB) or sham operation was performed, as also described elsewhere [6]. In brief, under isoflurane anesthesia a midline laparotomy was carried out. Then, the intestinal tract was gently placed aside and the peritoneal layer was dissected in order to gain access to the retroperitoneal space. The abdominal aorta between the right renal artery and the superior mesenteric artery was carefully cleaned from the surrounding connective tissue. A blunted 22-gauge needle was utilized to constrict the aorta above the right renal artery. After AB was completed, the intestines were placed back to the abdominal cavity and the abdominal muscle layer was sutured in single interrupted fashion. Finally, the skin wound was closed by applying surgical clips. Following surgery, analgesia was provided by subcutaneously administered buprenorphine in the dose of 0.05 mg/kg. Sham-operated animals were subjected to the same surgical procedure, except the aortic constriction.

2.3 Experimental groups

Following surgeries, the rats were divided into 6 experimental groups:

- Sham-wk6 group (n = 9): after sham operation the rats were followed-up for 6 weeks;
- AB-wk6 group (n = 13): after AB the rats were followed-up for 6 weeks;
- Sham-wk12 group (n = 9): after sham operation the rats were followed-up for 12 weeks;
- AB-wk12 group (n = 13): after AB the rats were followed-up for 12 weeks;
- Sham-wk18 group (n = 10): after sham operation the rats were followed-up for 18 weeks;
- AB-wk18 group (n = 13): after AB the rats were followed-up for 18 weeks, respectively.

2.4 Echocardiography

Echocardiography was carried out using the Vevo® 2100 imaging system (FujiFilm VisualSonics Inc., Toronto, Ontario, Canada) equipped with a 21-MHz linear probe according to the previously described protocol [6]. Repetitive measurements were performed in the AB-wk18 and Sham-wk18 groups at baseline and 3, 6, 9, 12, 15 and 18 weeks after AB/Sham operation. On M-mode recording images, LV internal diameters (LV end-diastolic diameter [LVEDD] and LV end-systolic diameter [LVESD]) and anterior wall thicknesses (PWT) in diastole (d) and systole (s) were measured and LV mass index (LV mass/body weight) were calculated.

2.5 Pressure–volume analysis

LV P–V analysis was performed according to a previously described protocol with minor modifications [6]. At the end of the experimental period (at week 6, 12 or 18 respectively), rats were anesthetized 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% O2. Pancuronium was administered intraperitoneally (in the dose of 2 mg/kg) 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), maximal slope of systolic pressure increment (dP/dt max) and
diastolic pressure decrement (dP/dt\text{min}), LV end-diastolic volume (LVESV), LV end-diastolic volume (LVEDV), stroke volume (SV), ejection fraction (EF), heart rate (HR), cardiac output (CO), stroke work (SW) were computed and calculated. Furthermore, the integrative index of arterial afterload, arterial elastance \(E_a\) calculated by the following equation: \(E_a = LVESV/SV\) [11], and the time constant of LV pressure decay \(\tau_a\) (according to the Glanz method [12]) were also assessed.

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. By this maneuver, the following load-independent contractility indexexes were calculated: the slope of the end-diastolic \(P-V\) relationship (ESPVr, according to the parabolic curvilinear model [13]), preload recruitable stroke work (PRSW) and the slope of the \(dP/dt_{\text{max}}\)-end-diastolic volume relationship (\(dP/dt_{\text{max}}\)-EDV).

To characterize myocardial stiffness, the slope of the end-diastolic \(P-V\) relationship (EDPVR) was derived.

To assess LV net performance, ventriculo-arterial coupling (VAC) was determined as the ratio of \(E_a\) and ESPVR (VAC = \(E_a/\text{ESPVr}\)) [14]. Parallel conductance was calculated, and volume calibration of the conductance system was performed as previously described [2]. After completion of the hemodynamic measurements, animals were euthanized by exsanguination. After exsanguination, the organs were perfused in situ with cold (4 °C), oxygenated Ringer solution to eliminate erythrocytes from myocardial tissue. Then, heart weights and tibial lengths were quickly measured. The ratio of heart weight-to-tibial length was calculated to assess the extent of LVH.

2.6. Measurement of permeabilized cardiomyocyte mechanical parameters

In each experimental groups, deep-frozen heart tissue samples from 6 animals were used for cardiomyocyte measurements (\(N = 6\)). In each groups, a total of 10–12 cardiomyocytes were measured (\(n = 10–12\)). Samples were mechanically isolated in isolating solution ([iso contained: KCi (100 mM), EGTA (2 mM), MgCl2 (1 mM), Na2ATP (4 mM), imidazole (10 mM), pH = 7.0) containing phenylmethylsulfonyl fluoride (PMSF, 0.5 mM, Sigma–Aldrich), leupeptin (40 μM, Sigma–Aldrich), and E-64 (10 μM, Sigma–Aldrich) protease inhibitors at 4 °C. Cardiomyocytes were permeabilized with 0.5% Triton X-100 detergent for 4 min to remove all membranous structures. Single isolated and permeabilized cardiomyocytes were mounted between two thin stainless steel needle by means of silicone adhesive (DAP 100% all-purpose silicone sealant; Baltimore, MD, USA) at 15 °C. The needles were connected to a highly sensitive force transducer (SensoNor, Horten, Norway) and an electromagnetic length controller (Aurora Scientific Inc., Aurora, Canada). Activating and relaxing solutions were mainly used during the force measurement procedure. The activating solution contained: CaEGTA (7 mM), KCl (37.34 mM), N,N,N,N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid (BES, 10 mM), MgCl2 (6.24 mM), Na2ATP (6.99 mM), Na2CrP (15 mM), pH = 7.2 resulting in a \([\text{Ca}^{2+}]\) of \(pCa = 4.75\) (pCa: -lg\([\text{Ca}^{2+}]\)). The relaxing solution contained the same components apart from containing EGTA instead of CaEGTA, pH = 7.2 resulting in a \([\text{Ca}^{2+}]\) of \(pCa = 9\). Both solutions were supplemented with protease inhibitors. Solutions with intermediate calcium concentrations were prepared by mixing relaxing and activating solutions (pCa 5.4–7.0). The sarcomere length (SL) was adjusted to 2.3 μm. The active force (\(F_{\text{active}}\)) and \([\text{Ca}^{2+}]\)-sensitivity of force production were determined in activating solutions with different \([\text{Ca}^{2+}]\) concentrations. When the maximal active force (\(F_{\text{max}}\)) had been reached, a quick release-restretch maneuver (30 ms) was applied in the activating solution. The length of the myocyte was reduced by 20% followed by quick re-stretching the cell back to its initial length (release-restretch maneuver).

The Ca2+-independent passive tension (\(F_{\text{passive}}\)) was approximated by shortening of the preparations to 80% of the original lengths in relaxing solution for 8s. The cell was then exposed to a series of solutions containing intermediate pCa to construct the pCa-force relationship. \(F_{\text{active}}\) at submaximal Ca2+ levels were normalized to the \(F_{\text{active}}\) at maximal Ca2+ level (4.75, \(F_{\text{max}}\)). The relation between the \(F_{\text{active}}\) and pCa was fitted to a modified Hill equation: \(F_{\text{total}} = F_{\text{max}}[\text{Ca}^{2+}]^{nHill/(pCa50 + [\text{Ca}^{2+}]^{nHill}) + F_{\text{passive}}\text{, where } F_{\text{max}}\text{ is the maximal force, } F_{\text{passive}}\text{ is the passive force, } F_{\text{total}} = F_{\text{max}} + F_{\text{passive}}\). \(K_{50}\) is the calculated Ca2+ concentration, nHill is a constant the slope and the midpoint of the sigmoidal relationship, respectively. pCa50 illustrated the Ca2+ concentration at which half of the maximal force was produced. The values of absolute forces were normalized to myocyte cross sectional area and expressed in kN/m².
described protocol [7]. Briefly, LV myocardium was homogenized in a lysis buffer (RLT buffer; Qiagen, Hilden, Germany). RNA was isolated using 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 (Thermo Scientific™, Waltham, MA, USA). 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. Reverse transcription reaction (1 μg total RNA of each sample) was completed using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed with the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in duplicates of each sample in a volume of 10 μl in each well containing cDNA (1 μl), TaqMan Universal PCR MasterMix (5 μl), and a TaqMan Gene Expression Assay (0.5 μl) (Applied Biosystems) for the following genes: atrial natriuretic peptide (ANP; assay ID: Rn00664637_g1), β-type myosin heavy chain (β-MHC; assay ID: Rn00568328_m1), α-type myosin heavy chain (α-MHC; assay ID: Rn00568304_m1). Gene expression data were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; assay ID: Rn01775763_g1), and expression levels were calculated using the CT (ΔΔCT). All results are expressed as values normalized to a positive calibrator (a pool of cDNA from all samples of the Sham-wk6 group [2−ΔΔCT]).

2.10. Statistics

All values are expressed as mean ± standard error of the mean. The distribution of the datasets was tested by D’Agostino-Pearson omnibus or Shapiro-Wilk normality test.

An unpaired two-sided Student’s t-test in case of normal distribution or Mann-Whitney U test in case of non-normal distribution was used to compare two independent groups.

Two-way analysis of variance (ANOVA) with the factors “time” and “aortic banding” were carried out to compare six independent groups. For values of $P_{\text{interaction}}$ $P_{\text{aortic banding}}$ and $P_{\text{time}}$ see Suppl. Table 1. Prior to two-way ANOVA, those datasets that failed to show normal distribution were logarithmically transformed. Tukey post hoc test was utilized to detect intergroup differences.

Repeated-measures one-way ANOVA or Friedman test was performed for comparing data of the echocardiographic measurements at different time points (week 3, 6, 9, 12, 15 and 18) within a group. To examine intergroup differences, Holm-Sidak or Dunn post hoc test was carried out.

According to the distribution of the datasets, Pearson (in case of normal) or Spearman (in case of not-normal) correlation test was performed to detect correlations between $p\text{Ca}_{50}$ and ESPVR, between $p\text{Ca}_{50}$ and PRSW and between $p\text{Ca}_{50}$ and $dP/dt_{\text{max-EDV}}$. Western immunoblot assays were performed in triplicate. Intensities of protein bands were quantified by determining the area under the intensity curves by a Gaussian fit using ImageJ (National Institutes of Health, Bethesda, MD, USA) and MagicPlot (Saint Petersburg, Russia) software.

A $P$ value of $<0.05$ was used as a criterion for statistical difference. Furthermore, two additional categories ($P<0.01$ and $P<0.001$) were introduced to indicate the strength of the observed statistical difference.

2.11. Data availability

The raw data from the current study will be made available by the corresponding author upon reasonable request.

### Table 1

LV and myofilament function in aortic-banded and sham-operated rats at different time points.

| Week 6 | | Week 12 | | Week 18 | |
|--------|--------|--------|--------|--------|
| SBP, mmHg | 148 ± 4 | 215 ± 4 | 138 ± 5 | 215 ± 5 | 150 ± 5 |
| DBP, mmHg | 116 ± 3 | 150 ± 2 | 110 ± 4 | 154 ± 4 | 120 ± 4 |
| MAP, mmHg | 127 ± 4 | 172 ± 2 | 119 ± 4 | 174 ± 4 | 140 ± 4 |
| $E_{\text{e}}$, mmHg/ml | 0.75 ± 0.06 | 1.20 ± 0.08* | 0.68 ± 0.05 | 1.33 ± 0.10,** | 0.84 ± 0.05 | 1.54 ± 0.16,** |
| HR, beats/min | 355 ± 7 | 369 ± 9 | 354 ± 5 | 366 ± 7 | 379 ± 7 | 357 ± 5 |
| $dP/dt_{\text{max}}$, mmHg/s | 8018 ± 314 | 10,781 ± 283 | — | 8425 ± 365 | 10,692 ± 374 | 9689 ± 322 | 10,810 ± 518 |
| $dP/dt_{\text{max}}$, mmHg/s | — | — | — | — | — | — | — |
| $dP/dt_{\text{min}}$, mmHg/s | 10,781 ± 283 | 10,692 ± 374 | — | 9689 ± 322 | 10,810 ± 518 | — | — |
| LVEDV, μl | 268 ± 16 | 305 ± 14 | 286 ± 23 | 320 ± 20 | 283 ± 18 | 327 ± 14 |
| LVESV, μl | 175 ± 15 | 194 ± 12 | 178 ± 17 | 231 ± 11 | 160 ± 11 | 241 ± 11,** |
| SV, μl | 188 ± 16 | 173 ± 10 | 195 ± 11 | 163 ± 12 | 175 ± 10 | 151 ± 15 |
| CO, ml/min | 66.7 ± 6.1 | 62.9 ± 3.0 | 69.4 ± 4.5 | 59.4 ± 4.1 | 66.3 ± 4.4 | 53.7 ± 5.4 |
| SW, mmHg*μl | 17.314 ± 1300 | 25.194 ± 1873 | 18.404 ± 988 | 22.005 ± 2677 | 17.674 ± 1296 | 20.405 ± 1855 |
| EF, % | 58 ± 3 | 51 ± 2 | 57 ± 2 | 44 ± 2,## | 55 ± 2 | 41 ± 3,### |
| $\text{Tau}$, ms | 14.2 ± 0.4 | 18.4 ± 0.9,## | 12.8 ± 0.6 | 19.4 ± 0.6,## | 13.0 ± 0.3 | 21.7 ± 1.2,## |
| EDPRV, mmHg/μl | 0.038 ± 0.005 | 0.038 ± 0.007 | 0.028 ± 0.004 | 0.042 ± 0.006 | 0.014 ± 0.003 | 0.032 ± 0.004,## |
| VAC | 0.50 ± 0.08 | 0.45 ± 0.06 | 0.54 ± 0.06 | 0.76 ± 0.08,## | 0.57 ± 0.10 | 0.87 ± 0.08,## |
| $F_{\text{max}}$, kN/m² | 15.3 ± 0.8 | 13.6 ± 0.7 | 14.2 ± 0.5 | 13.1 ± 0.8 | 13.6 ± 0.8 | 15.5 ± 1.0 |

Values are expressed as mean ± standard error of the mean. AB indicates aortic-banding; SBP: systolic arterial blood pressure; DBP: diastolic arterial blood pressure; MAP: mean arterial pressure; $E_{\text{e}}$: arterial elastance; HR: heart rate; $dP/dt_{\text{max}}$: maximal slope of systolic pressure increment; $dP/dt_{\text{min}}$: maximal slope of diastolic pressure decrement; LVEDV: LV end-diastolic volume; LVESV: LV end-systolic volume; SV: stroke volume; CO: cardiac output; SW: stroke work; EF: ejection fraction; Tau: time constant of LV pressure decay according to the Glantz' method; EDPRV: end-diastolic pressure-volume relationship; VAC: ventriculo-arterial coupling; $F_{\text{max}}$: Ca²⁺-saturated maximal force; *: $P<.05$ vs. age-matched sham.

### Table 2


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3. Results

3.1. Echocardiographic follow-up during the development of PO-induced LVH

From week 3 until the end of the experimental period, AWTd, PWTd and LVmassindex were increased in the AB-wk18 group compared to the sham-wk18 group, indicating the development of LVH (Fig. 1B-D). Furthermore, at week 12, week 15 and week 18, LVEDD was also increased in the AB-wk18 group compared to the sham-wk18 group, suggesting chamber dilatation (Fig. 1E).

3.2. Pathological hypertrophy and fibrosis markers in PO-induced LVH

In the AB-wk6, AB-wk12 and AB-wk18 groups, HW/TL and CD were increased compared to the corresponding sham groups (Fig. 2B–C). The myocardial mRNA expression levels of β/α-MHC ratio and ANP were also elevated in the AB groups compared to their corresponding sham groups, indicating reactivation of the fetal gene program (Fig. 2D–E). Furthermore, assessment of the myocardial collagen area revealed increased interstitial fibrosis in the AB-wk12 and AB-wk18 groups compared to the sham-wk12 and sham-wk18 groups, respectively (Fig. 2F).

3.3. Myofilament function

pCa50 increased significantly in the AB-wk6 group compared to the sham-wk6 group, indicating increased myofilament Ca2+ sensitivity (Fig. 3). This pCa50 value was also significantly higher in the AB-wk6 group than in the AB-wk12 and AB-wk18 groups (Fig. 3). Myofilament Ca2+ sensitivity in the AB-wk12 and AB-wk18 groups did not differ from their corresponding sham groups (Fig. 3).

Furthermore, no significant differences could be observed in Fmax among the experimental groups (Table 1).

3.4. Site-specific phosphorylation of cardiac troponin-I and myosin binding protein-C

Phosphorylation of cTnI at Ser-22/23 and Ser-43 did not differ among the study groups (Fig. 4A–B). There was also no difference in the phosphorylation status of cMyBP-C at Ser-282 (Fig. 4D). In contrast, phosphorylation of cTnI at the PKC-specific Thr-144 site was significantly increased in the AB-wk6 group compared either to the sham-wk6 group or the AB-wk12 and AB-wk18 groups (Fig. 4C).

3.5. LV function

Hemodynamic results obtained from P–V analysis are summarized in Figs. 5–7 and Table 1.

3.5.1. Arterial loading

SBP, DBP, MAP and Ea were elevated in the AB groups compared to the corresponding sham groups, confirming the presence of increased PO proximal to the aortic constriction (Table 1).

3.5.2. Load-dependent systolic parameters

The AB-wk6 group was associated with preserved systolic performance. Accordingly, no difference could be observed in load-dependent systolic parameters (EF, SV, CO) between the AB and the sham group at week 6 (Table 1). In contrast, in the AB-wk12 and AB-wk18 groups, EF decreased significantly, while SV and CO showed a tendency towards decreased values compared to the corresponding sham groups (Table 1).

3.5.3. Load-independent contractility parameters

In the AB-wk6 group, ESPVR, PRSW and dP/dtmax-EDV increased significantly compared to the sham-wk6 group, indicating increased LV contractility (Figs. 5A–B, 6A–B, 7A–B). This contractility augmentation diminished in the AB-wk12 and AB-wk18 groups. Accordingly, the load-independent contractility parameters were not different in the AB-
Fig. 2. Pathological hypertrophy markers at different stages of pressure overload-induced LVH.
Representative photomicrographs of hematoxylin and eosin (magnification 200×, scale bar: 40 μm) and picrosirius red staining (magnification 50×, scale bar: 200 μm) are shown demonstrating enlargement of cardiomyocytes and interstitial collagen accumulation in the aortic-banded (AB) groups (A). The development of pressure overload-induced left ventricular myocardial hypertrophy (LVH) was confirmed by increased heart weight-to-tibial length ratio (HW/TL) (B) and cardiomyocyte diameter (CD) (C) in the AB groups at week 6, 12 and 18 compared to the corresponding sham groups. The pathological nature of LVH was reflected by markers of the fetal gene program (beta-to-alpha myosin heavy chain ratio [β/α-MHC] and atrial natriuretic peptide [ANP]) (D-E). Furthermore, in the AB groups at week 12 and 18, the extent of collagen area increased significantly compared to the corresponding sham groups. *: P < .05. **: P < .01. ***: P < .001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Myofilament Ca^{2+} sensitivity in pressure overload-induced LVH.

pCa-force relationship of permeabilized cardiomyocytes revealed increased myofilament Ca^{2+}-sensitivity (pCa_{50}) in the aortic-banded (AB) group at week 6 compared to the corresponding sham group and also to the AB groups at week 12 and 18. Experimental values are derived from 6 animals (N = 6) and 10–12 cardiomyocytes (n = 10–12) per group. ***: P < .001.
wk12 and AB-wk18 groups compared to sham-wk12 and sham-wk18 groups, but they were significantly decreased compared to the AB-wk6 group (Figs. 5A–B, 6A–B).

3.5.4. Ventriculo-arterial coupling

In the AB-wk6 group, the enhanced LV contractility (increased ESPVR) counterbalanced the elevated afterload (increased Ea), therefore VAC did not differ from the corresponding sham group (Table 1). In contrast, in the AB-wk12 and AB-wk18 groups, the lack of compensatory LV contractility augmentation (reduced ESPVR values compared to AB-wk6) along with the elevated afterload (increased Ea) resulted in contractility-afterload mismatch. Thus, the values of VAC were significantly higher in the AB-wk12 and AB-wk18 groups compared to that of the AB-wk6 group (Table 1).

3.5.5. Diastolic function

Tau increased significantly in the AB-wk6, AB-wk12 and AB-wk18 groups compared to their corresponding sham groups (Table 1). Furthermore, the slope of EDPVR was also elevated in the AB-wk18 group compared to the sham-wk18 group (Table 1).

3.6. Correlation between myofilament Ca$^{2+}$ sensitivity and LV contractility parameters

Strong correlations were detected between the load-independent LV contractility parameters (ESPVR, PRSW and dP/dtmax-EDV) and myofilament Ca$^{2+}$ sensitivity (pCa50) (Figs. 5–7).

4. Discussion

The present paper is the first that demonstrates a strong correlation between myofilament Ca$^{2+}$ sensitivity of permeabilized cardiomyocytes (pCa50) and P–V analysis derived LV contractility parameters (ESPVR, PRSW, dP/dtmax-EDV) in a rat model of PO-induced LVH. Our results indicate that changes in myofilament Ca$^{2+}$ sensitivity might underlie the alterations in LV contractility during the development and progression of PO-induced LVH.

4.1. Structural and molecular characterization of AB-induced LVH

In the current study the abdominal AB rat model was used to investigate PO-induced remodeling of the LV. Consistently with our previous experiences with this model [4,6,7,16], AB evoked the development of marked LVH, as indicated by echocardiographic data (increased wall thicknesses and LVmassindex) (Fig. 1B–D), post mortem measurement (increased HW/TL) and histological analysis (increased CD) (Fig. 2A–C). The pathological nature of LVH was confirmed by the reactivation of the fetal gene program (enhanced β/α-MHC ratio and ANP mRNA levels) (Fig. 2D–E) [17]. All of these structural and molecular alterations were already present after 6 weeks of AB. In addition,
at later time points (week 12 and week 18) AB was also associated with chamber dilatation and progressive accumulation of interstitial collagen content (Fig. 1E, Fig. 2F), which is also in good agreement with prior findings [18].

4.2. Longitudinal assessment of LV function in PO-induced LVH by P–V analysis

Currently, P–V analysis represents the gold standard method to reliably measure in vivo LV hemodynamics [2]. Of particular interest, the assessment of the P–V loops at different preloads enables us to calculate LV contractility parameters, independently of loading conditions. Therefore, this technique is extremely valuable in pathological states, where the robust alterations in pre- and/or afterload (e.g. AB-induced chronic PO) make the conventional load-dependent parameters inappropriate to characterize LV function. For these reasons, P–V analysis was utilized in the present investigation as well, to explore timeline alterations in LV hemodynamics at different stages of PO-induced LVH.

**Early stage of PO-induced LVH.** The early stage of PO-induced LVH is typically associated with impaired diastolic function (predominantly prolonged active relaxation) [19], and preserved systolic performance. Although, at this stage, the conventional load-dependent systolic indexes show no alteration, assessment of P–V analysis-derived load-independent indexes revealed robust augmentation of LV contractility [4,6,16]. This increment in LV contractility allows the LV to compensate for the increased afterload, thereby maintaining an optimal interaction between the LV and the arterial system. Based on our previous experiences with the abdominal AB model [4,16], week 6 was chosen as a time point to investigate the early stage of LVH. According to our expectations, in the AB group at this time point diastolic dysfunction was already present (indicated by prolonged Tau), but the load-dependent systolic indexes (e.g. EF) were preserved (Table 1). To characterize LV contractility independently from extrinsic conditions, three load-independent, gold standard parameters (ESPVR, PRSW and dP/dt max-EDV) were calculated from P–V analysis data [2]. All of these indexes confirmed augmented LV contractility in the AB group (Figs. 4B, 5B, 6B). Furthermore, assessment of VAC ratio confirmed an adequate matching between LV contractility and the afterload of the connecting arterial system (Table 1). Therefore, from a hemodynamic point of view, the AB group at week 6 met the criteria of an early stage of PO-induced LVH.

**Advanced stages of PO-induced LVH.** In case of PO-induced pathological LVH, the contractility augmentation provides only temporary adaptation. Accordingly, after a certain period of time, the initially increased LV contractility regresses, despite of the persistently elevated PO [5,6]. In animal models of chronic PO-induced LVH, the time point when LV systolic dysfunction is expected to develop is dependent on the species of the experimental animal (mice versus rat), the location of the banding suture (thorax versus abdomen) and the severity of the applied constriction (severe versus moderate) [20]. Our experience from previous consecutive studies with the abdominal AB rat model indicates, that an early transition from “compensated” LVH to systolic dysfunction takes place after 12 weeks of chronic PO in male AB rats [6]. Thus, in the current study, week 12 and week 18 were selected to study advanced stages of LVH. At these time points, the P–V analysis derived contractility indexes were substantially reduced compared to that of the early stage of LVH (AB-wk6 group) (Fig. 4B, 5B, 6B). The decompensation of the hypercontractile state along with increased arterial loading condition (Ea) resulted in contractility-afterload mismatch (impaired VAC ratio) (Table 1). Therefore, at these time points the net systolic performance was decreased, which was reflected by a slight reduction in load-dependent systolic parameters (e.g. EF) (Table 1). Furthermore, the AB-wk18 group was also associated with marked

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**Fig. 5.** Correlation between the slope of the end-systolic pressure-volume relationship and myofilament Ca\(^{2+}\) sensitivity during the progression of PO-induced LVH. Assessment of the slope of the end-systolic pressure-volume relationship (ESPVR) revealed increased left ventricular (LV) contractility in the aortic-banded (AB) group at week 6 compared to the corresponding sham group and also to the AB groups at week 12 and 18 (A-B). Alterations in ESPVR and pCa50 showed a strong correlation among all the study groups (C) and also among only the AB groups (D). No correlation could be detected among the Sham groups (E). LVH indicates left ventricular hypertrophy; PO: pressure overload. **: \(P < .01\). ***: \(P < .001\).
impairment of both aspects (active relaxation and passive stiffness) of diastolic function (Table 1).

4.3. Myofilament function in PO-induced LVH and its correlation to global LV function

The force generation of cardiomyocytes is eventually dependent on sarcomere function [21]. Therefore, it is reasonable to hypothesize that certain alteration might occur in myofilament function during the development and progression of PO-induced LVH that underlie the observed changes in LV contractility. This is further supported by the fact that former studies reported increased, unaltered and decreased cardiac myocyte function at different stages of PO-induced pathological LVH [8–10,22]. However, these studies share common limitations that warrants the need for further experimentations. First of all, most of the previous investigations analyzed myofilament function at only one specific time point (cross sectional studies). Consequently, we have limited data regarding the timeline of sarcomere changes in PO-induced LVH. Furthermore, none of the mentioned investigations assessed LV contractility in the same experimental model. Hence, our knowledge how the reported changes of myofilament function appear on global LV hemodynamics is also limited. Therefore, in the present investigation, we aimed to detect myofilament function and LV contractility from the same experimental animals at different stages of PO-induced LVH.

Early stage of PO-induced LVH. As we pointed it out above, the early stage of PO-induced LVH (AB-wk6 group) was associated with increased contractility (ESPVR, PRSW, dP/dtmax-EDV) on the global LV level. In the same experimental group, we observed enhanced Ca2+-sensitivity (pCa50) and unaltered Fmax of permeabilized LV cardiomyocytes (Fig. 3 and Table 1). Therefore, our results indicate that increased myofilament Ca2+-sensitivity held responsibility for the contractility augmentation on the cellular level during the early-phase of PO-induced LVH. Although, this kind of relation between myofilament Ca2+-sensitivity and LV contractility in pathological LVH has not been postulated yet, numerous groups have reported increased sarcomere Ca2+-sensitivity in pathological LVH with preserved systolic function [8,23]. Furthermore, to define the underlying molecular mechanism for the increased myofilament Ca2+-sensitivity, we analyzed the phosphorylation status of specific regulatory sites of cTnI (Ser-23/24, Ser-43 and Thr-144) and cMyBP-C (Ser-282). These measurements revealed a robust increment in the phosphorylation of the PKC-dependent Thr-144 site of cTnI in the AB-wk6 group (Fig. 4C). In line with our results, it has been previously reported that pseudo-phosphorylation of cTnI at Thr-143/144 (Thr-143 in human and Thr-144 in mouse/rat) potentially enhances myofilament Ca2+-sensitivity [24,25]. Interestingly, the hyperphosphorylation of Thr-143 site did not affect the maximal force development in human permeabilized cardiomyocytes [24], which finding is also in good agreement with our results demonstrating unaltered Fmax in the AB-wk6 group (Table 1). Considering the fact that no alterations occurred at other sites of cTnI (Ser-22/23 and Ser-43) and cMyBP-C (Ser-282), it could be hypothesized that hyperphosphorylation of PKC-specific Thr-144 site of cTnI might have predominantly contributed to the increased myofilament Ca2+ sensitivity in the AB group at week 6.
Advanced stages of PO-induced LVH. At more advanced stages of PO-induced LVH, the compensatory increment in LV contractility declined, as indicated by \( P_eV \) analysis (Figs. 5B, 6B, 7B). In parallel, we found that the cellular (enhancement of myofilament Ca\(^{2+}\)-sensitivity) and molecular (cTnI hyperphosphorylation at PKC-specific Thr-144 site) adaptations also diminished in the AB groups at week 12 and 18 (Figs. 3, 4C). Therefore, in our longitudinal study, alterations of sarcomere Ca\(^{2+}\)-sensitivity as well as cTnI phosphorylation at Thr-144 followed very precisely the changes of LV contractility. Previous studies conducted on advanced stages of PO-induced LVH found normal or decreased Ca\(^{2+}\)-sensitivity in isolated and skinned single cardiomyocyte preparations. For instance, in a spontaneously hypertensive rat strain with evident signs of HF, neither pCa\(_{50}\) nor F\(_{\text{max}}\) differed from the control’s level [22]. These results are in good agreement with the present findings, demonstrating the lack of cellular compensation to the increased PO in advanced stages of LVH. Furthermore, in a TAC-induced end-stage HF rat model, both components of myofilament function (pCa\(_{50}\)), F\(_{\text{max}}\)) were substantially decreased [9,10]. It is important to note that the aortic arch was constricted to a very severe level for a relatively long experimental period (36 weeks) in the latter studies. Therefore, it could be hypothesized that in this particular TAC model, the status of LVH and HF reached a more advanced level compared either to our abdominal AB or the spontaneously hypertensive rat models.

Based on our findings and the previous literature data, we hypothesize that increment in myofilament Ca\(^{2+}\)-sensitivity (due to hyperphosphorylation of the PKC-specific Thr-144 site of cTnI) represents an early but only temporary adaptation to increased PO. Accordingly, the initially increased sarcomere Ca\(^{2+}\)-sensitivity regresses during the transition from compensated LVH to early-stage HF and it substantially reduces at HF.

5. Conclusion

The present paper found that the early-stage of PO-induced LVH is associated with increased LV contractility on the intact organ level, and enhanced myofilament Ca\(^{2+}\)-sensitivity (due to hyperphosphorylation of the PKC-specific Thr-144 site of cTnI) on the sarcomere level. This early functional adaption compensates for the increased afterload resulting in maintained ventriculo-arterial coupling and preserved systolic function. However, at more advanced stages of PO-induced LVH, the initial augmentation of LV contractility as well as the increased myofilament Ca\(^{2+}\)-sensitivity decline, leading to impaired ventriculo-arterial coupling and reduced systolic performance. These data indicate, that changes in sarcomere Ca\(^{2+}\)-sensitivity might underlie the alterations in LV contractility during the development and progression of PO-induced LVH.

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Conflict of interest

None.

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