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Myocardial Mechanics in a Rat Model with Banding and Debanding of the Ascending Aorta

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Background: Aortic banding and debanding models have provided useful information on the development and regression of left ventricular hypertrophy (LVH). In this animal study, we aimed to evaluate left ventricular (LV) deformation related to the development and regression of LVH.

Methods: Minimally invasive ascending aorta banding was performed in rats (10 Sprague Dawley rats, 7 weeks). Ten rats underwent a sham operation. Thirty-five days later, the band was removed. Echocardiographic and histopathologic analysis was assessed at pre-banding, 35 days of banding and 14 days of debanding.

Results: Banding of the ascending aorta created an expected increase in the aortic velocity and gradient, which normalized with the debanding procedure. Pressure overload resulted in a robust hypertrophic response as assessed by gross and microscopic histology, transthoracic echocardiography [heart weight/tibia length (g/m); 21.0 ± 0.8 vs. 33.2 ± 2.0 vs. 26.6 ± 2.8, $p < 0.001$]. The circumferential (CS) and radial strains were not different between the groups. However, there were significant differences in the degree of fibrosis according to the banding status (fibrosis; 0.10 ± 0.20% vs. 5.26 ± 3.12% vs. 4.03 ± 3.93%, $p = 0.003$), and global CS showed a significant correlation with the degree of myocardial fibrosis in this animal model ($r = 0.688$, $p = 0.028$).

Conclusion: In this animal study, simulating a severe LV pressure overload state, a significant increase in the LV mass index did not result in a significant reduction in the LV mechanical parameters. The degree of LV fibrosis, which developed with pressure overload, was significantly related to the magnitude of left ventricular mechanics.

KEY WORDS: Left ventricular hypertrophy · Aortic banding · Debanding.

Introduction

Banding of the ascending aorta in rodents is the most commonly used method for generating a small animal model of aortic stenosis (AS), even though the left ventricular hypertrophy (LVH) produced by acute, severe pressure overload is unlike the slow, progressive pressure overload in patients with AS.[1)] Aortic banding causes LVH and reactive interstitial fibrosis, which have a diffuse distribution within the intersti-

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tium, but fibrosis can also be more specifically perivascular, which is observed in patients with AS. Recently, a reproducible mouse model of reverse remodeling by banding and debanding of the ascending aorta was reported.[1-4] In AS patients, longitudinal strain was decreased but circumferential strain (CS) increased (perhaps as a compensatory mechanism) before aortic valve replacement (AVR). These abnormalities were reversed after AVR.[5-8] Two-dimensional speckle-tacking echocardiography (STE) strain has become the leading method for assessing subclinical left ventricular (LV) systolic abnormalities, because it overcomes the limitations of conventional echocardiography in murine models.[9][10] This study aimed to evaluate the LV mechanics and associated pathology in a banding and debanding model with a two-dimensional STE.

Methods

Animal study protocol

The ascending aorta banding procedure was performed in 20 rats. Among them, 10 rats were euthanized 5 weeks later. The other 10 rats underwent a debanding operation and were then sacrificed 14 days later. Sham operations were performed in additional 10 rats and were sacrificed 7 weeks later.

Seven-week-old male Sprague Dawley rats were anaesthetized with ketamine (80 mg/kg, intraperitoneally) and xylazine (8 mg/kg, intraperitoneally) and then orotracheal intubations were performed with a 20-gauge IV catheter. Mechanical ventilation was maintained with a Mini-Vent ventilator (Harvard Apparatus, Holliston, MA, USA) containing 2% isoflurane and 98% O_2 at a tidal volume of 350 μL and respiratory frequency of 160 min^{-1}.

The ascending aorta banding procedure was performed in 20 rats through upper hemi-sternotomy with careful thymus separation. Then, the ascending aorta was freely dissected. Banding was performed with a Teflon (DuPont Pharmaceuticals, Wilmington, DE, USA) felt supported 5-0 silk ligation around the ascending aorta and a 18-G blunted needle. The Teflon felt that supported aortic banding was helpful for the subsequent debanding operation. The sternum was fixed and the muscle layers and skin were closed with 4-0 silk sutures in three layers. The sham operation was performed as the same thoracotomy procedure without ligation in another 10 rats (Fig. 1). Following surgery, ketoprophen (5 mg/kg, intramuscular, once a day) and gentamycin (5 mg/kg, intramuscular, once a day) were administered over a period of 5 days.

