Submission ID #: 60036
Scriptwriter Name: Bridget Colvin
Project Page Link: http://www.jove.com/files_upload.php?src=18311028

Title: Studying Left Ventricular Reverse Remodeling by Aortic Debanding in Rodents

Authors and Affiliations: Patrícia Goncalves-Rodrigues*, Daniela Miranda-Silva*, Adelino F Leite-Moreira, and Inês Falcão-Pires
*These authors contributed equally to the work

Unidade de Investigação Cardiovascular, Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto

Corresponding Author:
Inês Falcão Pires
ipires@med.up.pt

Email addresses for Co-authors:
rodrigues13patricia@gmail.com
daniela.miranda143@gmail.com
amoreira@med.up.pt

Author Questionnaire:

1. Microscopy: Does your protocol require JoVE to film through your microscope? N

2. Does your protocol demonstrate software usage? Y

If yes, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured files to your project page.

We will install the OBS program as you suggest.

3. Which steps from the protocol section below are the most important for viewers to see?

2.2.1., 2.6.2., 3.2.1.-3.2.3., 3.4.1.

4. What is the single most difficult aspect of this procedure and what do you do to ensure success?

The most important step are two steps, the aorta dissection for the banding, 2.6.2, and the debanding procedure, 3.2.1, 3.2.2 and 3.2.3.

5. Will the filming need to take place in multiple locations (greater than walking distance)? N

Section - Introduction

Videographer: Interviewee Headshots are required. Take a headshot for each interviewee.

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length (30 words maximum) and clarity.**

NOTE: Authors reshot all interviews, please use the new footage.

1.1. **Daniela Miranda-Silva**: Aortic debanding is a useful experimental model for studying myocardial reverse remodeling in rodents and for unravelling novel insights into the mechanisms of cardiac hypertrophy regression and diastolic function recovery **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.2. **Patricia Gonçalves-Rodrigues**: This method facilitates the evaluation of cardiac reverse remodeling *in vivo* and the collection of biological samples for *in vitro* and molecular studies at different timepoints during the disease progression **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

1.3. **Daniela Miranda-Silva**: This technique mimics the progression of myocardial remodeling and reverse remodeling and allows exploring the mechanisms of incomplete myocardium recovery **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.4. **Patricia Gonçalves-Rodrigues**: This protocol provides insights into the mechanisms underlying complete and incomplete myocardial reverse remodeling in chronic pressure overload-related pathologies, such as hypertension or aortic stenosis **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics title card: (for human subjects or animal work, does not count toward word length total)

1.5. Procedures involving animal subjects have been approved by the Portuguese Animal Committee (DGAV).

Section - Protocol

2. Aortic Banding Preparation and Post-Operative Care

2.1. Connect the orotracheal tube to the ventilator to initiate the mechanical ventilation **[1]** and adjust the ventilator to a frequency of 160 breaths/minutes and a tidal volume of 10 milliliters/kilogram **[2]**.

2.1.1. Talent connect tube to ventilator. Use footage from New shoot: 2.1.1. 00:00:05 to 00:00:15. *Videographer: Important step*

2.1.2. Talent adjusting ventilator Use footage from New shoot: 2.1.2. 00:00:06 to 00:00:11.

2.2. Before beginning the band procedure, confirm a lack of response to pain reflex in a 6-8-week-old C57BL/J6 (C-fifty-seven black six) mouse **[1-TXT]** and place the mouse at dorsal recumbency on an inclined plane for intubation **[2]**.

2.2.1. WIDE: Talent pinching toe Use footage from New shoot: 2.2.1. **TEXT: Anesthesia: 8% -> 2-3% sevoflurane**

2.3. Shave and apply depilatory cream from the neckline to the mid-chest level of the mouse **[1]** and apply ointment to the animal's eyes **[2]**.

2.3.1. Cream being applied to shaved skin Use footage from New shoot: 2.3.1. 00:00:16 to 00:00:19.

2.3.2. ECU: Ointment being applied Use footage from New shoot: 2.3.2. 00:00:04 to 00:00:10.

2.4. After placing the rectal probe and the oximeter for temperature and blood oxygenation monitoring, place the mouse in the right-lateral decubitus on a heating pad **[1]** and tape the limbs to the magnetic fixator retraction system to maintain the animal in the correct position during the procedure **[2]**.

