

Myocardial reverse remodeling after pressure unloading is associated with maintained cardiac mechanoenergetics in a rat model of left ventricular hypertrophy

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Submitted 26 January 2016; accepted in final form 10 June 2016

Ruppert M, Korkmaz-Icöz S, Li S, Németh BT, Hegedűs P, Brlecic P, Mátyás C, Zorn M, Merkely B, Karck M, Radovits T, Szabó G. Myocardial reverse remodeling after pressure unloading is associated with maintained cardiac mechanoenergetics in a rat model of left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol* 311: H592–H603, 2016. First published June 24, 2016; doi:10.1152/ajpheart.00085.2016.—Pressure unloading represents the only effective therapy in increased afterload-induced left ventricular hypertrophy (LVH) as it leads to myocardial reverse remodeling (reduction of increased left ventricular mass, attenuated myocardial fibrosis) and preserved cardiac function. However, the effect of myocardial reverse remodeling on cardiac mechanoenergetics has not been elucidated. Therefore, we aimed to provide a detailed hemodynamic characterization in a rat model of LVH undergoing pressure unloading. Pressure overload was induced in Sprague-Dawley rats by abdominal aortic banding for 6 (AB 6th wk) or 12 wk (AB 12th wk). Sham-operated animals served as controls. Aortic debanding procedure was performed after the 6th experimental week (debanded 12th wk) to investigate the regression of LVH. Pressure unloading resulted in significant reduction of LVH (heart weight-to-tibial length ratio: 0.38 ± 0.01 vs. 0.58 ± 0.02 g/mm, cardiomyocyte diameter: 18.3 ± 0.1 vs. 24.1 ± 0.8 μ m debanded 12th wk vs. AB 12th wk, $P < 0.05$), attenuated the extracellular matrix remodeling (Masson's score: 1.37 ± 0.13 vs. 1.73 ± 0.10 , debanded 12th wk vs. AB 12th wk, $P < 0.05$), provided protection against the diastolic dysfunction, and reversed the maladaptive contractility augmentation (slope of end-systolic pressure-volume relationship: 1.39 ± 0.24 vs. 2.04 ± 0.09 mmHg/ μ l, $P < 0.05$ debanded 12th wk vs. AB 6th wk, $P < 0.05$). In addition, myocardial reverse remodeling was also associated with preserved ventriculoarterial coupling and increased mechanical efficiency (50.6 ± 2.8 vs. $38.9 \pm 2.5\%$, debanded 12th wk vs. AB 12th wk, $P < 0.05$), indicating a complete functional and mechanoenergetic recovery. According to our best knowledge, this is the first study demonstrating that the regression of LVH is accompanied by maintained cardiac mechanoenergetics.

pressure overload-induced cardiac hypertrophy; pressure unloading; cardiac contractility; cardiac mechanoenergetics; diastolic dysfunction; pressure-volume analysis

NEW & NOTEWORTHY

Our experiment demonstrates for the first time that the regression of left ventricular hypertrophy by pressure unloading not only leads to preserved diastolic function, and reversed maladaptive contractility augmentation, but also to maintained cardiac mechanoenergetics. Therefore, pressure unloading at a relatively early time point could result in complete functional recovery.

HYPERTENSION AND AORTIC STENOSIS are clinical syndromes associated with sustained pressure overload of the left ventricle (LV) and affect more than one-third of the population in the United States, thus representing major health problems (26).

Cardiomyocytes possess the ability to sense the mechanical stimulus of pressure overload, and, when it chronically persists, they try to adapt to the altered circumstances (32). The response reaction consists of increased synthesis of sarcomeric proteins and enlargement of individual cardiomyocytes, resulting in reduced wall stress (18). However, in the long term, the remodeling process also includes reactivation of the fetal gene program (15), extracellular matrix (ECM) remodeling (10), and decreased capillary density (39). All of these alterations collectively lead to ventricular dilatation with progressive decline in both the systolic and the diastolic performance.

The initial step of the hypertrophic program, namely the myocardial wall thickening and the subsequent normalization of wall tension, was previously regarded to be advantageous and required to preserve the functionality of the heart (3, 12). However, this long-standing hypothesis has been reconsidered, since it was recently demonstrated that wall stress normalization may not be necessary for the adaptation to sustained pressure overload (14). Therefore, the development of LV hypertrophy (LVH) with increment in cardiac contractility is considered to be pathological, as in the long term it entails the possibility of functional deterioration (22). In particular, impaired myocardial mechanoenergetics have been reported in hypertensive patients with LVH (11, 23), as well as in animal models of chronic pressure overload of the LV (33).

Currently, the only effective, clinically available therapy in pressure overload-induced LVH is represented by antihypertensive therapy (in the case of arterial hypertension) or aortic valve replacement (AVR) surgery/intervention (in the case of aortic stenosis). This means that only the termination of the pathological stimulus of hemodynamic overload is able to ensure an adequate therapeutic response and evoke the regression of the preestablished hypertrophy at an early stage, a phenomenon called myocardial reverse remodeling. Indeed

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numerous clinical trials and animal studies provided evidence that significant regression of LV mass and LV diameters is achievable following pressure unloading therapy (8, 16, 36, 41). Furthermore, despite the fact that myocardial fibrosis often persists years after AVR, the clinical experience shows that surgeries performed at early time points are capable to attenuate the collagen accumulation in the myocardium (42).

On the contrary, the functional consequences of reverse remodeling are still not entirely clear. In recent years, clinical studies utilizing noninvasive, echocardiographic approaches demonstrated that pressure unloading therapy is associated with improved midwall fractional shortening (FS) (29, 44). Furthermore, the restoration of maladaptive contractility augmentation has already been observed in a rat model of banding and debanding of the ascending aorta (7). However, the effect of myocardial reverse remodeling on cardiac mechanoenergetics has not been elucidated. To answer this question, precise hemodynamic investigations are needed.

Recently, the technique of pressure-volume (P-V) analysis became the “gold standard” method to accurately assess in vivo cardiac function in animal models (28). The detailed analysis of the P-V loops enables a unique possibility to gain sensitive contractility and mechanoenergetic parameters independently from vascular loading conditions. Thus, in the present study, we aimed to provide a detailed hemodynamic characterization of a rat model with LVH undergoing pressure unloading therapy, utilizing a P-V conductance system. Additionally, the time course of the fibrotic alterations in the ECM and the functional changes of the heart were also investigated.

METHODS

Ethical Approval and 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 “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication no. 86-23, revised 1996). This investigation was approved by the appropriate ethics review committee. Male Sprague-Dawley rats ($n = 50$) (160–180 g; Charles River, Sulzfeld, Germany) were housed in a room with constant temperature of $22 \pm 2^\circ\text{C}$ with 12:12-h light-dark cycles. During the 12-wk-long observation period, the animals were allowed access to standard laboratory rat diet and water ad libitum.

Abdominal Aortic Banding and Debanding

Pressure overload was induced by abdominal aortic banding, as also described elsewhere (25). General anesthesia was evoked with 5% of isoflurane gas in a chamber and maintained by inhalation from a connected tube with 1.5–2% of isoflurane in O_2 . The animals were placed in a supine position on a heating pad to maintain the core temperature at 37°C , measured via a rectal probe. The surgery was performed in midline laparotomy under sterile conditions. The abdominal aorta was constricted at the suprarenal level by a 2-0 silk suture to the external diameter of a 22-gauge needle. 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.

After the 6th experimental week, in a group of aortic-banded animals, the narrowing suture was cut and removed from the abdominal aorta. The prompt drop of blood pressure was confirmed by

simultaneous invasive blood pressure measurement via the carotid artery during the mentioned debanding procedure in previous pilot studies.

Experimental Groups and Design

Six weeks later after the aortic banding or sham operation, the animals were divided into five experimental groups:

1) Aortic-banded (AB) 6th wk group ($n = 8$): these animals underwent aortic banding procedure and a 6-wk-long follow-up period.

2) Sham-operated 6th wk group ($n = 8$): these animals underwent sham operation and a 6-wk-long follow-up period to serve as a control for the first group.

3) AB 12th wk group ($n = 8$): these animals underwent aortic banding procedure and a 12-wk-long follow-up period.

4) Sham-operated 12th wk group ($n = 8$): these animals underwent sham operation and a 12-wk-long follow-up period to serve as a control for the third group.

5) Debanding 12th wk group ($n = 10$): these animals underwent aortic banding procedure, after the 6th wk the banding suture was removed, and they were followed up until the 12th wk.