The debanding operation was performed at 5 weeks after aortic banding for 10 rats. The pre- and post-operative procedure was same as the banding operation. Right-sided, muscle-saving thoracotomy for the debanding operation was performed in the second intercostal space. After the banding site was identified, the debanding procedure was performed with cutting the banded silk on the Teflon felt by which aortic rupture was avoided. The thymus was repositioned, and the chest was closed in three layers. For the sham operation group, a similar thoracotomy procedure was performed. The remained 10 rats in the aortic banding model were killed 35 days after the banding operation. Debanding group was sacrificed 14 days after the debanding operation. Sham group was killed 7 weeks after sham operation. The chest was opened, and the hearts were excised.

The study protocol was approved by the Ethics Committee of Daejeon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Daejeon, and Republic of Korea

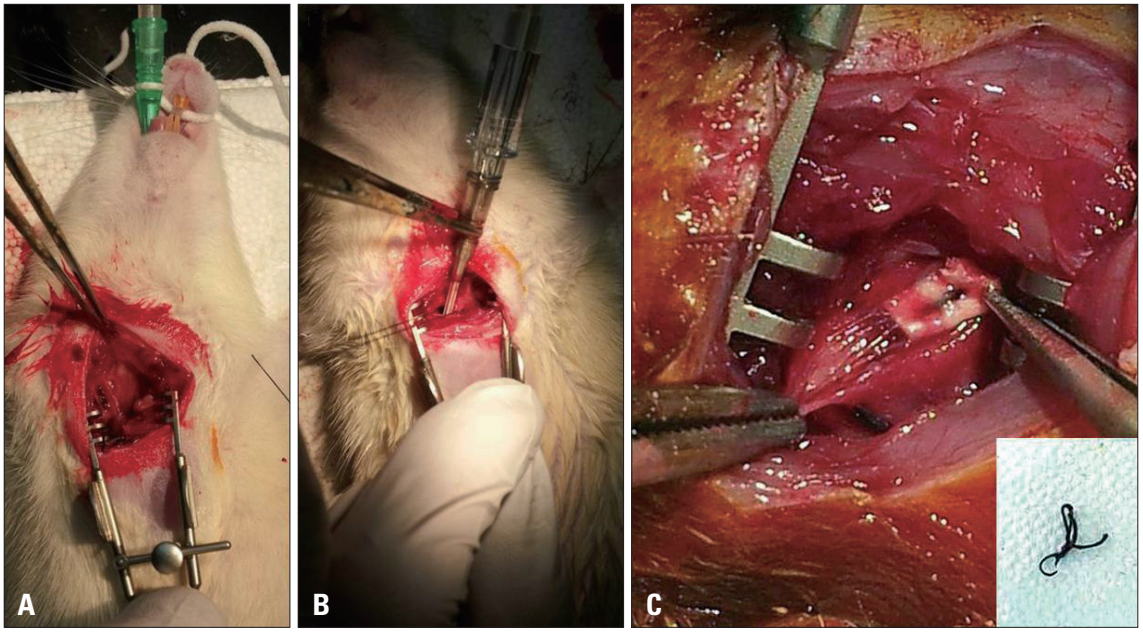

Fig. 1. Photograph of the operation procedure showing the minimally invasive ascending aorta banding procedure via upper hemi-sternotomy (A and B). The Teflon-felt supported banding technique was helpful for the subsequent debanding operation (C).

(DMDDJ-AP-2012-020).

Echocardiography

Echocardiography was performed three times in all subjects at baseline, 5 weeks post banding, and then 2 weeks post debanding. Effective banding procedures were confirmed with echocardiographic findings of a maximal peak velocity of the ligation site more than 3 m/sec for the banding group. Relieved constriction of the ascending aorta after debanding procedure was also documented in echo-Doppler findings.