2.4.1. Talent placing mouse onto heating pad in the correct position *Videographer: More Talent than mouse in shot*

2.4.2. Limb being taped NOTE: 2.4.1 – 2.4.2 combined. Use footage from new shoot: 2.4.1 + 2.4.2. 00:00:26 to sec 00:00:39

2.5. Desinfect the chest with consecutive 70% alcohol and providone-iodine solution scrubs **[1]**. Then, open the skin, muscle and ribs in order to perfom the band to the aorta **[2-TXT]**.

2.5.1. Chest being wiped, with alcohol and povidone-iodine solution containers visible in frame Use footage from new shoot: 2.5.1
2.5.2. Skin incision Use footage from new shoot: New shoot: 2.5.2. 00:00:11 to 00:00:26
2.5.3. Muscle separation Use footage from new shoot: 2.5.3. 00:00:08 to 00:00:41.
2.5.4. Aorta with no suture Use footage from new shoot: 2.5.4
2.5.5. Representative shot of Talent applying band Use footage from new shoot: 2.5.5. 00:00:17 to 00:00:41. NOTE: banding of aorta is at 0:36 – 0:40. **TEXT: See text for banding application details**

2.6. At the end of the procedure, apply povidone-iodine solution to the skin suture site and administer analgesia twice daily for 2-3 days **[1-TXT]**.

2.6.1. Mouse being injected, with stock buprenorphine container label visible in frame Use footage form original shoot (corresponds to minute 02:24 in video article for review sent initially: https://www.jove.com/v/60036/studying-left-ventricular-reverse-remodeling-aortic-debanding?status=a62042k)
TEXT: Analgesia: buprenorphine 0.1 mg/kg s.c.

2.7. Then inject sterile saline intraperitoneally to prevent dehydration in cases of significant bleeding during the surgery **[1]** and place the animal in an incubator with monitoring until full recumbency **[2]**.

2.7.1. Talent placing mouse into incubator Use footage from new shoot: 2.7.1. Use sec 00:00:02 to sec 00:00:06.

3. Aortic Debanding

3.1. Seven weeks after the banding surgery, gently dissect the tissues, adhesions, and fibrosis around the aorta **[1-TXT]** in half of the banded animals until the constriction becomes visible **[2]**. NOTE: Use original footage for steps 3.1 – 3.2 (02:48 to 03:13 from https://www.jove.com/v/60036/studying-left-ventricular-reverse-remodeling-aortic-debanding?status=a62042k)

3.1.1. WIDE: Talent dissecting tissues *Videographer: More Talent than mouse in shot*
TEXT: Remove loose suture in 1/2 BA-operated animals

3.2. Carefully dissect the aorta **[1]** and separate the suture from the vessel **[2]**. Then use scissors to cut the suture **[3]**.

3.2.1. Shot of dissected aorta/being dissected *Videographer: Important/difficult step*
3.2.2. Suture being separated *Videographer: Important/difficult step*
3.2.3. Suture being cut *Videographer: Important/difficult step*

3.3. **Patricia Gonçalves-Rodrigues**: Note that a prolonged occlusion of the ascending aorta during banding or debanding may lead to lung edema and the excessive activation of inflammatory pathways **[1]**. NOTE: If no new statement 3.3 was submitted, use the original footage.

3.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

3.4. Close the chest wall with a simple interrupted or continuous 6-0 polypropylene sutures using the minimum number of stitches possible **[1-TXT]** and close the skin with a 6-0 silk-polypropylene suture in a continuous suture pattern. Perform the post-operative care as demonstrated for banding **[2]**.

3.4.1. Chest wall suture(s) being placed Use footage from new shoot: 3.4.1. 00:00:52 to 00:00:58. *Videographer: Important step* **TEXT: Tighten last chest suture when lungs inflated to avoid pneumothorax**
3.4.2. Skin suture(s) being placed Use footage from new shoot: 3.4.2. 00:00:31 to 00:00:47.

~~3.5. Then perform the post-operative care as demonstrated for banding **[1]**.~~

4. Echocardiography

NOTE: Use original footage from this section but record new VO.

4.1. Every 2 to 3 weeks after the surgery, remove the fur from the neckline to the mid-chest level as demonstrated **[1-TXT]** and place the animal on a heating pad in the supine position **[2]**.

4.1.1. Talent applying cream *Videographer: More Talent than mouse in shot* **TEXT: Anesthesia: 5% -> 2.5% sevoflurane**
4.1.2. Talent placing mouse onto pad *Videographer: More Talent than mouse in shot*

4.2. Place ECG electrodes onto the exposed skin **[1]** and confirm the presence of a good ECG trace and a heart rate between 300-350 beats per minutes **[2]**.