To investigate the time course of the morphological and functional alterations of the hearts, serial echocardiography was performed after the 3rd, 6th, 9th, and 12th wk. At the end of the experimental period (6th wk or 12th wk, respectively) invasive hemodynamic measurement was carried out by using the method of LV P-V analysis. Then the rats were euthanized, the hearts were removed, and the heart weights were immediately measured.

Echocardiography

Anesthesia was induced by 5% of isoflurane in a chamber. Then, in a supine position, the animals were placed on an automatic heating pad to maintain the body temperature at 37°C and were connected to a funnel to keep the anesthesia by inhalation of 1.5–2% isoflurane gas in O_2 . To gain an appropriate acoustic window, the animals' thorax was shaved, and a prewarmed ultrasound gel was also utilized. After stabilization for 10 min (anesthesia standardization), transthoracic echocardiography was performed by using an HDI 5000 CV echocardiography machine (ATL Ultrasound, Philips, Bothell, WA) equipped with a 10-MHz linear probe. Two-dimensional parasternal long-axis and short-axis images, as well M-mode recordings at the midpapillary muscle level, were assessed. The study was completed by analyzing the digital images in a blinded fashion using an image analyzing software (HDI Lab, Philips, Bothell, WA).

From these images, the following parameters were measured: LV anterior wall thickness (AWT), LV posterior wall thickness (PWT) in diastole (index: d) and systole (index: s); LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters. End-diastolic and end-systolic time points were defined as the largest and the smallest LV dimensions. All values were calculated as the average from three consecutive cardiac cycles.

LV mass was calculated to estimate the myocardial weight using the Devereux formula: $\text{LV mass (g)} = \{[(\text{LVEDD} + \text{AWT}_d + \text{PWT}_d)^3 - \text{LVEDD}^3] \times 1.04\} \times 0.8 + 0.14$ (13). In addition, relative wall thickness (RWT) was calculated to assess LV geometry, as the following: $\text{RWT} = (\text{AWT}_d + \text{PWT}_d) / \text{LVEDD}$. Furthermore, LV volumes were estimated according to the Teichholz method: $\text{LVEDV} = [7 / (2.4 + \text{LVEDD})] \times \text{LVEDD}^3$, $\text{LVESV} = [7 / (2.4 + \text{LVESD})] \times \text{LVESD}^3$ (38), where LVEDV is LV end-diastolic volume and LVESV is LV end-systolic volume.

Cardiac function was assessed by the following parameters: stroke volume (SV) as $\text{LVEDV} - \text{LVESV}$, cardiac output (CO) as $\text{SV} \times \text{heart rate}$, FS as $[(\text{LVEDD} - \text{LVESD}) / \text{LVEDD}] \times 100$, and ejection fraction (EF) as $(\text{LVEDV} - \text{LVESV} / \text{LVEDV}) \times 100$. To avoid the influence of body weight differences, SV and CO were normalized to body weight, leading to the parameters of SV index and cardiac index.

Invasive Hemodynamic Measurement

P-V analysis was performed as previously described (30). After the experimental period (6th or 12th wk, respectively), the rats were anesthetized with pentobarbital sodium (60 mg/kg ip), tracheotomized, and intubated to facilitate breathing. The animals were placed on an automatic heating pad in a supine position, and the body temperature was maintained (measured via a rectal probe) at 37°C during the whole procedure. A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2F microtip P-V catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization for 5 min, arterial blood pressure was recorded. Then the catheter was guided to the LV under pressure control. After 5-min stabilization period, signals were continuously recorded at a sampling rate of 1,000 samples/s using a P-V conductance system (MPVS-Ultra, Millar Instruments) connected to the PowerLab 16/30 data acquisition system, (AD Instruments, Colorado Springs, CO), stored, and displayed on a personal computer by the LabChart5 Software System (AD Instruments).

With the use of a special P-V analysis program (PVAN, Millar Instruments) systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), heart rate, LVEDV, LVESV, LV end-systolic pressure, LV end-diastolic pressure (LVEDP), EF, SV, arterial elastance (E_a), time constant of LV pressure decay (τ_G) [according to the Glanz method (43)], stroke work (SW), and P-V area (PVA) were computed and calculated. The total peripheral resistance (TPR) was computed by the following equation: $TPR = MAP/CO$.

To detect load-independent sensitive contractility parameters, the P-V loops were also registered at transiently decreasing preload, which was achieved by the occlusion of the inferior caval vein. The slope (end-systolic elastance, E_{es}) of the end-systolic P-V relationship (ESPVR) and the preload recruitable SW (PRSW) were calculated as reliable LV contractility indexes. The slope of the LV end-diastolic P-V relationship (EDPVR) was defined as a precise index of LV stiffness.

Furthermore, ventriculoarterial coupling (VAC) ratio (as E_a/E_{es}) and mechanical efficiency (Eff) (as SW/PVA) were calculated to assess the mechanoenergetics of the heart.

To determine the exact volume of the chamber, the so-called parallel conductance has to be extracted from the total conductance directly measured by the catheter. For this reason, at the end of the experiment, 0.1 ml hypertonic saline was injected intravenously, and, from the shift of the pressure volume loops, parallel conductance was calculated and used for the volume correction. The volume calibration of the conductance system was performed as previously described (28).

Histology

At the end of the experiment, after the blood samples were collected, all rats were euthanized via rapid exsanguination by dissecting the inferior caval vein. Then the hearts were harvested, fixed in buffered paraformaldehyde solution (4%), and embedded in paraffin. Transverse, transmural, 5- μ m thick slices of the ventricles were sectioned and placed on adhesive slides. To detect cardiomyocyte diameter (CD), the sections were stained with hematoxylin and eosin. 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 one sample. To assess the amount of cardiac fibrosis, Masson's trichrome staining was performed. The myocardial collagen content was determined by semiquantitative morphometry scoring of the sections, according to the followings: 0, absent; 1, slight; 2, moderate; 3, intense. Twenty randomly selected visual fields of the LV were assessed, and the mean value represents one sample. The evaluations were conducted by an investigator blinded to the experimental design.

Biochemical Analysis

Blood was collected from the inferior caval vein in Lithium-Heparin-Gel Monocuvette tubes and immediately centrifuged, and the plasma was separated. Plasma level of high-sensitivity cardiac troponin T was determined using standard automated clinical assays.

Western Blotting

Heart tissue samples were lysed by RIPA buffer (Melford, Ipswich, UK) to release the proteins of interest. This was followed by Bradford measurement to determine the protein concentrations. Then, a total of 20 μ g/30 μ l protein homogenates were denaturated at 70°C, separated on sodium dodecyl sulfate polyacrylamide electrophoresis gels, and transferred to polyvinylidene fluoride membranes (Millipore, Darmstadt, Germany) in semidry conditions. After transfer, the membranes were washed and blocked for 1 h in 5% of BSA in Tris-buffered saline-Tween 20 at room temperature to reduce the nonspecific bindings of the antibodies. Then the membranes were incubated overnight at 4°C with the following primary antibodies: anti- α -smooth muscle actin antibody (1:1,000 dilution, Abcam, Cambridge, UK), anti- Na^+ /Ca²⁺ exchanger 1 (NCX1) antibody (1:2,000 dilution, Abcam), anti-sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase 2 (SERCA2) antibody (1:1,000 dilution, Abcam), anti-matrix metalloproteinase (MMP)-2 (1:1,000 dilution, Abcam), and anti-MMP-9 (1:1,000 dilution, Abcam). The blots were washed to remove excessive primary antibody binding and incubated with horseradish peroxidase conjugated secondary antibody (1:5,000 dilution, Santa Cruz Biotechnology, Heidelberg, Germany) for 1 h at room temperature. Glyceraldehyde-3-phosphate dehydrogenase housekeeping protein was used as loading control and protein normalization. The immunoreactive protein bands were developed using the enhanced chemiluminescence system (PerkinElmer, Rodgau-Juegesheim, Germany). The intensity of the immunoblot bands was detected with Chemi-smart 5100 (Peglab, Biotechnologie, Erlangen, Germany).

Cardiac mRNA Analysis

Cardiac mRNA analysis was performed as previously described (27). Briefly, LV tissue was homogenized in a lysis buffer (RLT buffer; Qiagen, Hilden, Germany); RNA was isolated from the ventricular samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen), according to the manufacturer's instructions, and quantified by measuring optical density at 260 nm. RNA purity was ensured by obtaining a 260-to-280-nm optical density ratio $\sim$ 2.0. Reverse transcription reaction (1 μ g 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) in triplicates of each sample in a volume of 10 μ l in each well containing cDNA (1 μ l), TaqMan Universal PCR MasterMix (5 μ l), and a TaqMan Gene Expression Assay (0.5 μ l) (Applied Biosystems) for the following genes: myocardial fibrosis markers, such as fibronectin 1 (FN1; assay ID: Rn00569575_m1), collagen type 1 α 1 (Col1a1; assay ID: Rn01463848_m1), collagen type 3 α 1 (Col3a1; assay ID: Rn01437681_m1), transforming growth factor- β 1 (TGF- β 1; assay ID: Rn00572010_m1); and a regulator of mitochondrial function: peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α ; assay ID: Rn00580241_m1). Gene expression data were normalized to ribosomal protein L27 (RPL27; assay ID: Rn00821099_g1), and expression levels were calculated using the CT comparative method ($2^{-\Delta CT}$). All results are expressed as values normalized to a positive calibrator (a pool of cDNA from all samples of the sham 6th wk group).

Statistics

All values are expressed as means $\pm$ SE. The normal distribution of our data was tested using the Shapiro-Wilk test. In the case of

normally distributed datasets, one-way ANOVA, followed by Tukey's post hoc test, was used for detecting differences among groups. Datasets failed to show normal distribution were analyzed by the nonparametric Kruskal-Wallis test, followed by Dunn's post hoc test. Repeated-measures one-way ANOVA, followed by Tukey's post hoc test, was performed for comparing data of the echocardiographic measurements at four time points within a group. A P value of <0.05 was used as a criterion of statistical significance.

RESULTS

Effect of Myocardial Reverse Remodeling on LVH

Due to the chronic pressure overload, in the 6th wk AB group, a robust hypertrophy developed, which was confirmed by significantly increased LV mass, CD, and heart weight-to-tibial length ratio (HW/TL) (Figs. 1 and 2 and

Table 1). In this group, the LVEDV and LVESV were not statistically different from the control group, while the RWT significantly increased, confirming concentric form of hypertrophy (Table 1). In the 12th wk AB group, the hypertrophy showed evidence of chamber dilatation, as observed in the declining trend of RWT and in the significantly increased LVEDV and LVESV compared with the control groups (Fig. 2 and Table 1).

Pressure unloading was able to reverse the preestablished hypertrophy almost to the control level. Correspondingly, the LV mass, the CD, and the HW/TL were significantly decreased compared with the 12th wk and also the 6th wk AB groups and did not differ from the control group (Figs. 1 and 2). Furthermore, the LVEDV and the LVESV showed also a marked decrease compared to the AB group (Table 1).

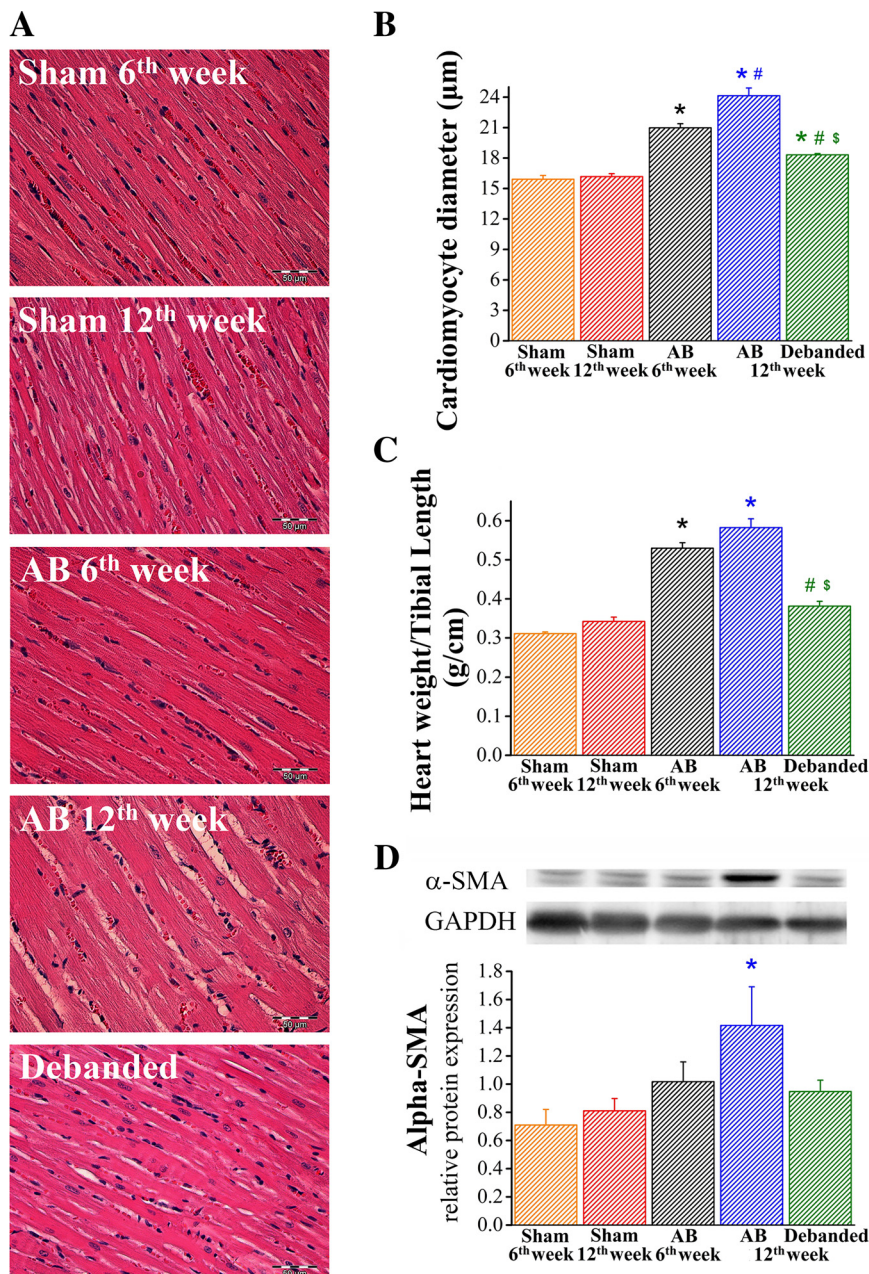


Fig. 1. Effect of pressure unloading on left ventricular hypertrophy. In the 6th wk aortic-banded (AB) group, a massive left ventricular hypertrophy was detectable, which showed further progression for the 12th wk, as reflected by the significantly increased cardiomyocyte diameter (A and B) and heart weight-to-tibial length ratio (C). Pressure unloading resulted in almost complete regression of the preestablished hypertrophy. A: the representative photomicrographs were stained with hematoxylin and eosin. Magnification, $\times 20$; scale bar, 50 μm . D: Western blot analysis of α -smooth muscle actin (α -SMA) showed that the significantly increased protein expression level in the 12th wk AB group was normalized to the control level by pressure unloading. * $P < 0.05$ vs. age-matched sham. # $P < 0.05$ vs. AB 6th wk. \$ $P < 0.05$ vs. AB 12th wk.

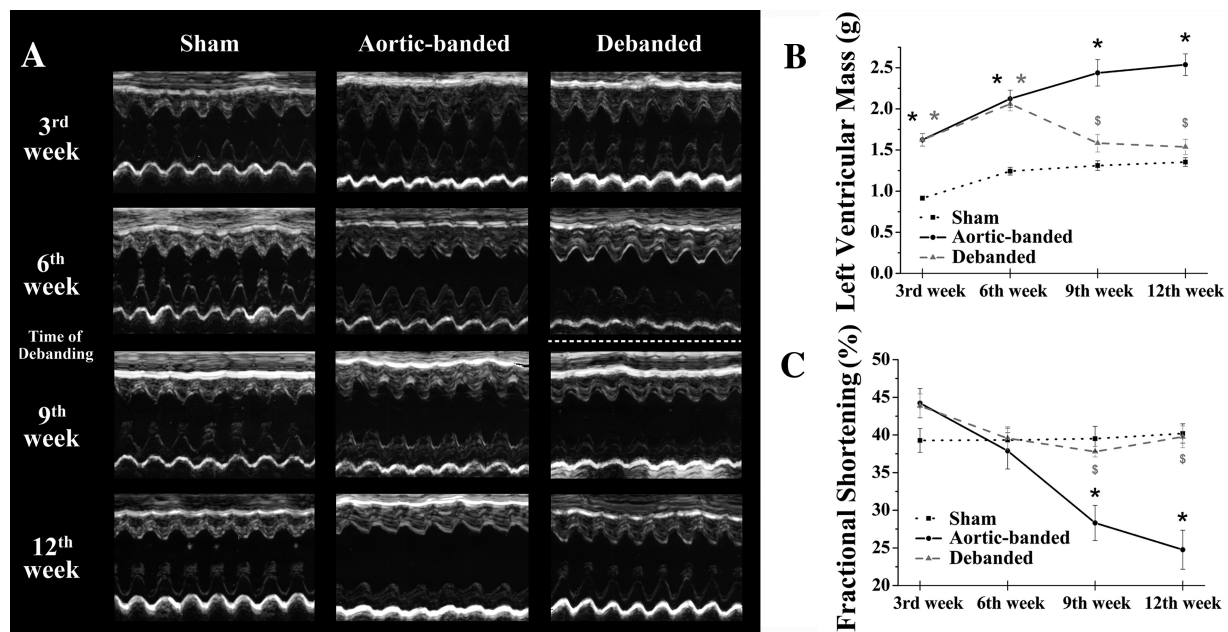


Fig. 2. Pressure unloading protects the heart from functional decompensation. *A*: representative M-mode echocardiographic images at the midpapillary muscle level are shown, presenting the temporal development of left ventricular hypertrophy and its regression by pressure unloading. *B*: left ventricular mass showed significant increase after the 6th wk of pressure overload; however, the debanding procedure resulted in almost complete regression for the 12th wk. *C*: furthermore, pressure unloading was able to provide significant protection against the functional deterioration, as indicated by the preserved fractional shortening values. * $P < 0.05$ vs. sham. \$ $P < 0.05$ vs. aortic-banded.

To detect the pathological nature of the hypertrophy, we also investigated the cardiac expression level of α -smooth muscle actin, which was significantly increased in the 12th wk AB group and decreased in the debanded 12th wk group (Fig. 1).

Pressure Unloading Is Associated with Preserved Cardiac Function and Restored Cardiac Contractility

Basic hemodynamic parameters. In line with the expectations, after the 3rd and 6th experimental week, the systolic cardiac function was preserved in the pressure overloaded groups, according to the echocardiographic measurements (Fig. 2, Table 1). The decompensation of the hypertrophy in the AB group was first detectable at the 9th wk (Fig. 2, Table 1), as represented by significantly decreased EF, FS, SV, SV index, and CO. On the contrary, pressure unloading was able to provide significant protection against the decompensation process and preserved all of these parameters at the control level (Fig. 2, Table 1). Regarding the basic hemodynamic parameters, the results from P-V analysis were in parallel with the echocardiographic findings. Correspondingly, in the 6th wk AB group, no significant differences were detected in EF and SV compared with the control group. However, the 12th wk AB group was associated with significantly decreased EF compared with the control groups and also to the 6th wk AB group (Table 2). Furthermore, the active relaxation constant τ_G showed a strong rising tendency in the 6th wk AB group, while in the 12th wk AB group it reached the level of statistical significance (Table 2). The LVEDP was significantly elevated only in the 12th wk AB group (Table 2). In the debanded 12th wk group, EF, SV, τ_G , and LVEDP showed almost complete regression to the controls level (Table 2).

Pressure unloading. The aortic banding procedure resulted in increased pressure overload as reflected by the elevated

systolic blood pressure, diastolic blood pressure, MAP, E_a , and TPR in the 6th wk and 12th wk AB groups (Table 2). In the debanded 12th wk group, all of these parameters were significantly reduced by removing the narrowing suture after the 6th wk (Table 2).

Load-independent sensitive parameters derived from P-V analysis at different preloads. Representative cardiac indexes derived from P-V analysis during transient occlusion of the inferior caval vein are shown in Fig. 3. In the 6th wk AB group, the slope of the ESPVR was steeper than observed in the controls, suggesting enhanced contractility. Inversely, in the 12th wk AB group, the slope significantly decreased from the 6th wk state, verifying the functional decompensation of the hypertrophy (Table 2). In the debanded 12th wk group, the regression of LVH was accompanied by the reduction of the enhanced contractility. PRSW also showed increased systolic performance in the 6th wk AB group, and its deterioration in the 12th wk AB group was also detectable. However, the changes in this parameter did not reach the level of statistical significance (Table 2). The slope of the EDPVR was significantly increased in the 12th AB group compared with the control group, indicating increased myocardial stiffness (Table 2).

Mechanoenergetics. VAC ratio was significantly increased in the 12th wk AB group, calling attention to impaired LV mechanoenergetics. Furthermore, Eff was significantly reduced in the 12th wk AB group. The effectiveness of LV function was restored by pressure unloading (Fig. 4, A–F).

Effect of Pressure Unloading on the Expression of Mitochondrial Regulator and Ca^{2+} Handling Factors

The mRNA level of PGC-1 α and the protein expression levels of NCX1 and SERCA2 significantly decreased in the

Table 1. Echocardiographic parameters during the 12-wk-long experimental period (temporal development of left ventricular hypertrophy and its regression by pressure unloading)

	Sham	AB	Debanded
<i>n</i>	8	8	10
3rd wk			
HR, beats/min	350 ± 14	362 ± 10	358 ± 13
LV mass, g	0.91 ± 0.03	1.62 ± 0.07*	1.62 ± 0.08*
RWT	0.48 ± 0.01	0.67 ± 0.02*	0.65 ± 0.03*
LVEDV, μ l	283.7 ± 11.0	305.6 ± 6.4	316.2 ± 11.6
LVESV, μ l	91.5 ± 8.4	82.8 ± 6.8	85.6 ± 7.9
SV, μ l	192.2 ± 7.7	222.8 ± 9.6*	230.6 ± 6.4*
SVI, μ l/g	0.54 ± 0.03	0.63 ± 0.04	0.61 ± 0.03
CO, ml/min	67.1 ± 3.1	78.6 ± 3.6	82.6 ± 3.0*
CI, μ l·min ⁻¹ ·g ⁻¹	186.9 ± 7.8	223.8 ± 14.6	220.0 ± 12.9
EF, %	68.0 ± 2.1	72.8 ± 2.4	73.2 ± 1.8
FS, %	39.3 ± 1.6	44.2 ± 1.9	43.9 ± 1.6
6th wk			
HR, beats/min	340 ± 12	366 ± 18	361 ± 12
LV mass, g	1.24 ± 0.05†	2.12 ± 0.1*	2.00 ± 0.11*
RWT	0.50 ± 0.03	0.63 ± 0.02*	0.65 ± 0.01*
LVEDV, μ l	356.8 ± 12.8†	393.2 ± 14.2†	384.0 ± 15.9†
LVESV, μ l	118.5 ± 4.5	138.2 ± 19.4	125.8 ± 13.5†
SV, μ l	238.4 ± 14.8	255.0 ± 9.6	258.2 ± 7.9
SVI, μ l/g	0.51 ± 0.03	0.57 ± 0.03	0.53 ± 0.01
CO, ml/min	81.0 ± 6.1	93.8 ± 7.0	91.2 ± 12.0
CI, μ l·min ⁻¹ ·g ⁻¹	172.2 ± 12.9	207.5 ± 14.2	193.7 ± 10.3
EF, %	66.4 ± 1.9	65.5 ± 3.4	67.9 ± 1.9
FS, %	39.3 ± 1.6	37.9 ± 2.4	39.6 ± 1.6
Time of debanding			
9th wk			
HR, beats/min	339 ± 18	352 ± 12	336 ± 11
LV mass, g	1.31 ± 0.06†	2.44 ± 0.16*†‡	1.58 ± 0.11 ^S
RWT	0.47 ± 0.02	0.63 ± 0.02*	0.55 ± 0.03* ^S
LVEDV, μ l	378.1 ± 10.7†	443.3 ± 26.5*†	367.2 ± 15.5 ^S
LVESV, μ l	118.6 ± 8.5	225.1 ± 28.4*†‡	125.1 ± 6.2 ^S
SV, μ l	259.5 ± 10.2†	218.2 ± 7.4*	242.1 ± 10.7
SVI, μ l/g	0.49 ± 0.02	0.42 ± 0.02*†‡	0.50 ± 0.02 ^S
CO, ml/min	88.9 ± 6.3	76.5 ± 1.7	81.3 ± 4.2
CI, μ l·min ⁻¹ ·g ⁻¹	167.8 ± 9.4	146.7 ± 5.3†‡	166.3 ± 6.9†
EF, %	67.8 ± 2.0	50.5 ± 3.5*†‡	65.9 ± 0.9 ^S
FS, %	39.5 ± 1.7	27.2 ± 2.3*†‡	37.8 ± 0.7 ^S
12th wk			
HR, beats/min	334 ± 13	360 ± 9	373 ± 93
LV mass, g	1.35 ± 0.05†	2.54 ± 0.14*†‡	1.54 ± 0.09‡ ^S
RWT	0.47 ± 0.02	0.57 ± 0.02*	0.5 ± 0.02†‡
LVEDV, μ l	399.0 ± 8.1†‡	466.2 ± 34.8*†	395.7 ± 17.0† ^S
LVESV, μ l	125.2 ± 7.0	256.0 ± 37.1*†‡	127.4 ± 11.2 ^S
SV, μ l	273.9 ± 7.9†‡	210.1 ± 14.5*	268.3 ± 9.2 ^S
SVI, μ l/g	0.48 ± 0.02	0.38 ± 0.03*†‡	0.49 ± 0.02† ^S
CO, μ l/min	91.4 ± 4.7†	75.5 ± 5.4*	99.9 ± 2.8 ^S
CI, μ l·min ⁻¹ ·g ⁻¹	160.5 ± 6.4	136.8 ± 10.5†‡	183.5 ± 6.0 ^S
EF, %	68.7 ± 1.6	46.5 ± 11.4*†‡	68.1 ± 1.8 ^S
FS, %	40.2 ± 1.3	24.8 ± 2.6*†‡	39.7 ± 1.5 ^S

Values are means ± SE; *n*, no. of rats. AB, aortic banded; HR, heart rate; LV, left ventricular; RWT, relative wall thickness; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV, stroke volume; SVI, stroke volume index; CO, cardiac output; CI, cardiac index; EF, ejection fraction; FS, fractional shortening. *P* < 0.05 vs. *sham, ^S AB, †3rd wk, ‡6th wk.

12th AB group compared with the control group. However, pressure unloading therapy efficiently restored the expression level of the mitochondrial regulator and the Ca²⁺ handling factors to the control level (Fig. 4, G–I).

ECM Remodeling Is Decelerated by Pressure Unloading

The extent of myocardial collagen accumulation did not differ in the 6th wk AB group compared with the controls, as

revealed by Masson's trichrome staining (Fig. 5, A and B). However, at this time point, significantly increased mRNA levels of FN1, Col1a1, Col3a1, and TGF- β ₁ were detected (Fig. 5, E–H). In the 12th wk AB group, cardiac fibrosis was significantly increased (Fig. 5, A and B). In contrast, the mRNA levels of FN1, Col1a1, Col3a1, and TGF- β ₁ were substantially decreased compared with the 6th wk AB group (Fig. 5, E–H). Pressure unloading successfully decreased the mRNA levels of FN1, Col1a1, Col3a1, and TGF- β ₁ (Fig. 5, E–H). However, myocardial reverse remodeling only partially inhibited the collagen accumulation in the ECM, since the value of the debanded 12th wk group was significantly lower than that of the 12th wk AB group, but statistically higher than observed in the 6th wk AB group (Fig. 5, A and B). Furthermore, the 12th wk AB group was associated with significantly increased expressions of MMP-2 and the MMP-9 compared with the control groups. However, the expression levels were significantly reduced in the debanded 12th wk group (Fig. 5, C and D).

Pressure Unloading Inhibits Myocardial Ischemia and Necrosis

The plasma level of high sensitive troponin T was significantly increased in the 6th (401 ± 95 vs. 103 ± 10 pg/ml, AB 6th wk vs. sham 6th wk, *P* < 0.05) and in the 12th wk AB groups (459 ± 125 vs. 129 ± 27 pg/ml, AB 12th wk vs. sham 12th wk, *P* < 0.05), indicating myocardial ischemia and necrosis. Pressure unloading provided protection and significantly reduced the plasma level of the cardiac necroenzyme (126 ± 27 vs. 458 ± 125 pg/ml, debanded 12th wk vs. AB 12th wk, *P* < 0.05).

DISCUSSION

The aim of the present study was to characterize the mechanoenergetic alterations in a rat model of LVH undergoing pressure unloading therapy using LV P-V analysis method. According to our best knowledge, this is the first study demonstrating that the regression of LVH by pressure unloading leads not only to reversed myocardial contractility augmentation and restored diastolic function, but also to maintained cardiac mechanoenergetics.

According to Laplace's law, when the pathological stimulus of sustained pressure overload emerges, the myocardial wall thickness increases to normalize wall tension (18). This maladaptive response reaction, also known LVH, is characterized by increased expression of sarcomeric proteins with subsequent enlargement of the cardiomyocytes, ECM remodeling (10), and decreased capillary density (39). The reversibility of these alterations has been already proven by numerous previous works, since they demonstrated that the regression of preestablished pathological hypertrophy, a phenomenon called myocardial reverse remodeling, is achievable after pressure unloading therapy (4, 16, 41). In our study, we also found that cardiomyocyte hypertrophy confirmed by significantly increased CD and HW/TL was restored almost to the healthy control level (Fig. 1) after marked decrease in pressure overload (Table 2). In addition, clinical studies reported that a significant reduction in LV mass and in LVEDD is feasible in only a few weeks following AVR surgery (19). In accordance with this finding, in our rodent model we observed that, 3 wk

Table 2. Detailed characterization of cardiac function by pressure-volume analysis

	Sham 6th wk	Sham 12th wk	AB 6th wk	AB 12th wk	Debanded 12th wk
<i>n</i>	8	8	8	8	8
SBP, mmHg	144 ± 3	141 ± 4	225 ± 7*	209 ± 10*	172 ± 4*#
DBP, mmHg	117 ± 2	117 ± 2	158 ± 4*	159 ± 5*	134 ± 3*#
MAP, mmHg	126 ± 3	125 ± 3	180 ± 4*	176 ± 7*	147 ± 3*#
HR, beats/min	442 ± 14	388 ± 15	432 ± 17	445 ± 14	430 ± 12
E _a , mmHg/μl	1.6 ± 0.1	1.8 ± 0.2	2.5 ± 0.2	3.1 ± 0.3*	2.3 ± 0.3 [§]
TPR, mmHg·ml ⁻¹ ·min	3.4 ± 0.2	4.8 ± 0.4	5.6 ± 0.6	7.2 ± 0.6*	4.8 ± 0.7 [§]
LVEDV, μl	152.8 ± 6.1	151.4 ± 7.0	146.3 ± 10.4	177.6 ± 10.9	157.6 ± 11.2
LVESV, μl	66.3 ± 6.5	79.1 ± 5.5	67.1 ± 9.8	119.9 ± 9.5*#	78.6 ± 8.4 [§]
LVESP, mmHg	139.9 ± 1.7	120.2 ± 6.4	200.1 ± 9.3*	196.6 ± 7.2*	168.6 ± 4.1*#
LVEDP, mmHg	1.9 ± 0.3	2.7 ± 0.4	2.4 ± 0.5	4.5 ± 0.4*#	3.2 ± 0.3 [§]
EF, %	57.6 ± 3.0	48.0 ± 2.5	56.02 ± 4.1	32.8 ± 6.8*#	49.8 ± 3.2 [§]
SV, μl	86.6 ± 3.4	72.3 ± 4.9	79.2 ± 4.1	57.7 ± 4.9	79.0 ± 8.3
τ _G , ms	9.8 ± 0.4	11.5 ± 0.6	14.5 ± 1.5	16.1 ± 1.6*	12.5 ± 0.6
E _{es} , mmHg/μl	1.19 ± 0.09	1.17 ± 0.17	2.04 ± 0.09*	1.57 ± 0.14#	1.39 ± 0.24 [#]
PRSW, mmHg	86.03 ± 4.45	77.22 ± 9.24	104.54 ± 7.22	76.11 ± 7.41	91.16 ± 9.28
Slope of EDPVR, mmHg/μl	0.034 ± 0.008	0.036 ± 0.004	0.068 ± 0.011	0.081 ± 0.028*	0.058 ± 0.015

Values are means ± SE; *n*, no. of rats. AB, aortic banded; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; TPR, total peripheral resistance; LVEDV, left ventricular (LV) end-diastolic volume; LVESV, LV end-systolic volume; LVESP, LV end-systolic pressure; LVEDP, LV end-diastolic pressure; EF, ejection fraction; SV, stroke volume; τ_G, time constant of LV pressure decay; E_{es}, slope of the end-systolic pressure-volume relationship; PRSW, preload recruitable stroke work; EDPVR, end-diastolic pressure-volume relationship. *P* < 0.05 vs. *age-matched sham, #AB 6th wk, \$AB 12th week.

after the debanding procedure, the regression almost reached its full extent, as evidenced by the significantly reduced LV mass and LVEDV parameters (Table 1). The regression was further justified by the reversion of the pathologically altered gene expression profile, the so-called “fetal gene program” (15). The increased expression of the α-smooth muscle actin (a member of this gene family) in pressure-overload induced pathological hypertrophy was previously demonstrated (5) and was further verified in our investigation. However, pressure unloading therapy efficiently reduced its expression, indicating successful regression on the molecular level as well (Fig. 1). Our findings are in correspondence with previous literature data demonstrating restored mRNA expression of skeletal muscle α₁-actin during myocardial reverse remodeling (1, 4).

Despite clinical studies showed that myocardial fibrosis often persists years after AVR (40), it was also demonstrated that, in patients whose intraoperative biopsy samples revealed less interstitial fibrosis, AVR provided better functional improvement and attenuated collagen accumulation in the myocardium (42). In the present study, compensated hypertrophy (6th wk AB) was associated with normal collagen content. However, in line with the literature (17, 37), substantially increased mRNA levels of FN1, Col1a1, Col3a1, and TGF-β₁ were observed, indicating activated profibrotic signaling already in the 6th wk AB group (Fig. 5, E–H). In the 12th wk AB group, these profibrotic transcriptional alterations manifested in enhanced myocardial interstitial fibrosis, as revealed by Masson’s trichrome staining. At this time point, the mRNA levels of FN1, Col1a1, Col3a1, and TGF-β₁ were significantly decreased compared with the 6th wk AB group (Fig. 5, E–H). Pressure unloading at the 6th wk effectively reduced the expression of the profibrotic genes and provided protection against the increased collagen accumulation (Fig. 5). However, in line with the previous findings (16), myocardial reverse remodeling only attenuated the process, and the extent of cardiac fibrosis was significantly higher in the debanded 12th wk group than that observed in control groups (Fig. 5, A and B). In addition, the protein expression levels of MMP-2 and

MMP-9 were elevated in only the 12th wk AB group and remained unchanged in the pressure-unloaded hearts (Fig. 5, C and D). The role of MMPs in the transition from LVH to decompensated heart failure has already been explored by different research groups (9, 35). Therefore, it appears that decreased expression of MMPs in the pressure-unloaded animals may have contributed to the prevention of chamber dilatation.

The correlation between LVH and myocardial ischemia has already been proposed. Indeed, it was demonstrated that the decreased capillary density (39) and the reduced angiogenesis (34), along with the exaggerated cardiomyocyte growth during the development of LVH, could result in an imbalance between the oxygen supply and demand, which could contribute to the progression of heart failure (34). In our experiment, pressure overload-induced LVH was associated with significantly increased cardiac troponin T levels, indicating myocardial ischemia. However, the plasma concentration of the necroenzyme was restored to the control level after pressure unloading.

In contrast to the morphological aspects, the functional consequences of myocardial reverse remodeling are still not entirely explored. Recently, a paradigm shift took place in the consideration of the nature of LVH. According to the original hypothesis, the initial myocardial wall thickening and wall tension normalization are adaptive reactions in response to sustained pressure overload, as they are required to maintain adequate cardiac function and avoid the manifestation of congestive heart failure (3, 18). Nevertheless, this theory has been reconsidered, since it was demonstrated in a genetically modified murine model that normalization of wall stress may not be necessary to preserve cardiac performance (14). Therefore, the manifestation of LVH with increment in cardiac contractility does not entail functional benefits, but, as the Framingham Heart Study pointed it out, it increases the risk for cardiovascular mortality (24). Although pressure-unloading therapy represents the only effective treatment in increased afterload-induced LVH, the consequences of myocardial reverse remodeling on cardiac

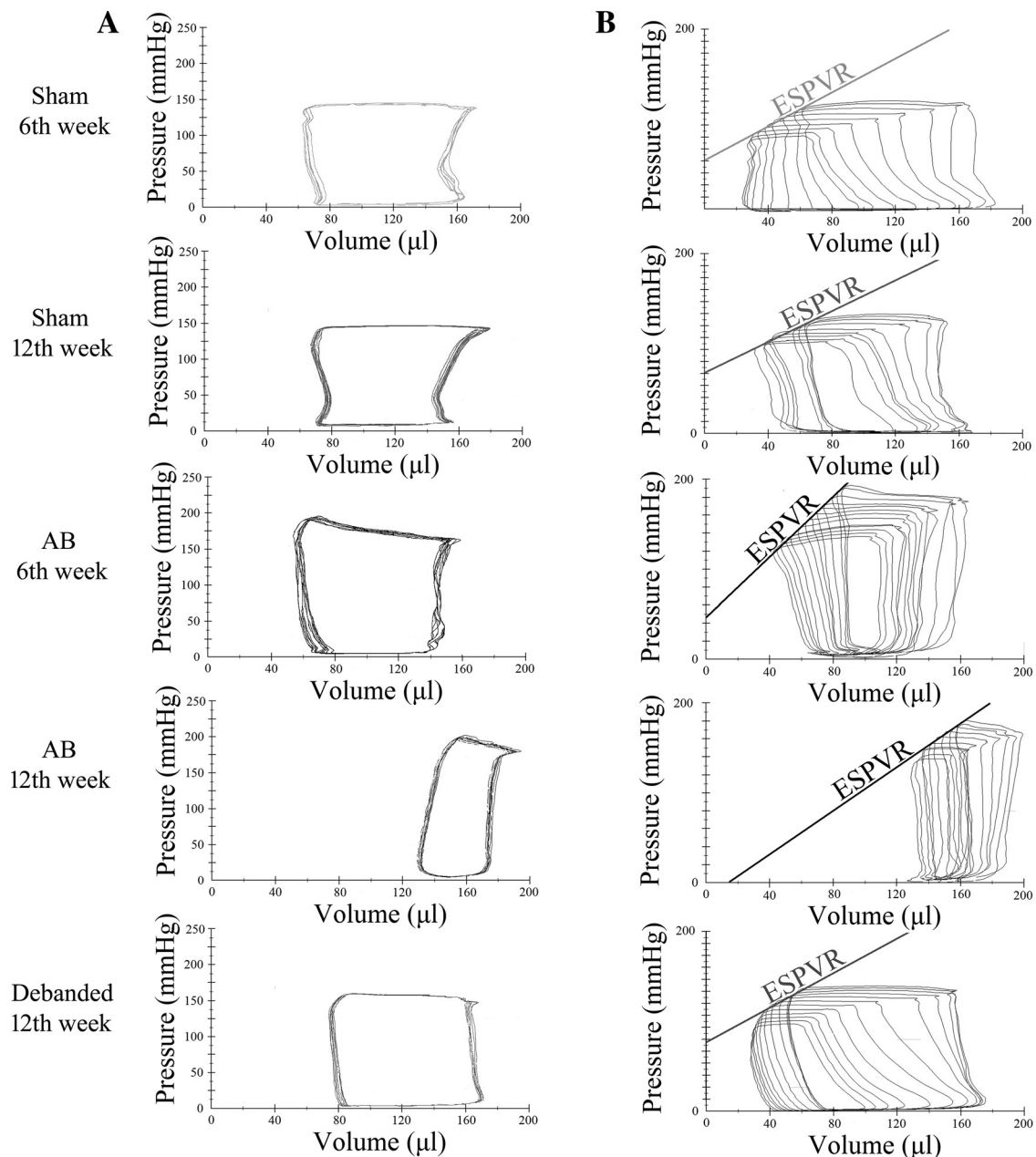


Fig. 3. Left ventricular pressure-volume analysis. Representative left ventricular pressure-volume (P-V) loops indicating in vivo cardiac function are shown. *A*: original recordings of steady-state P-V loops in the 12th aortic-banded (AB) group demonstrate rightward shift of the loops, implying chamber dilatation and decompensation of cardiac function. Pressure unloading restores the P-V loops to the original position and preserves its width, thus maintaining adequate cardiac function. *B*: original recordings were obtained at different preloads during transient vena cava occlusion and show steeper slope of the end-systolic P-V relationship (ESPVR) in the 6th wk AB group, suggesting enhanced contractility. The slope of ESPVR decreases in the 12th wk AB group. Furthermore, in the debanded 12th wk group, the slope also becomes less steep, suggesting that the regression of preestablished hypertrophy is accompanied by the reduction of the enhanced contractility.

mechanoenergetics have not been elucidated. Therefore, to address this issue, we performed in vivo LV P-V analysis.

Systolic Function and Cardiac Contractility

In our study, in the 6th wk AB group, a hemodynamically well-compensated LVH developed with preserved preload-dependent systolic indexes, as confirmed by the echocardiographic (Table 1) and the invasive hemodynamic measurements (Table 2) as well. In line with the literature (22), these hearts were associated with augmented cardiac contractility, as

reflected by the significantly increased ESPVR (E_{es}) and tentatively increased PRSW (Fig. 3 and Table 2). Nevertheless, the observed increase in cardiac contractility proved to be not only a functional compensation, but also the consequence of a pathological reaction (manifestation of pathological LVH in response to sustained pressure overload), since it progressed to ventricular dilatation and functional deterioration after the 9th and the 12th experimental weeks, as indicated by echocardiography and P-V analysis (Figs. 2 and 3 and Tables 1 and 2). Myocardial contractility (E_{es} , PRSW) in particular substan-

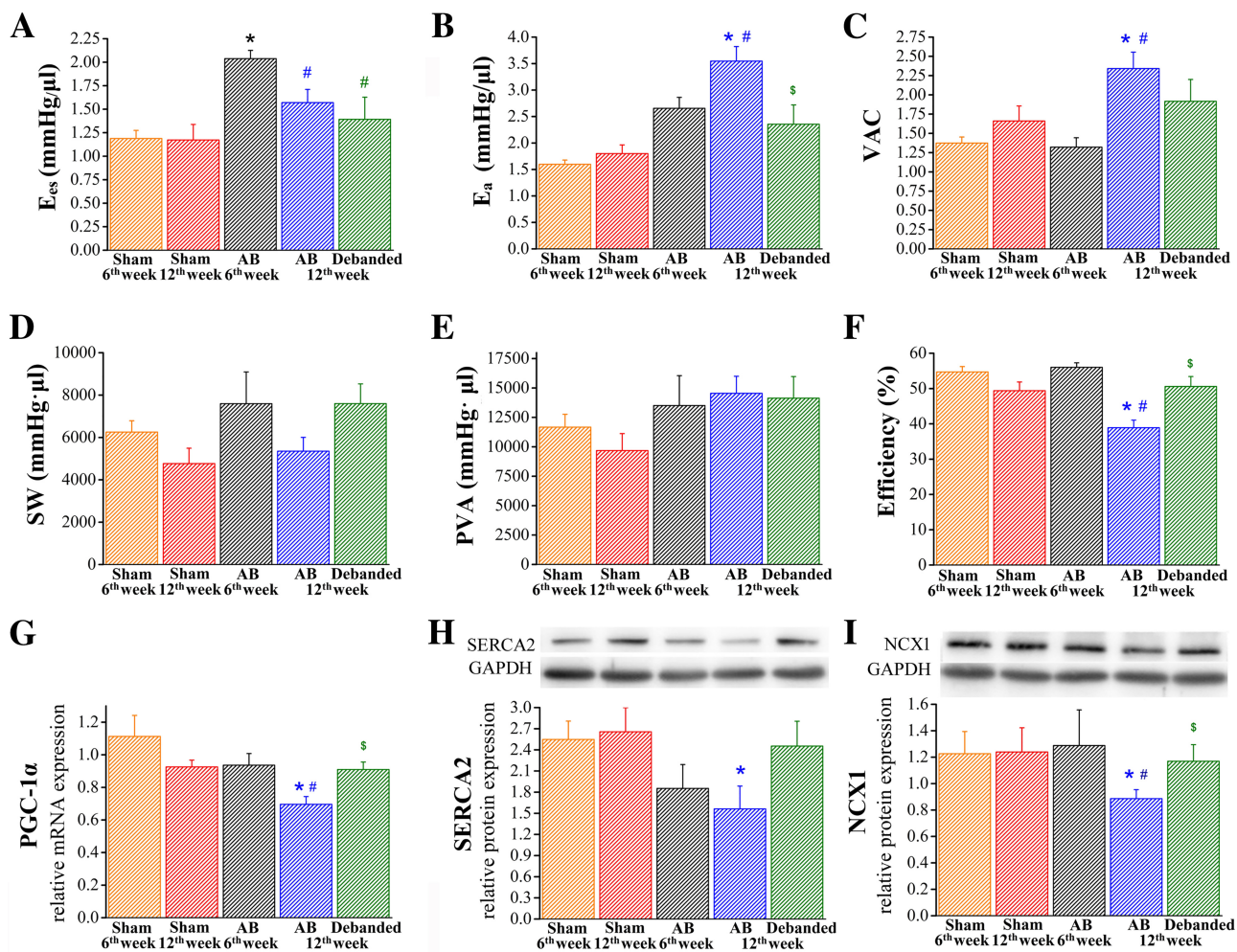


Fig. 4. Effect of myocardial reverse remodeling on cardiac mechanoenergetics. In the 12th wk aortic-banded (AB) group, ventriculoarterial coupling ratio (VAC; C) significantly increased, as a result of the reduced LV contractility (E_{es} ; A) and the increased arterial elastance (E_a ; B). C: myocardial reverse remodeling resulted in reduced VAC, leading to a more appropriate matching between the LV and the arterial system. Furthermore, in the 12th wk AB group, the decreased stroke work (SW; D) and the increased pressure-volume area (PVA; E) led to significant reduction in mechanical efficiency (F). However, pressure unloading therapy successfully restored the SW in the debanded 12th wk group (D), leading to maintained mechanical efficiency (F). G: the mRNA level of peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α) significantly decreased in the 12th wk AB group. On the contrary, myocardial reverse remodeling was associated with restored PGC-1 α expression. Western blot analysis of the Na^+/Ca^{2+} exchanger 1 (NCX1; I) and the sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase 2 (SERCA2; H) revealed that the disturbed Ca^{2+} homeostasis in the 12th wk AB group was restored to the control value by pressure unloading therapy. * $P < 0.05$ vs. age-matched sham. # $P < 0.05$ vs. AB 6th wk. \$ $P < 0.05$ vs. AB 12th wk.

tially decreased in the 12th wk AB group under the same pressure overload, as observed in the compensated hypertrophy group (6th wk AB) (Fig. 3 and Table 2).

On the other hand, repeated echocardiographic measurements demonstrated that pressure-unloading therapy provided protection against the gradual deterioration of LV function and maintained adequate EF and FS after the 9th and the 12th wk as well (Fig. 2 and Table 1). Moreover, the detailed examination of LV function by P-V analysis in the 12th wk debanded group showed that the sensitive load-independent contractility parameters (E_{es} , PRSW) significantly regressed compared with the 6th wk AB group (Fig. 3 and Table 2), which is in accordance with previous findings of Chen et al. (7).

Although the reduction of increased cardiac contractility could be observed both in the 12th wk debanded and 12th wk AB groups, completely different underlying processes could be proven: pressure unloading by debanding was associated with the regression of pathological LVH (Fig. 1), restoration of

normal cardiac contractility, and preserved EF and SV (Table 1), whereas ventricular dilatation and progression toward the development of heart failure could be documented 9 and 12 wk after aortic banding, as reflected by impaired contractility and reduced EF and SV.

Mechanoenergetics

Imbalance between energy production and consumption was suggested in heart failure, which may contribute to the progression of the pathophysiological state (21). In our study, P-V analysis was used to characterize mechanoenergetic changes in LVH and after pressure unloading. We defined VAC ratio (E_a/E_{es}), which is an important determinant of net cardiac performance and energetics (20). In human failing hearts, it was postulated that VAC significantly increases as a result of the reduced LV contractility (E_{es}) and increased E_a (2). This was also reflected by our investigation (Fig. 4, A–C). However,

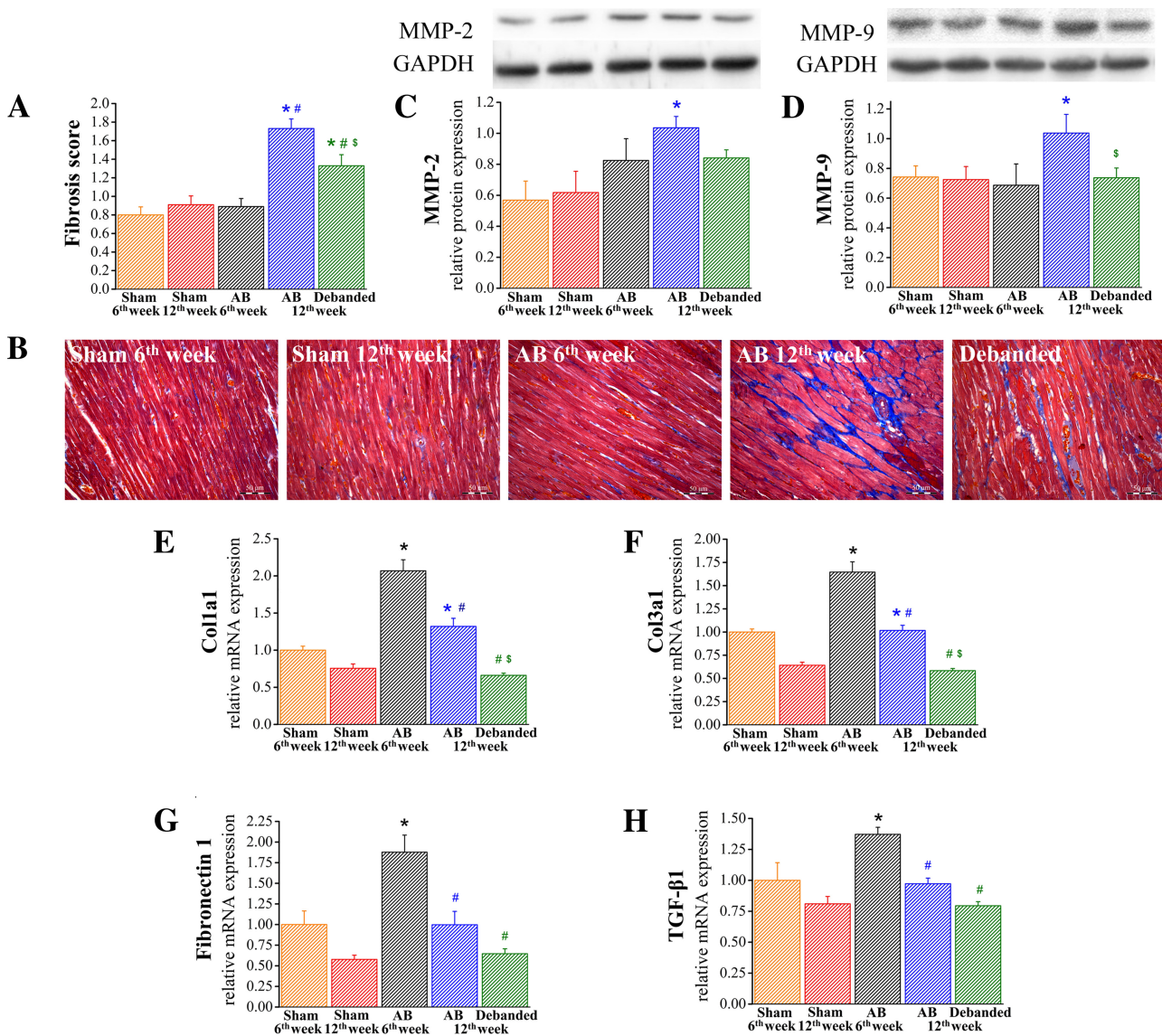


Fig. 5. Alterations of the ECM following pressure unloading. *B*: representative microphotographs of the left ventricular myocardium after Masson's trichrome staining show physiologically low amount of blue collagen staining in the sham 6th wk, sham 12th wk, and in the aortic-banded (AB) 6th wk groups. *A* and *B*: considerable increase in the collagen content is observable in the 12th wk AB group, and it only partially diminishes due to pressure unloading. The expression of matrix metalloproteinase-2 (MMP-2; *C*) and MMP-9 (*D*) were elevated in the 12th wk AB group and remained unchanged in all the other groups. On the gene expression level, increased mRNA levels of fibronectin 1 (FN1; *G*), collagen type 1 α 1 (Colla1; *E*), collagen type 3 α 1 (Col3a1; *F*), and transforming growth factor- β 1 (TGF- β 1; *H*) were observed, indicating activated profibrotic signaling already in the 6th wk AB group. In the 12th wk AB group, the profibrotic transcriptional alterations significantly decreased compared with the 6th wk AB group, but the mRNA level of Colla1 and Col3a1 remained still higher than in the sham 12th wk group. Pressure unloading resulted in complete restoration of the transcriptional alterations. * $P < 0.05$ vs. age-matched sham. # $P < 0.05$ vs. AB 6th wk. \$ $P < 0.05$ vs. AB 12th wk.

while in the mentioned human study decreased E_{es} was the main determinant of the increased VAC ratio, in our experiment the increment in E_a was more dominating. At the same time, myocardial reverse remodeling resulted in reduced VAC, leading to a more appropriate matching between the LV and the arterial system (Fig. 4, *A–C*). Furthermore, gradual deterioration of E_{eff} in hypertensive heart disease (11, 23) and in congestive heart failure was previously reported (2) and was also justified in our investigation. In the 6th wk AB group, the parallel increase in PVA and SW led to adequate E_{eff} . In contrast, in the 12th wk AB group, the functional decompensation of LVH was associated with decreased SW and further increased PVA that manifested in impaired E_{eff} (Fig. 4, *D–F*).

Significantly decreased expressions of SERCA2 and PGC-1 α were detected in the 12th AB group, which might underlie the observed impaired mechanoenergetics. Disturbed Ca^{2+} homeostasis was previously reported to result in impaired cardiac mechanoenergetics, while genetic transfer of SERCA2a led to its restoration. (33). Furthermore, downregulation of PGC-1 α , the master regulator of myocardial energy metabolism, has been demonstrated in pathological hypertrophy and in heart failure, indicating mitochondrial dysfunction and a metabolic shift from fatty acid to glucose utilization (27, 31). In our study, myocardial reverse remodeling restored the expression of SERCA2 and PGC-1 α to the control level and inhibited the deterioration in SW and maintained E_{eff} (Fig. 4). The improve-

ment in Eff implies that a greater portion of the total myocardial oxygen consumption is devoted to cardiac contractions in the pressure unloaded hearts. In addition, the reduction of the cardiac troponin T levels in the debanded 12th wk group may indicate that the oxygen supply was also improved besides the increased efficacy of energy consumption. This finding provides evidence for the first time that the regression of LVH by pressure unloading is accompanied by maintained cardiac mechanoenergetics.

Diastolic Function

In LVH, the impaired active relaxation and the increased myocardial stiffness are both responsible for the diastolic dysfunction (45). In our investigation, the active relaxation constant τ_G showed already a strong rising tendency in the 6th wk AB group and reached the level of statistical significance in the 12th wk AB group (Table 2). During active relaxation, the dissociation of actomyosin cross bridges occurs as a result of the reduction of intracellular Ca^{2+} concentration (6). The diastolic Ca^{2+} elimination is mainly fulfilled by SERCA2 and NCX1 (6). In accordance with the functional measurements, the expression levels of these proteins were significantly reduced due to 12 wk of pressure overload (Fig. 4, *H* and *I*). Pressure unloading efficiently increased the expression of SERCA2 and NCX1, resulting in restored active relaxation (Table 2). One of the main determinants of myocardial stiffness is the collagen accumulation in the ECM (10). This observation was precisely reflected by our investigation, since the index of myocardial stiffness, the slope of EDPVR, along with the Masson's score significantly increased in the 12th wk AB group and regressed by pressure unloading (Table 2).

In conclusion, our findings demonstrate for the first time that myocardial reverse remodeling leads not only to restored diastolic dysfunction, and reversed maladaptive contractility augmentation, but also to maintained cardiac mechanoenergetics. Therefore, pressure unloading at a relatively early time point could result in complete functional recovery besides its favorable effects on cardiomyocyte hypertrophy and myocardial fibrosis.

Limitations

Since M-mode echocardiography derived absolute volume calculations do not exactly represent the true LV volume values, LVEDV, LVESV, SV, and EF derived from the more reliable and more precise P-V analysis method were not identical with the echocardiographic data. However, both techniques showed the same differences among the experimental groups.

ACKNOWLEDGMENTS

The excellent technical assistance of Patricia Kraft, Tobias Mayer, Karin Sonnenberg, Lutz Hoffmann, and Viktória Gregor is greatly acknowledged.

GRANTS

This study was supported by the Land Baden-Württemberg, Germany; by the Medical Faculty of the University of Heidelberg, Germany (to S. Korkmaz-Icöz); by the Hungarian Scientific Research Fund (OTKA 105555 to B. Merkely); and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (to T. Radovits).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.R., S.K.-I., S.L., P.H., B.M., M.K., T.R., and G.S. conception and design of research; M.R., S.K.-I., S.L., B.T.N., P.B., C.M., M.Z., and T.R. performed experiments; M.R., S.K.-I., B.T.N., P.H., C.M., M.Z., T.R., and G.S. analyzed data; M.R., S.K.-I., T.R., and G.S. interpreted results of experiments; M.R., S.L., P.H., P.B., and C.M. prepared figures; M.R., S.K.-I., P.H., P.B., C.M., B.M., M.K., T.R., and G.S. drafted manuscript; M.R., S.K.-I., P.B., B.M., T.R., and G.S. edited and revised manuscript; M.R., S.K.-I., S.L., B.M., M.K., T.R., and G.S. approved final version of manuscript.

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