Animals were anesthetized with ketamine (80 mg/kg, intraperitoneally) and xylazine (8 mg/kg, intraperitoneally) during echocardiographic examinations. To obtain a physiologically relevant depth of sedation during echocardiography, the heart rate was maintained at approximately 270–320 beats per minute. Echocardiography was performed using a 15 MHz linear array transducer (Sequoia Acuson system, Mountain View, CA, USA). An acoustic capture B-mode cine clip (120 Hz) (Sequoia Acuson system, Mountain View, CA, USA) with electrocardiographic gating provided 20–30 frames/beat (≥ 110 frames/s) using the smallest possible depth and sector size.[11] Off-line analysis was performed with velocity vector imaging (VVI) using Syngo SC2000 workplace (Siemens Medical Solutions Inc., Mountain View, CA, USA). M-mode images from the parasternal long axis view were used to measure conventional echocardiographic parameters [LV end-diastolic dimension, LV end-systolic dimension, interventricular septal dimension, LV mass, fractional shortening (FS), and LV ejection fraction (EF)].[12] B-mode clips were acquired in the mid-level region of the parasternal short axis view. The epicardial and endocardial LV borders were manually traced and accurate tracings were verified for more than 3 cycles at end-systole to measure the peak CS and radial strain (RS).[11][13] A single investigator who was blinded to the animal groups performed all image acquisitions and offline measurements.

Histopathological analysis

After euthanasia, the hearts were removed, fixed in 4% formaldehyde and embedded in paraffin. Three mid-ventricular sections were stained with hematoxylin-eosin for histological analysis, Periodic acid-Schiff (PAS) strain for determining for size of cardiomyocyte, and Masson's trichrome for determining fibrosis. The histopathologic evaluation of each slides was performed blinded.

Image J version 1.44 (US National Institutes of Health, Bethesda, MD, USA) was used to quantitatively measure fibrosis by determining the blue-stained area in each section. The cardiomyocyte size was measured in each 40X-magnified section area on PAS stained slides.

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS version 18, SPSS Inc., Chicago, IL, USA). The data are presented as the mean value ± standard deviation.

Comparisons among three groups were made using one-way analysis of variance with post-hoc Tukey tests. The relationships between fibrosis and the peak CS were examined using Pearson's correlation coefficient and linear regression analysis. A p value < 0.05 was considered statistically significant.

Results

Compared with the sham operation group, all rats in the banding group had a significantly higher LV mass index after 5 weeks of the aortic banding procedure. The debanding group had significant dilatation of the LV chamber dimension, lower EF and lower FS compared to the banding group (Table 1).

Table 1. Echocardiographic and histological measurements

Variables	Sham group (n = 10)	Banding group (n = 10)	Debanding group (n = 10)	p-value
Age, weeks	14	12	14	
Weight, g	417.2 ± 2.8	363 ± 8.7	414 ± 1.6	< 0.001
Heart weight/tibia length (g/m)	21.0 ± 0.8	33.2 ± 2.0*	26.6 ± 2.8†	< 0.001
Conventional echocardiography findings				
IVS, mm	1.37 ± 0.13	2.22 ± 0.50*	1.65 ± 0.17†	< 0.001
LVEDD, mm	7.0 ± 0.60	6.6 ± 0.75	7.6 ± 0.58†	0.004
LVESD, mm	4.3 ± 0.48	3.7 ± 0.73	4.8 ± 0.53†	< 0.001
LV mass, g	1.2 ± 0.15	1.48 ± 0.17*	1.34 ± 0.14	0.006
EF, %	73.0 ± 5.9	80.9 ± 7.6	70.1 ± 6.2†	< 0.001
FS	37.6 ± 5.1	44.3 ± 7.5	35.7 ± 4.6†	< 0.001
Histological findings				
IVS, mm	1.28 ± 0.07	2.7 ± 0.43*	2.1 ± 0.34†	< 0.001
LV free-wall thickness	1.37 ± 0.15	3.06 ± 0.47*	2.36 ± 0.26†	< 0.001

*p < 0.05 vs. sham group, †p < 0.05 vs. banding group. IVS: interventricular septal dimension, LVEDD: left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, LV: left ventricular, EF: ejection fraction, FS: fractional shortening

Two-dimensional STE showed no difference in the global peak CS and RS between the sham, banding, and debanding groups (-25.7 ± 6.0 vs. -23.7 ± 5.8 vs. -23.7 ± 5.0, p = 0.661 for CS and 29.8 ± 7.9 vs. 29.0 ± 8.6 vs. 32.6 ± 9.5, p = 281 for RS) (Table 2). In the histological analysis, the size of the myocyte was significantly larger in the banding group than in the sham and debanding groups and was reversed after debanding (Table 2, Fig. 2 and 3). However, myocardial fibrosis was prevalent in the banding and debanding groups compared to the sham group (Table 2, Fig. 3). The 5-weekbanding procedure provoked minimal fibrosis that was lower than approximately 12%. The global peak CS was significantly correlated with the fibrosis severity in the banding group (r = 0.688, p = 0.028) (Fig. 4 and 5). However, there were no correlations between the global peak RS and fibrosis in the banding group (r = -0.618, p = 0.057) (Fig. 4 and 5).

DISCUSSION

In this animal study simulating a severe LV pressure overload state, there was a significant increase in the LV mass index, which was reversible after debanding. Unlike for reversible LVH, myocardial fibrosis did not recover with the debanding procedure 2 weeks later. Quantitative measurement of fibrosis after banding was significantly related to the magnitude of LV mechanics. A mouse model of banding and debanding of the ascending aorta was used to investigate reverse cardiac remodeling. In the pressure overload state, myocytes undergo hypertrophy and proliferated fibroblasts increase extracellular matrix deposition, including collagen accumulation with the activation of bioactive molecules.[14)15)] Fibrosis induced LV pressure overload states included reactive interstitial fibrosis. Cardiomyocyte hypertrophy and reactive fibrosis are associated with increased ventricular stiffness and deformation that could be determined by myocardial mechanics.[8)16)17)] STE, such as CS and RS, have been used to better characterize the regional and global myocardial systolic and diastolic function than EF. In the present study, peak CS was correlated with myocardial fibrosis at banding and was not recovered within the 2 weeks after debanding. Peak RS was not significantly correlated with myocardial fibrosis in present study. Also, Peng et al.[9)] reported that CS efficiently detected the progression of fibrotic changes in mouse model with transverse aortic banding operation. On the other hand, RS showed little concordance with transverse aortic banding induced fibrosis.

Longitudinal strain was decreased in severe AS patients with normal EF. However, CS was increased before AVR and then reversed after AVR according to LV compensation.[5-8)] The longitudinal muscle fiber are located in the subendocardium, which is easily affected by increased intracardiac pressures. In addition, due to a larger radius curvature, longitudinal fibers experience greater stress compared to circumferential fibers. Therefore, longitudinal strain analysis could be important in

Table 2. Myocardial mechanics and histological measurements

Variables	Sham group (n = 10)	Banding group (n = 10)	Debanding group (n = 10)	p-value
Frames/beat	26.4 ± 0.89	27.4 ± 8.0	28.7 ± 2.9	0.414
Heart rate	265.4 ± 28.1	275.0 ± 47.5	275.0 ± 26.7	0.452
Two dimensional STE findings				
Global peak circumferential strain, %	-25.7 ± 6.0	-23.7 ± 5.8	-23.7 ± 5.0	0.661
Global peak radial strain, %	29.8 ± 7.9	25.0 ± 8.6	32.6 ± 9.5	0.281
Histological findings				
Cardiomyocyte size, μm^2	2999 ± 543	8523 ± 2934*	4798 ± 819†	< 0.001
Fibrosis, %	0.10 ± 0.20	5.26 ± 3.12*	4.03 ± 3.93*	0.003

*p < 0.05 vs. sham group, †p < 0.05 vs. banding group. STE: speckle-tracking echocardiography