4.2.1. Electrode(s) being placed
4.2.2. LAB MEDIA: 4.2.2. ECG Trace.jpg: Shot of ECG trace and heart rate

4.3. Monitor the temperature **[1]** and apply echo gel to the chest region **[2]**.
4.3.1. Shot of temperature rated out
4.3.2. Gel being applied

4.4. Place the mice in dorsal recumbency slightly turned to the right **[1]** and start the echocardiograph using the appropriate settings **[2]**.

4.4.1. Talent adjusting animal position *Videographer: More Talent than mouse in shot*
4.4.2. Talent adjusting settings

4.5. Next, position an ultrasound probe over the thorax **[1]** and assess the pressure gradient across the aorta **[2-TXT]**.

4.5.1. Probe being placed over thorax (Long axis view)
4.5.2. LAB MEDIA: AO velocities bofore and after constriction.jpg: Shot of pressure

gradient **TEXT: Assess 7 wk after banding + 2 wk debanding**

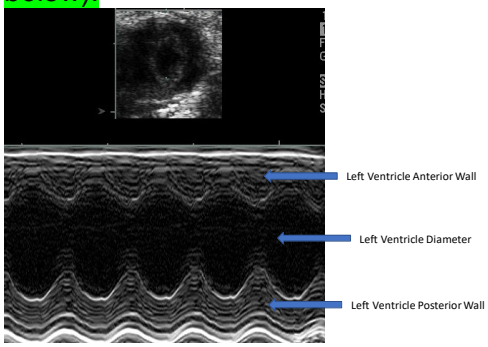
4.6. Record two-dimensional, guided images of the aorta showing the presence or absence of the ascending aorta constriction to anatomically visualize the efficacy of the banding and debanding **[1]**.

4.6.1. LAB MEDIA: Show "4.7.1 Long Axis-aortic dimension" followed by " 4.5.2 Ao velocities before and after aortic constriction". Allow more time for the second image. In legend of 2nd picture, please put "**aortic flow velocities**"

4.7. To assess the hypertrophy, position the probe at a left ventricle short axis at the papillary muscle level **[1]** and press the M-mode tracing button to visualize the left ventricle anterior wall, left ventricle diameter, and left ventricle posterior wall in diastole and systole **[2]**.

4.7.1. Probe being placed in Short Axis View

4.7.2. LAB MEDIA: Show image "MM", label it "**Left Ventricular Dimensions**", and emphasize the labelled parts of the image as they are mentioned in VO (see arrows below).

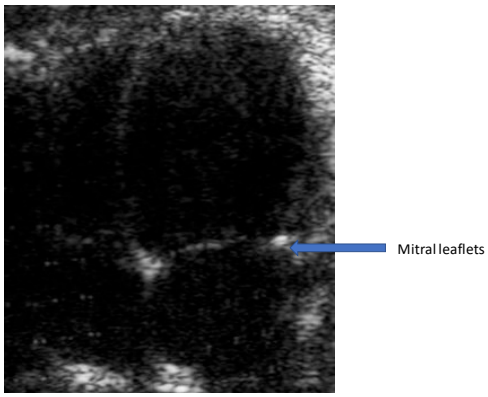


4.8. Then, assess the systolic function and calculate the ejection fraction and fractional shortening according to standard protocols **[1]**.

4.8.1. Representative shot of Talent assessing function/calculating data

4.9. To assess the diastolic function, determine the peak of the pulsed-wave Doppler of the early and late mitral flow velocity using an apical 4-chamber view just above the mitral leaflets **[1]** and record the lateral mitral annular myocardial early diastolic and peak systolic velocities using pulsed-tissue doppler imaging and the apical 4-chamber view **[2-TXT]**.
NOTE from orginal postshoot: The video for this step has the name 4.6. This step must continue as the last one in section 4 ("Echocardiography (ECG)").)

4.9.1. LAB MEDIA: 4-chambers view.jpg.

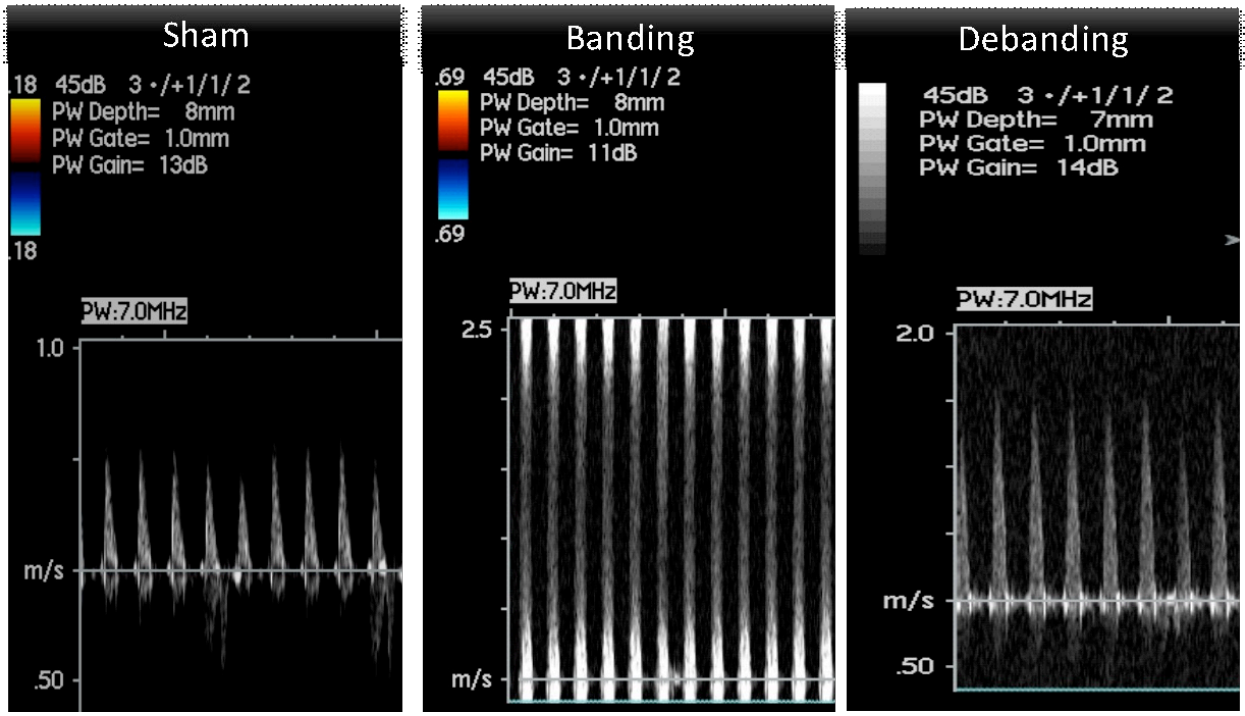


4.9.2. SCREEN: To be provided by Authors: Shot of TDI-pulsed apical 4-chamber view **TEXT: Record and average ≥ 3 consecutive heartbeats for each parameter assessment** NOTE: I'm not entirely sure what should go here, but if I had to guess I'd say 4.10.3. E ans A waves trace.jpg

Section – Results

5. Results: Representative Echocardiographic, Pressure Volume, and Histological Assessments of Cardiac Structure and Function

5.1. The success of aortic constriction can be verified by an increased left ventricle end-systolic pressure and by Doppler aortic flow velocities greater than 2.5 meters/seconds **[1]**. NOTE: When showing Figure 5, leave group names above the pictures ("Sham", "Banding", "Debanding"), see below:



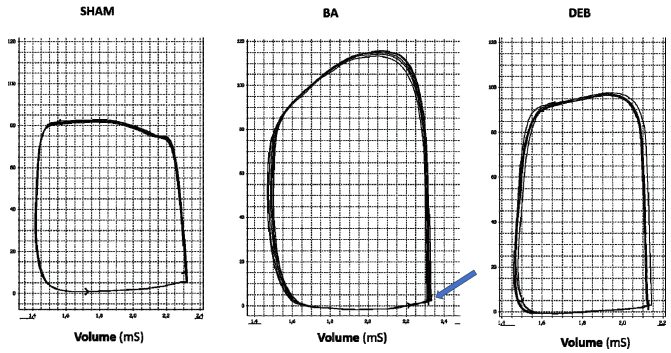
5.1.1. LAB MEDIA: Figure 5A: JoVE Video Editor please emphasizes Banding graph

5.2. Compared to sham-operated animals, banding-induced left ventricle hypertrophy is demonstrated by an increased left ventricle mass **[1]** and an impaired diastolic function, as evident by higher filling pressures **[2]**, left ventricular end-diastolic pressure **[3]**.