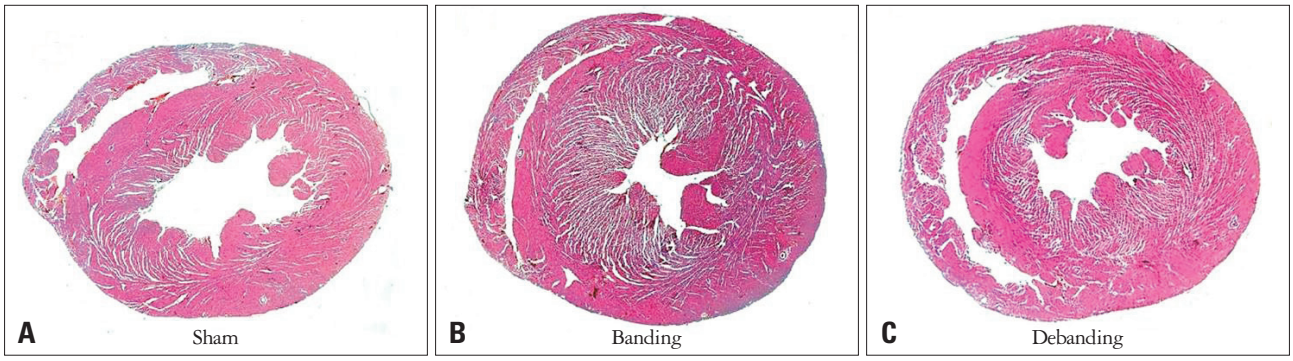

Fig. 2. Gross findings of the left ventricles detached from a sham rat (A), a rat that underwent the 5-week aortic banding procedure (B) and a rat that underwent the aortic debanding procedure (C) showing significant hypertrophy of the left ventricle in the aortic banding heart.

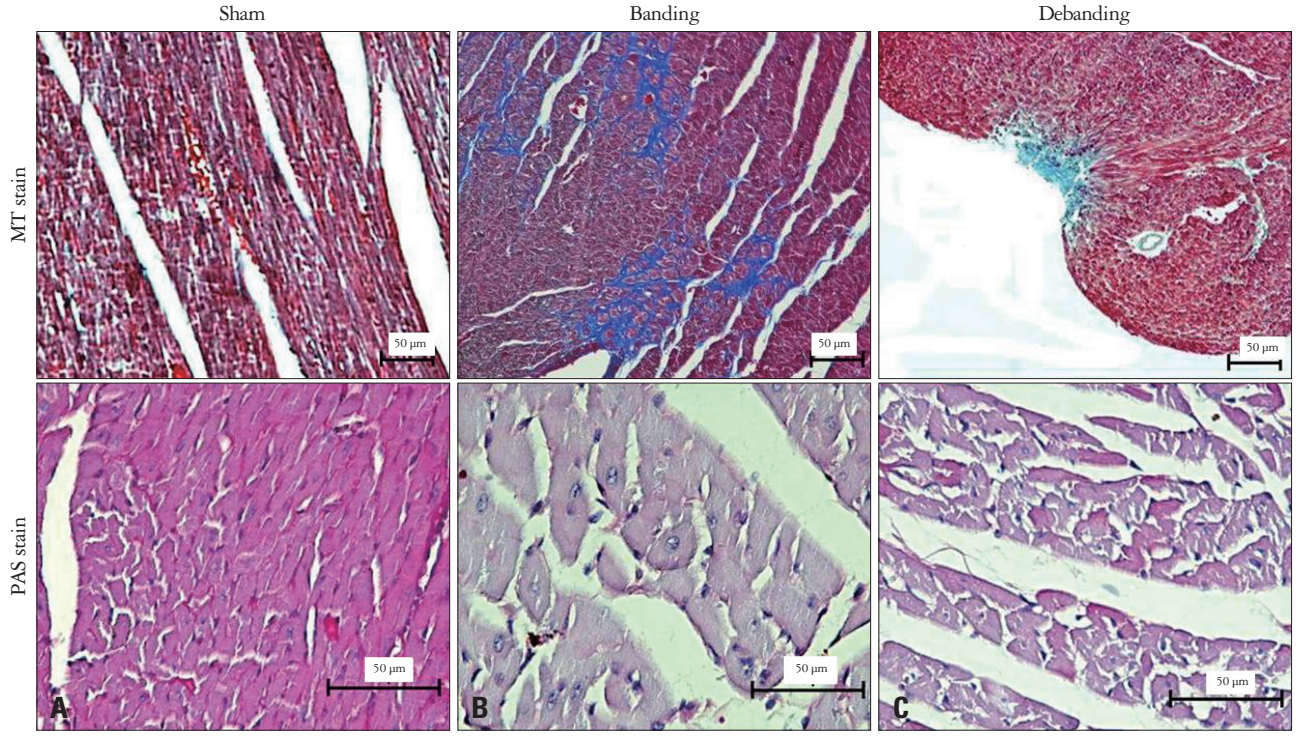

Fig. 3. Microscopic histopathology showing the myocardial staining for the sham group (A), 5-week banding group (B), and debanding group (C). Myocyte hypertrophy (PAS stain, × 400) with reactive fibrosis (MT stain, × 200) was obviously documented in the banding heart (B). However, in the debanding heart, myocardial fibrosis was documented (MT stain) without myocyte hypertrophy (PAS stain) (C). PAS stain: Periodic acid-Schiff stain, MT stain: Masson's trichrome stain.

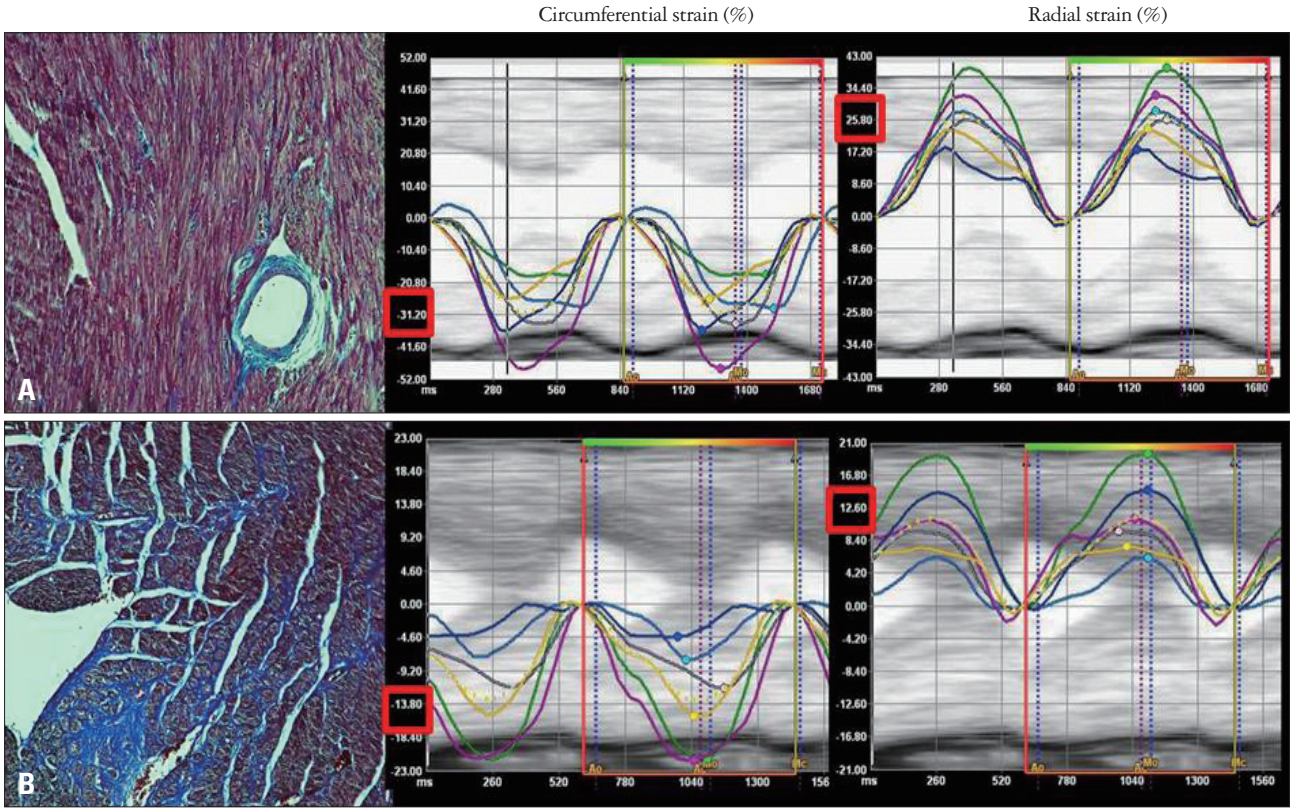

Fig. 4. Velocity vector imaging with electrocardiographic gating for 20–30 frames/beat showed the higher fibrotic myocardium (A) and lower circumferential and radial strain than the lower fibrotic myocardium (B).

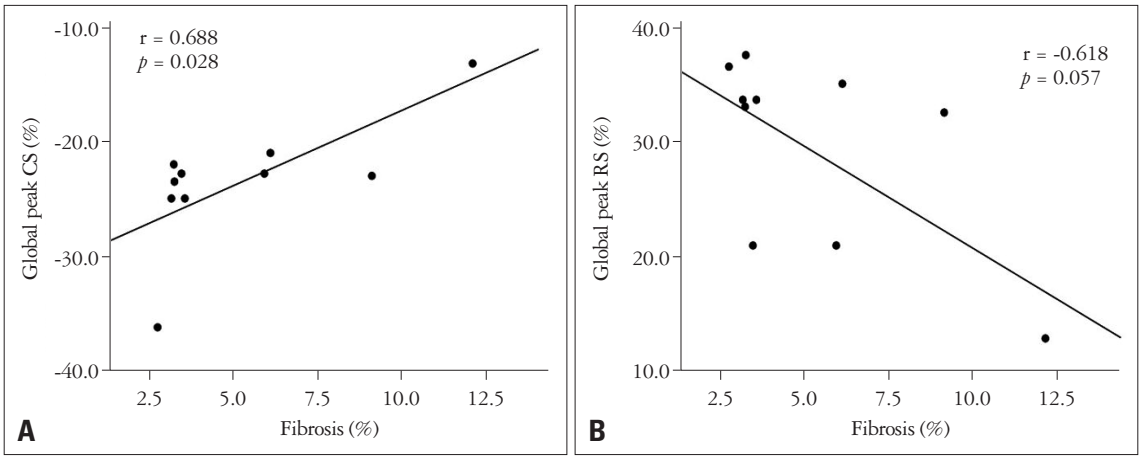

Fig. 5. Correlation of myocardial fibrosis with the peak global CS (A) and RS (B) had a significant negative correlation between myocardial fibrosis and the magnitude of peak global systolic CS (A). However, there was no significant correlation with the peak global systolic RS (B). CS: circumferential strain, RS: radial strain.

the pressure overload status. However, the longitudinal strain could not be analyzed because of the difficulty of window acquisition in murine model.[18] CS was not significantly changed after banding and debanding without a compensation mechanism. An explanation for this discrepant result may be that the severe constriction technique causes acute hemodynamic instability with a reduction in the EF and disturbances in the compensatory increase of CS. Additionally, CS might be a parameter that contributes to the maintenance of LV systolic performance.[18]

The debanding procedure had high mortality rate of more than 10% because of aortic rupture during dissection of periaortic adhesions.[2][3] In the present study, Teflon-felt supported banding of the ascending aorta could reduce the mortality by avoiding aorta injury during cutting of the banded silk. A decreased pressure gradient after the debanding operation was confirmed by non-invasive Doppler echocardiography.

In our study, reactive interstitial fibrosis was observed after the banding operation. Approximately 5% reactive fibrosis was developed in this study, which was a little higher than in the 4 week banding model, which had less than 2–3% fibrosis. Reactive fibrosis in the aortic banding model is a progressive worsening according to the banding duration until 6–12 months.[17] Additionally, interstitial fibrosis precedes irreversible replacement fibrosis and is reversible under agents blocking the renin-angiotensin system.[16][19] In our study, fibrosis was not significantly reversible within 2 weeks after debanding, there was no medication and there was a longer banding duration than previous studies.[2][3][9]

Several limitations of this study should be considered. The sample size was a small and the frame rate relative to the heart cycle duration was lower than in previous studies performed in humans and animals. Higher frame rates were reported using different instrumentation and algorithms.[20] However, a validation study comparing the findings with magnetic resonance imaging showed the feasibility using a VVI and used the same instrument as in the present study.[11] Changes in the imaging angle of incidence can result in capturing different fiber layers at different levels and may introduce variability. Echocardiography was performed in rats that were sedated with a ketamine injection that could decrease heart performance. However, the ketamine dosage was kept to a minimum to induced sedation alone. Additionally, all of the groups were treated with identical methods. The velocity of the banding and debanding site was not estimated with invasive carotid catheterization, but we used echocardiography to determine whether the banding and debanding model was appropriate.

In conclusion, left ventricular fibrosis, developed in a pressure overload condition was irreversible within 2 weeks after debanding and was significantly related to deterioration in LV mechanics. Future investigation is needed to evaluate the cutoff point of the quantitatively assessed degree of fibrosis matched with reversible impairment of LV mechanics in a pressure overloaded animal model.

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