5.2.1. LAB MEDIA: Figure 5B: JoVE Video Editor please emphasizes middle image (banding)

5.2.2. LAB MEDIA: Figures 5C, 5D, and 5E: JoVE Video Editor please emphasize middle graphs

5.2.3. LAB MEDIA: Figure 6. Add an arrow pointing out the region with the increase of pressure in diastole, like this:



~~5.2.4. LAB MEDIA: Figure 6: JoVE Video Editor please emphasize middle graph~~

5.3. Histologically, seven-weeks of aortic banding **[1]** induces significant cardiomyocyte hypertrophy and fibrosis **[2]**.

5.3.1. LAB MEDIA: Figure 7: JoVE Video Editor please emphasize left BA image and data bar

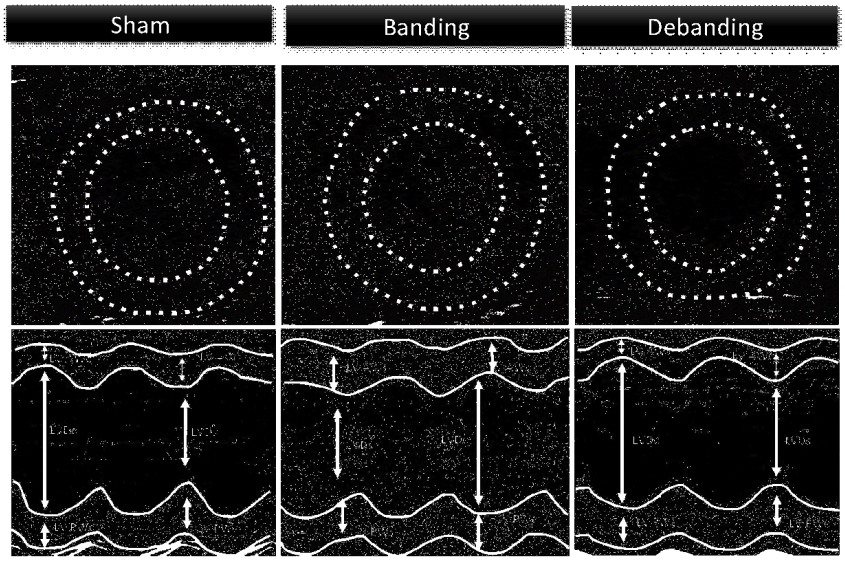
5.3.2. LAB MEDIA: Figure 7: JoVE Video Editor please emphasize right BA image and data bar

5.4. In mice subjected to debanding, a successful removal of the aortic stenosis can be verified by echo-Doppler velocities **[1]**.

5.4.1. LAB MEDIA: Figure 5A: JoVE Video Editor please emphasize Debanding graph

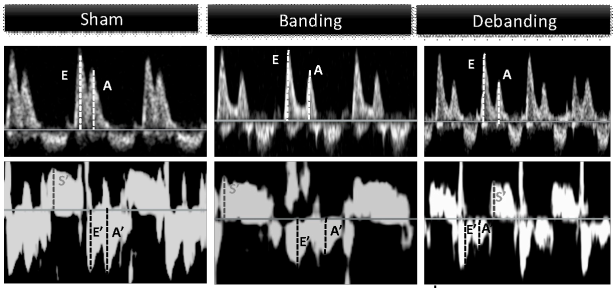
5.5. Overall, debanding promotes a significant decrease in the afterload and the left ventricle hypertrophy assessed by LV mass **[1]**.

5.5.1. LAB MEDIA: Figure 5B: JoVE Video Editor please emphasize right graph



5.6. Moreover, a normalization of the diastolic function **[1]** is also observed **[2]**.

5.6.1. LAB MEDIA: Figures 5C, 5D, and 5E: JoVE Video Editor please emphasizes right graphs



5.6.2. LAB MEDIA: Figure 6: JoVE Video Editor please emphasizes right graph

Section - Conclusion

6. Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

NOTE: Authors reshot all interviews, please use the new footage.

6.1. **Daniela Miranda-Silva**: (Step: 2.6., 3.2.) Manipulate carefully the aorta, avoiding prolonged aortic occlusion in order to guarantee that the blood oxygenation remains above 90% **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

6.2. **Patricia Gonçalves-Rodrigues**: This animal model allows to study myocardial remodeling and reverse remodeling at different timepoints of the disease progression and to evaluate the most dysregulated pathways at each timepoint **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

6.3. **Inês Falcão-Pires**: This procedure allows assessment of the therapeutic potential of promising drugs and to study the impact of several comorbidities in myocardial reverse remodeling and recovery **[1]**.

6.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera