Video Article

# Hemodynamic Characterization of Rodent Models of Pulmonary Arterial Hypertension

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## Abstract

Pulmonary arterial hypertension (PAH) is a rare disease of the pulmonary vasculature characterized by endothelial cell apoptosis, smooth muscle proliferation and obliteration of pulmonary arterioles. This in turn results in right ventricular (RV) failure, with significant morbidity and mortality. Rodent models of PAH, in the mouse and the rat, are important for understanding the pathophysiology underlying this rare disease. Notably, different models of PAH may be associated with different degrees of pulmonary hypertension, RV hypertrophy and RV failure. Therefore, a complete hemodynamic characterization of mice and rats with PAH is critical in determining the effects of drugs or genetic modifications on the disease.

Here we demonstrate standard procedures for assessment of right ventricular function and hemodynamics in both rat and mouse PAH models. Echocardiography is useful in determining RV function in rats, although obtaining standard views of the right ventricle is challenging in the awake mouse. Access for right heart catheterization is obtained by the internal jugular vein in closed-chest mice and rats. Pressures can be measured using polyethylene tubing with a fluid pressure transducer or a miniature micromanometer pressure catheter. Pressure-volume loop analysis can be performed in the open chest. After obtaining hemodynamics, the rodent is euthanized. The heart can be dissected to separate the RV free wall from the left ventricle (LV) and septum, allowing an assessment of RV hypertrophy using the Fulton index (RV/(LV+S)). Then samples can be harvested from the heart, lungs and other tissues as needed.

## Video Link

The video component of this article can be found at https://www.jove.com/video/53335/

## Introduction

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary vasculature associated with inflammatory cell infiltration, smooth muscle proliferation and endothelial cell apoptosis. These changes result in obliteration of pulmonary arterioles, subsequently leading to right ventricular (RV) dysfunction and heart failure. In order to understand the pathophysiology underlying PAH and RV failure in PAH, a number of different models, including genetic and pharmacologic models, for studying this disease have been developed (reviewed elsewhere[1,2]).

Of these models, the most popular are hypoxia-induced (Hx) PAH in the mouse and the monocrotaline (MCT) and SU5416-hypoxia (SuHx) models in the rat. In the mouse Hx model, mice are exposed to 4 weeks of hypoxia (either normobaric or hypobaric, corresponding to an altitude of 18,000 feet with a FiO2 of 0.10), with the resultant development of medial proliferation, increased RV systolic pressures and the development of RV hypertrophy[3]. MCT at a single dose of 60 mg/kg results in injury to pulmonary endothelial cells through an unclear mechanism that then results in the development of PAH[4]. SU5416 is a an inhibitor of the vascular endothelial growth factor receptors (VEGFR) 1 and 2 blocker, and treatment with a single subcutaneous injection of 60 mg/kg followed by exposure to chronic hypoxia for 3 weeks results in permanent pulmonary hypertension with pathologic changes similar to that seen in the human disease, with the formation of obliterative vascular lesions[5]. In the past years, several transgenic mouse models for pulmonary hypertension have been developed. These include knockout and mutations of the bone morphogenetic protein receptor 2 (*BMPR2*), as *BMPR2* gene mutations are found in both familial and idiopathic forms of PAH, heme oxygenase-1 knockout and IL-6 overexpression (reviewed elsewhere[1,2]).

These different rodent models of PH have different levels of pulmonary hypertension, RV hypertrophy and RV failure. While the hypoxia and various transgenic mouse models result in much milder PAH than the either rat model[1], it does allow testing of different genetic mutations and their associated molecular signaling pathways. The MCT model does result in severe PAH, although MCT appears to be toxic to endothelial cells in multiple tissues[4]. The SuHx model is characterized by vascular changes more similar to that seen in idiopathic PAH in humans, although requires both pharmacologic manipulation and hypoxia exposure. Moreover, in all of these models, there may be a disconnection between the histopathologic changes, pulmonary pressures and RV function associated with the development of PAH. This is in contrast to the human disease, where there is usually a proportionate relationship between histopathologic changes, the severity of pulmonary hypertension and the degree of RV failure. Thus, a comprehensive characterization of these rodent models of PH is required, and involves assessments of RV function (typically by echocardiography), hemodynamics (by cardiac catheterization) and histopathology of the heart and lungs (from tissue harvesting).

In this protocol, we describe the basic techniques used for hemodynamic characterization of PAH models in the rat and the mouse. These general techniques can be applied to any study of the right ventricle and pulmonary vasculature and is not limited to models of PAH. Visualizing the RV by echocardiography is relatively straightforward in rats, but is more challenging in mice due to their size and the complex geometry of the RV. Moreover, some surrogates used for quantifying RV function, such as TAPSE, pulmonary artery (PA) acceleration time and PA Doppler waveform notching, are not well validated in humans and correlate only weakly with assessment of pulmonary hypertension and RV function by invasive hemodynamics. Determination of the RV hemodynamics is best done with a closed-chest, to maintain the effects of a negative intrathoracic pressure with inspiration, although open chest catheterization with an impedance catheter allows determination of pressure-volume (PV) loops and a more detailed hemodynamic characterization. As with any procedure, developing experience with the procedures is critical to experimental success.

## Protocol

All procedures described follow the animal care guidelines of Duke University School of Medicine.

# 1. Prior to Starting the Procedure

Note: Prior to any animal procedures, ensure that appropriate institutional permission has been obtained. As with all procedures, use appropriate pain medication to ensure that there is no animal suffering.

1. Flush catheters with heparinized sterile saline (100 U/ml) to ensure the patency. Mark a point from the tip of the catheter equivalent to the length from the heart to the mid of the neck (approximately 4 cm for rats and 2 cm for mice).
2. Anesthetize the mouse or rat. Choices of anesthetic include isoflurane (induction 3-4%, maintenance 1.5% mixed with 100% oxygen), ketamine/xylazine (80-120/10 mg/kg) and pentobarbital (40-80 mg/kg)[6].
    1. For example, with ketamine:xylazine (80-120 mg/kg: 10-16 mg/kg IP for mice and 80-100 mg/kg: 5-10 mg/kg IP for rats), a single dose lasts for 20-50 min of anesthesia. For echocardiography, anesthetize mouse or rat with isoflurane (3-4% for induction and 1.5% for maintenance). Assess anesthetic depth by pinching the rodent in the surgical area to confirm that withdrawal reflexes are absent. Use veterinary ointment on eyes to prevent dryness while under anesthesia.
    Note: Different anesthetic agents can be used to obtain reliable results with proper use and optimization (reviewed elsewhere[6]). Our preference for catheterization is to use ketamine:xylazine. Overdose with ketamine/xylazine may profoundly decrease heart rate and cardiac function, so it is critical to maintain proper temperature and respiratory control. To maintain heart rate (> 400/min) in mice, we routinely perform bilateral vagotomy. The amount of ketamine/xylazine here will typically last 20-30 min, which is sufficient to perform either open- or closed-chest heart catheterization followed by euthanizing the animal.
3. Prepare the rat/mouse for the surgical procedure (**Figure 1**).
    1. Shave the fur from the chest (to allow echocardiography) and from the surgical region, in the right neck.
    2. Scrub the shaved surgical regions with a circular sweep from the center outwards using betadine, followed by cleansing with a 70% alcohol swab.
    3. Place the animal on a surgical platform with a warming pad underneath. Set the heating level to maintain a body temperature of 37-37.5 °C. Monitor body temperature with a rectal probe. Hypothermia can result in significant bradycardia and hyperthermia results in significant tachycardia.

# 2. Echocardiography

Note: A full description of rodent echocardiography is described elsewhere[7]. For the mouse, prior to anesthesia, images can be obtained on the awake, manually restrained animal. For the rat, anesthesia prior to echocardiography is preferred as rats are too large to be manually restrained while awake).

1. Parasternal Long Axis (PLAX) View.
    1. Place the animal in a supine position on the platform or restrain it manually.
    2. Select B Mode to project a 2D live image.
    3. Align the ultrasound transducer with a frequency of 40 MHz for mouse or 25 MHz for Rats to the left parasternal line, and then rotate the transducer counterclockwise 30° with the probe indicator pointing in the caudal direction (5 to 11 o'clock line position). Angle the transducer slightly (rocking along the short axis of the transducer in the same tomographic plane) to obtain a full LV chamber view in the center of the screen.
    4. Locate and view these anatomic structures (**Figure 2A**): the lumen of the left ventricle (LV); Interventricular septum (IVS); the lumen of the right ventricle (RV); Ascending aorta (AO); and Left atrium (LA).
    5. Switch to M Mode, once these above structures are clearly visualized. Place the indicator line through the widest part of LV lumen using AO as the reference point and also make the focus depth lie in the center of LV Chamber (**Figure 2B**). Make similar measurements of the RV by changing angulation of the transducer and obtaining M mode measurements.
    6. Use cine store to create a video loop to record the data for offline measurement (LV chamber dimension, FS and LV wall thickness).
    7. Obtain a doppler tracing of the aortic outflow in PW Doppler Mode by placing the PW cursor in the aorta and recording (**Figure 2C**).
2. Parasternal Short-axis View (PSAX) at the Aortic Level.
    1. Switch to B Mode.
    2. Rotate the transducer 90° clockwise from the parasternal long axis view to obtain the parasternal short-axis view (**Figure 3**). Move and angle the transducer toward the cranium to identify the aortic valve cross section view.

3. Identify the right ventricular outflow tract (RVOT) as a crescent-shaped structure localized to the upper right to the aorta, continued with pulmonary valve leaflets and pulmonary artery.
4. Hold steady at the same position manually. Switch to PW Doppler Mode.
   Note: A station platform for holding the rodent and probe can be used to minimize movement and variation in transducer position.
5. Place the sample volume proximal to the level of pulmonary valve in the center of the right ventricular outflow tract and then position the cursor parallel to the direction of blood flow through the vessel (**Figure 3B**).
   Note: It is important to adjust the sampling angle to the direction of blood flow or use the ultrasound software to correct for a change in angle. Without correction, the maximum angle to the vessel is 30°, which corresponds to ~ 15% underestimate of the velocity.
6. Adjust the scale (the velocity of blood flow) as needed to obtain a "good" Doppler envelope, which has white borders and a dark hollow inside indicating laminar blood flow (**Figure 3C**). Record the Doppler tracing.
   Note: A "bad" Doppler envelope does not accommodate sufficient white borders and a dark hollow.
7. If catheterization is not performed at this juncture, allow the rodent to recover if anesthesia was used. Do not leave the rodent unattended until it has regained sufficient consciousness to maintain sternal recumbency and do not return it to the company of other animals until it is fully recovered. If catheterization is performed, proceed to Section 3.

## 3. Right Heart Catheterization

1. Closed-chest approach for RV pressure measurement
   1. Setup:
      1. Connect the pressure transducer to input channel 1. In the software, set channel 1 for pressure and channel 2 for heart rate.
      2. To convert units to mmHg, record the baseline trace, perform a pressure calibration manually using a pressure gauge (if using a blood pressure transducer and PE tubing). Then perform units conversion under channel 1.
      3. To set heart rate, turn off the input of channel 2. Select cyclic measurements under channel 2 and choose channel 1 for source and rate for measurement.

   2. Place the mouse/rat underneath a dissection microscope with the focus in depth and a magnification of 5x.
   3. Incise the skin from the mandible to the sternum (**Figure 1**). Place a pair of retractors to each side of the incision to fully expose the cervical area.
   4. Bluntly dissect to separate the salivary glands to expose the right external jugular vein using fine blunt tip forceps (**Figure 4A, B**).
   5. Carefully isolate the right external jugular vein from the surrounding connective tissue.
   6. Place two pieces of silk suture (4-0 for rats; 6-0 for mice) underneath the right external jugular vein, ligate the vein distally (as close to the mandible as possible), and then tie a loose knot proximally (**Figure 4C**).
   7. Use iris scissors to make a small "nick" (cut) proximal to the distal tied knot.
   8. Hold the catheter with a forceps and insert the catheter into the cut of the vein, and then tighten the proximal knot.
      Note: We usually use polyethylene (PE)-10 tubing (~2 Fr size) for mice and PE-50 (~3 Fr size) for rats, which is connected to the regular pressure transducer through a 31 G or 21 G needle and calibrated. Mark the catheter with a marker at a length roughly corresponding to placement of the tip in the right ventricle. As an alternative to PE tubing, a micromanometer catheter can be used. Gently pulling the distal knot can help introduce the catheter.
   9. Gently push the catheter into the right heart and monitor the depth of advancement according to the mark. Monitor the pressure trace in the software to verify the catheter location and identify the RV pressure (**Figure 5**).
   10. Keep the catheter immobile and collect the data (toggle data recording next to the Start button) for 2 min.
   11. Proceed to sample collection (Section 4).

2. Open-chest Approach for RV P-V Loop Analysis.
   Note: P-V loop analysis of the right ventricle can't be performed with a closed-chest approach due to the stiffness of the conductance catheter, which does not pass from the SVC to the RA. Commercially available conductance catheters are designed for LV P-V loop analysis.
   1. In the software set Channel 1 for conductance; Channel 2 for pressure; and Channel 3 for heart rate.
   2. Intubate the rats with a 16 G Teflon tube and connect the tube to a mechanical ventilator. Calculate and set the ventilation parameters for mice or rats using the following formulas[6]: tidal volume ($V_t$, ml) = 6.2 x $M^{1.01(}$M = animal mass, kg); respiration rate (RR, $min^{-1}$) = 53.5 x $M^{-0.26}$(**Figure 6A**).
   3. Spread 70% alcohol onto the fur to reduce the spread of fur onto the surgical field.
   4. Make an incision below the xyphoid process and bilaterally dissect the skin with scissors towards the flank.
   5. Cut through the abdominal wall and open the abdominal cavity by bilateral dissection along the diaphragm.
   6. Open the diaphragm to expose the apex of the heart and bilaterally cut the rib cage (**Figure 6A**). Prevent evaporation and tissue drying by spraying saline into the thoracic and peritoneal cavities using a syringe.
      Note: We usually use a dissection scissor to open the abdominal cavity and rib cage. Bleeding is usually not significant, but if there is bleeding, electrocautery can be used.
   7. Carefully isolate the inferior vena cava (IVC) from the surrounding connective tissue.
   8. Place a piece of silk suture (4-0 for rats; 6-0 for mice) around the IVC, and then tie a loose knot (or thread the suture through a 16 G Teflon tube) (**Figure 6B**).
   9. Puncture the apical RV free wall with a 27-30 gauge needle parallel to the RV free wall and remove the needle. Be careful not to push the needle in more than 4 mm.
      Note: Alternatively, a small piece of PE-60 tubing can be used to guide puncture of the conductance catheter into the RV apex.
   10. Insert the conductance catheter tip through the stab wound on the apical RV free wall until all electrodes are inside the ventricle (**Figure 6C**).
   11. Monitor the pressure volume loop in the software and then adjust the position of the catheter to obtain consistently shaped loops that do not demonstrate significant respiratory variation (**Figure 7B, C**).
   12. Record baseline P-V loops (toggle data recording next to the Start button) for at least 10 sec to obtain a number of P-V loops.

13. Pull the suture placed around the IVC to alter the preloads and record the P-V loops. Analyze the data off-line and derive various parameters of RV systolic function (**Figure 7D**). This analysis has been described previously[8].
    Note: IVC can alternatively be occluded by forceps. Monitor the RV pressure trace to confirm the reduction of preload.
14. Perform saline and cuvette calibrations as previously described to allow a conversion from conductance units to volume units[6].
15. After recording the data, gently pull the catheter out and place the tip of the catheter immediately into a water bath with saline. Upon finishing, clean the catheter per manufacturer's instructions.

## 4. Collection of Heart and Lung Samples

Note: As the procedures here are described as terminal, the animal must be euthanized after either closed- or open-chest right heart catheterization.

1. Euthanize the mice by opening the thorax (bilateral thoracotomy) if a closed-chest approach was used, exanguination, or by turning off the ventilator after anesthetic overdose.
   Note: Cervical dislocation is not recommended.
2. To perform inflation perfusion of the lung, connect the inflation tubing onto a ringstand set to inflate the lung with a pressure of 20 $cmH_2O$ (but do not open the valve yet to inflate the lungs).
3. Bluntly dissect the trachea from surrounding muscle and connective tissue.
4. Place a piece of silk suture (4-0 for rats; 6-0 for mice) around the trachea, and then tie a loose knot.
5. Gently stretch the trachea by pressing the head and make a cut (70% of circumference) close to the mandible.
6. Keep the gentle stretch and insert the tracheal cannula (20 G for mice or 16 G for rats). Secure the cannula using the suture. Connect the cannula onto the inflation tubing and tie the suture around the cannula to prevent the backflow of fixatives.
7. Flush the lungs with PBS by using a 10 ml syringe to stab the RV free wall and inject toward the pulmonary artery. Nick the left atrium once the lungs start to blanch.
8. Harvest the heart by cutting at the root of the aorta.
9. Clamp the right lower lobe of lung using a mosquito hemostat and cut the right lower lobe. Place the pieces into microcentrifuge tubes and snap freeze in liquid nitrogen.
10. Inflate the lung with 10% buffered-neutral formalin for 5 min and remove the trachea cannula followed by ligating the trachea.
11. Dissect the lung out of the thorax and fix with 10% buffered-neutral formalin.
    Note: Alternatively, inflate the lung with optimal cutting media (OCT, diluted 1:1 with PBS) and freeze in undiluted OCT for later preparation of frozen sections.
12. Carefully separate the atria from the ventricles and isolate the right ventricle free wall by dissecting alongside the interventricular septum.
13. Weigh the RV and LV + septum (LV+S) to calculate a Fulton index (RV/LV+S)[9], which quantifies the degree of RV hypertrophy.
    Note: TheFulton Index varies in different models of PH. Rat[10]: control, 0.28 ±0.01; hypoxia-induced, 0.57 ±0.02; MCT-treated, 0.51 ±0.03. C57BL6/J mouse[11]: control, 0.26 ±0.01; SuHx (14 days), 0.40 ±0.02; SuHx (21 days), 0.43 ±0.01; SuHx (28 days), 0.44 ±0.03.
14. Snap freeze the RV and LV+S in liquid nitrogen or fix in 10% buffered-neutral formalin.

## Representative Results

As right heart catheterization in rodents is typically a terminal procedure that is not applicable to longitudinal follow-up, echocardiography is an excellent noninvasive alternative for screening and follow-up[12]. While pulmonary artery systolic pressure in human PAH on echocardiography is usually derived from tricuspid regurgitation that is usually straightforward to be obtained in the apical view, such a view is not reliably obtained in rodents, preventing the estimation of pulmonary artery systolic pressure by Doppler. However, a PSAX view at aortic level can be easily visualized in rodents, which enables to record and measure the pulmonary arterial Doppler tracing, the shape of which has been associated with the degree of pulmonary hypertension[12]. Representative results of the echocardiography studies are demonstrated in **Figure 3**. In this protocol, sonographers were blinded to the treatments or procedures that animals received. The results were analyzed off line.

Right heart catheterization and measurement of RVSP, which serves as an accuracy estimation of pulmonary artery systolic pressure in the absence of pulmonary stenosis, is the gold standard for quantification of PAH in rodent models[13,14]. In this protocol, both the closed-chest approach for RV pressure measurement (**Figure 5**) and open-chest approach for RV P-V loop analysis (**Figure 6, 7**) are presented[15,16]. Advantages of the closed-chest approach is less invasive than the open-chest approach and animals are more stable for a longer period[6]. Also, positive pressure ventilation is not required with this approach nor is the thorax opened, preserving the normal right-sided filling pressures associated with breathing and negative intrathoracic pressure. The open-chest approach allows the use of conductance catheters and the determination of PV loops, from which important parameters of RV function can be calculated. Thus, these approaches are complementary as they have different strengths and weaknesses.

In the closed-chest data shown from a mouse Hx model, the RVSP is elevated at 45 mmHg, consistent with significant pulmonary hypertension (**Figure 5**). In the open-chest data shown from a normal rat, the RVSP is significantly lower, at 27 mmHg (**Figure 7**). The relative volume units (RVU) of the X axis can be converted to volume units after cuvette calibration, followed by saline calibration to remove the component of the conductance due to the heart wall[6,8]. This then allows a calculation of important parameters of cardiac function, such as contractility (usually as assessed by the end-systolic elastance, $E_{es}$), diastolic function (from the end-diastolic pressure volume relationship), arterial elastance ($E_a$) and preload-recruitable stroke work, calculations of which are discussed elsewhere[6,8].

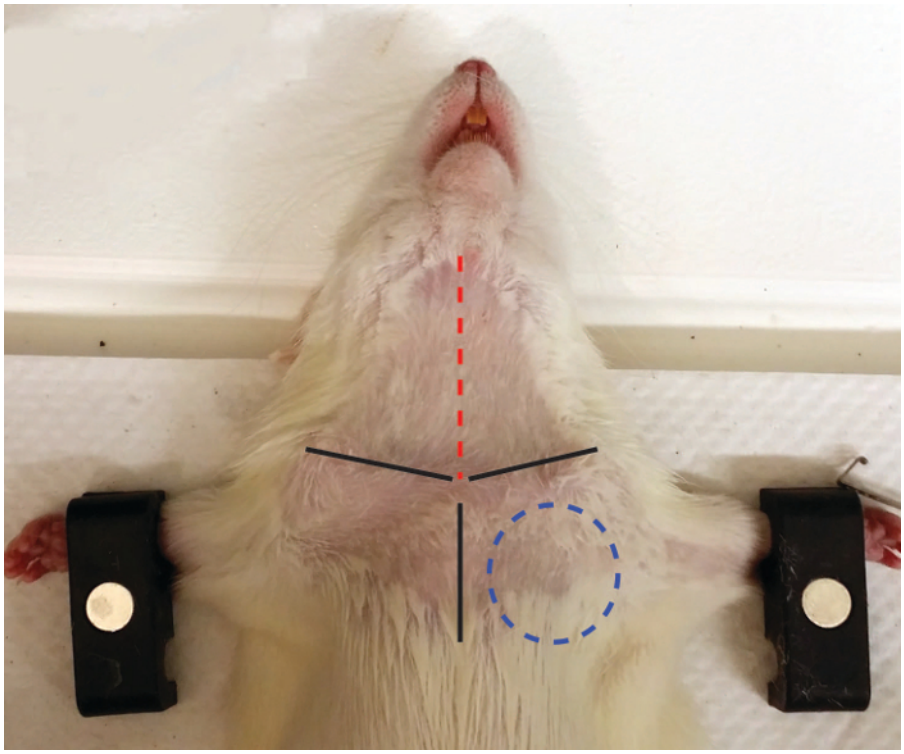

**Figure 1: Preparation of rodent for procedure.** Rats were anesthetized and the chest and neck were shaved. The red dash line denotes the incision that will be used for exposing the external jugular vein. Black lines represent the clavicles and sternum. The blue circle indicates the probe location for echocardiography.

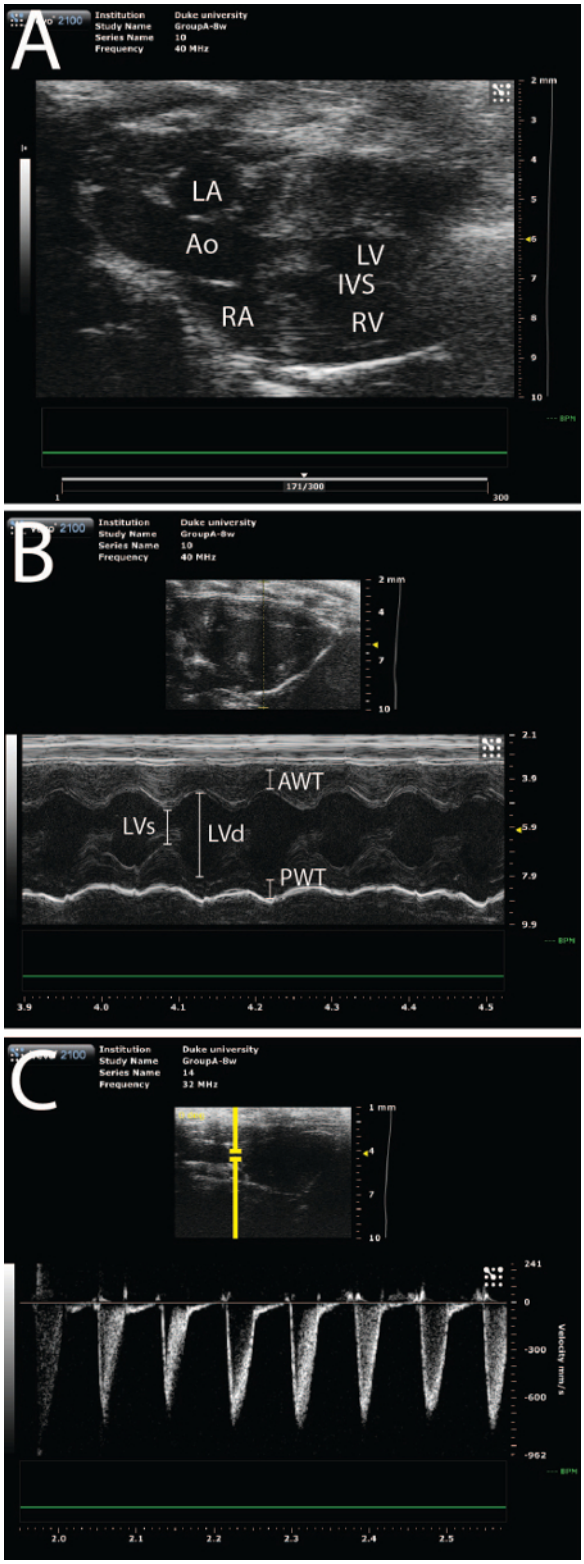


**Figure 2: Echo views of different anatomic structures.** These representative images are from a normal mouse. (**A**) Parasternal long axis (PLAX) view. LA: Left atrium; LV: The lumen of the left ventricle; IVS: Interventricular septum; RV: The lumen of the right ventricle; AO: Ascending aorta (AO). (NOTE: Different imaging orientation on PLAX may result from differing imaging conventions.) (**B**) M-mode of the LV with LV systolic (LVs) and diastolic (LVd) diameters, and anterior (AWT) and posterior wall thickness (PWT) noted. Fractional shortening is calculated as (LVd-LVs)/LVd. (**C**) PW Doppler of the aorta demonstrating an aortic outflow signal. Please click here to view a larger version of this figure.

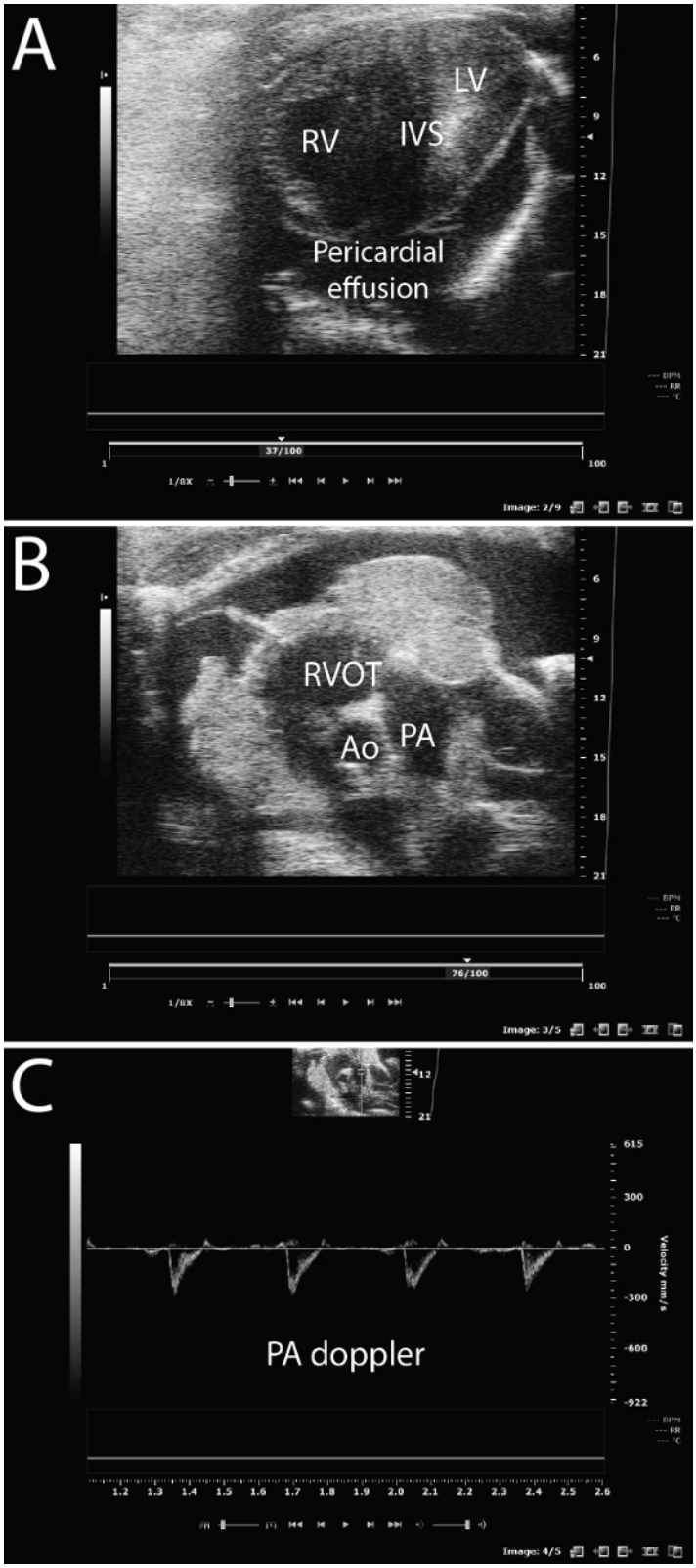


**Figure 3: Parasternal short-axis (PSAX) and RVOT views.** These representative images are from a rat with MCT PAH. (**A**) PSAX view at the mid-pap level of right ventricle. (**B**) PSAX view at the aortic level. RVOT: right ventricular outflow tract. PA: pulmonary artery. Ao: aorta. (**C**) PW Doppler Mode. The sample volume (yellow line) is placed in the center of the right ventricular outflow tract proximal to the level of pulmonary valve. Please click here to view a larger version of this figure.

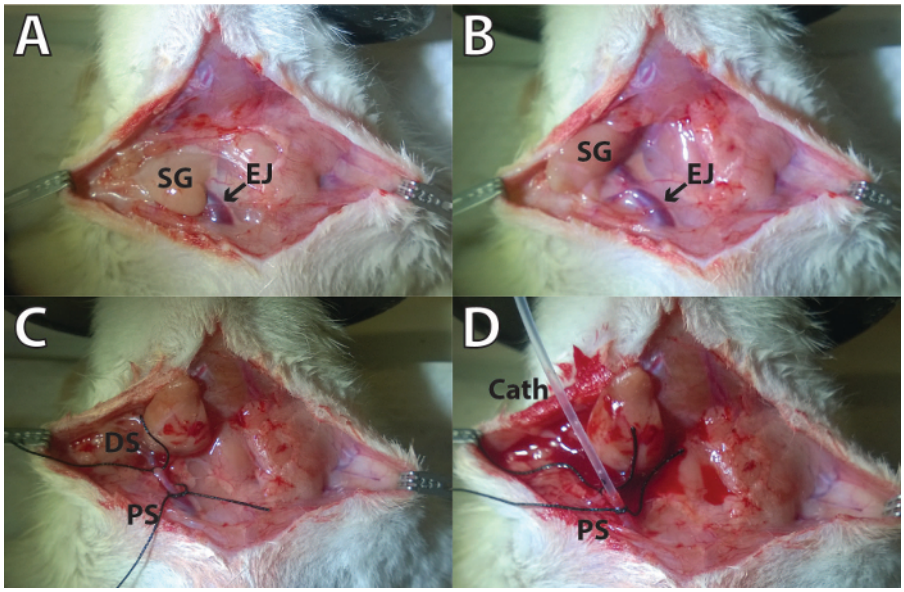


**Figure 4: Exposure of external jugular vein for catheterization of a rat.** (**A**) An incision from the mandible to the sternum was made and a pair of retractors was placed to each side of the incision to expose the cervical area. The salivary is gland (SG) is overlying the external jugular vein (EJ). (**B**) Bluntly dissect to separate the salivary glands and surrounding connective tissue to fully mobilize the right external jugular vein. (**C**) Place distal and proximal 4-0 silk suture around the right external jugular vein. (D) A PE-50 tube used as the pressure catheter was inserted into the right EJ. SG: salivary gland; EJ: external jugular vein; DS: Distal suture; PS: proximal suture; Cath: Catheter.

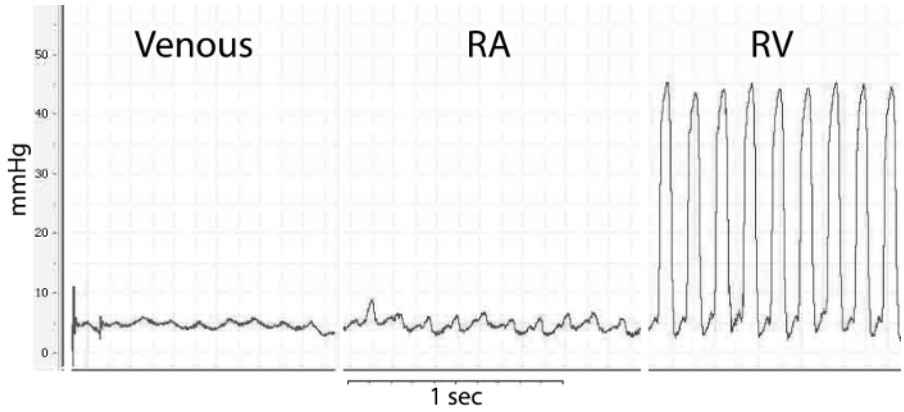


**Figure 5: Waveforms in different chambers during right heart catheterization.** Representative sample traces of pressure changes during right heart catheterization of a mouse with hypoxia-induced PAH. Panel left, middle, and right show pressure changes (mmHg) over time (sec) in superior vena cava (venous), right atrium (RA), right ventricle (RV).

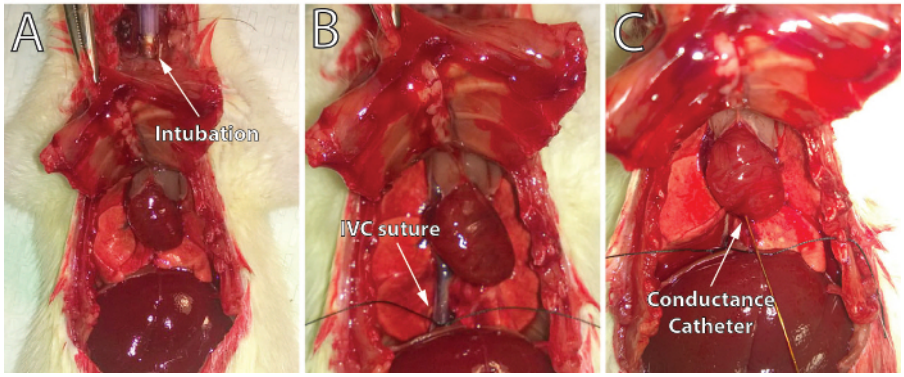


**Figure 6: Open-chest approach for RV catheter placement.** (**A**) View after intubation of the trachea, cutting through the abdominal wall, opening the diaphragm to expose the apex of the heart and bilaterally cut the rib cage. (**B**) Isolation and placement of a piece of suture around the IVC.; and (**C**) After insertion of the conductance catheter through the RV apical free wall.

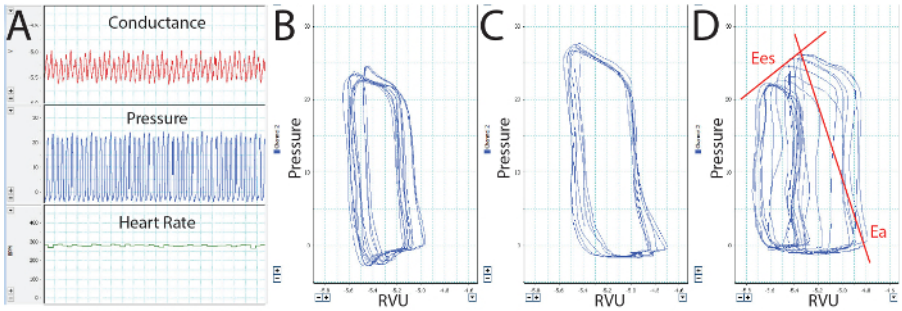


**Figure 7: Right ventricular pressure-volume loop analysis.** (**A**) Channels in the software demonstrating conductance (RVU - relative volume units), RV pressure (mmHg) and heart rate (BPM). Smoothing of 7-11 beats is required for obtaining good signal. (**B**) Placement of the conductance catheter in an area that is prone to changes in respiration results in PV loops that are variable. (**C**) Stable PV loops with proper placement of the conductance catheter. (**D**) Representative family of PV loops after relieving pressure on the inferior vena cava. This family of curves allows a calculation of end-systolic elastance ($E_{es}$ - a measure of cardiac contractility) and vascular elastance ($E_a$ - a measure of pulmonary vascular elastance). Please click here to view a larger version of this figure.

## Discussion

The protocols outlined here describe a comprehensive characterization of hemodynamics and right ventricular function in rodent models of pulmonary hypertension. While right heart catheterization as described here is a terminal procedure, the mortality associated with echocardiography is minimal, which allows for screening and follow-up of disease progression. However, similar to patients with PH having markedly increased mortality with anesthesia[17], in our experience, rats with severe PH do not tolerate anesthesia as well, due to decompensated right heart failure, decreased RV preload, and subsequently hypoxia-induced pulmonary vascular constriction. Therefore, slow induction and closely monitoring respiration are essential.

The video and illustrations demonstrate what our laboratory has found to be successful with regards to these procedures, although alternative approaches can also be utilized (reviewed in Pacher *et al.*[6]). In PH research, these techniques are required in order to properly characterize different models of disease and the effects of genetic or pharmacologic manipulations on them. These methods each have their inherent limitations: for echocardiography, views of the right ventricle are limited and are difficult to quantify; for closed-chest catheterization, only an estimate of PA pressure is provided by an RVSP and an assessment of RV function is limited to dP/dt; for open-chest catheterization, the thorax must be opened, which can have effects on venous return and right heart function.

Experience with these procedures is required to obtain reproducible and consistent results and there are a few critical steps where care is required. Determination of parameters from echocardiography requires standardized views, as small changes in probe angle can result in very different distance and Doppler measurements. During catheterization, introduction of a curve into the catheter can facilitate entry into the right ventricle; with polyethylene tubing, this can be done by heating the tubing gently; for micromanometer catheters, there are specialized catheters designed with a curve for this purpose. Dissection of the RV from the LV+S requires carefully en bloc harvest of the heart and the separation of the ventricles from the atria. As in any experiment, careful attention to detail with all of these procedures is critical for obtaining useful data. It is also important to be aware that animal respiration can affect the signal recording for pressure and PV loops. Identification and quantification of good and uniform pressure traces with consistently shaped loops are important in obtaining reliable data.

This protocol will be of utility in research with animal models of PH and the right ventricle. Such studies include models of RV pressure overload (pulmonary artery banding) and right heart failure. These different experimental approaches allow quantification of pulmonary hypertension and RV function that are complementary. Cardiac catheterization has been used for the assessment of RV pressures, and recently investigators have reported success in traversing the pulmonic valve in the rat with micromanometer catheters designed for the mouse, allowing a direct measurement of PA pressures[18]. Open-chest PV loops allow a quantification of RV function; for example, in the rat MCT model, losartan significantly reduced RV afterload, restored ventricular-arterial coupling and improved RV diastolic function[16]. Noninvasive parameters derived from echocardiography have been used to estimate stroke volume, cardiac output and an estimate of PA pressures[19,20]. In total, these different techniques allow an assessment of the hemodynamic severity of pulmonary vascular disease and the RV response.

## Disclosures

The authors have nothing to disclose.

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## References

1. Gomez-Arroyo, J. *et al.* A brief overview of mouse models of pulmonary arterial hypertension: problems and prospects. *Am J Physiol Lung Cell Mol Physiol.* **302**, L977-991 (2012).

2. Ryan, J. J., Marsboom, G., & Archer, S. L. Rodent models of group 1 pulmonary hypertension. *Handbook of experimental pharmacology.* **218**, 105-149 (2013).
3. Voelkel, N. F., & Tuder, R. M. Hypoxia-induced pulmonary vascular remodeling: a model for what human disease? *J Clin Invest.* **106**, 733-738 (2000).
4. Gomez-Arroyo, J. G. *et al.* The monocrotaline model of pulmonary hypertension in perspective. *Am J Physiol Lung Cell Mol Physiol.* **302**, L363-369 (2012).
5. Abe, K. *et al.* Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. *Circulation.* **121**, 2747-2754 (2010).
6. Pacher, P., Nagayama, T., Mukhopadhyay, P., Batkai, S., & Kass, D. A. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc.* **3**, 1422-1434 (2008).
7. Brittain, E., Penner, N. L., West, J., Hemnes, A. Echocardiographic Assessment of the Right Heart in Mice. *J. Vis. Exp.* (81), e50912 (2013).
8. Abraham, D. M., & Mao, L. Cardiac Pressure-Volume Loop Analyses Using Conductance Catheters in Mice. *J Vis Exp.* **In revision** (2015).
9. Vergadi, E. *et al.* Early macrophage recruitment and alternative activation are critical for the later development of hypoxia-induced pulmonary hypertension. *Circulation.* **123**, 1986-1995 (2011).
10. Mam, V. *et al.* Impaired vasoconstriction and nitric oxide-mediated relaxation in pulmonary arteries of hypoxia- and monocrotaline-induced pulmonary hypertensive rats. *J Pharmacol Exp Ther.* **332**, 455-462 (2010).
11. Wang, Z., Schreier, D. A., Hacker, T. A., & Chesler, N. C. Progressive right ventricular functional and structural changes in a mouse model of pulmonary arterial hypertension. *Physiol Rep.* **1**, e00184 (2013).
12. Thibault, H. B. *et al.* Noninvasive assessment of murine pulmonary arterial pressure: validation and application to models of pulmonary hypertension. *Circ Cardiovasc Imaging.* **3**, 157-163 (2010).
13. Abe, K. *et al.* Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. . *Circ Res.* **94**, 385-393 (2004).
14. Ma, W. *et al.* hypoxia chamer info--Calpain mediates pulmonary vascular remodeling in rodent models of pulmonary hypertension, and its inhibition attenuates pathologic features of disease. *J Clin Invest.* **121**, 4548-4566 (2011).
15. de Man, F. S. *et al.* Bisoprolol delays progression towards right heart failure in experimental pulmonary hypertension. *Circ Heart Fail.* **5**, 97-105 (2012).
16. de Man, F. S. *et al.* Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. *Am J Respir Crit Care Med.* **186**, 780-789 (2012).
17. Pritts, C. D., & Pearl, R. G. Anesthesia for patients with pulmonary hypertension. *Curr Opin Anaesthesiol.* **23**, 411-416 (2010).
18. Paulin, R. *et al.* A miR-208-Mef2 Axis Drives the Decompensation of Right Ventricular Function in Pulmonary Hypertension. *Circ Res.* **116**, 56-69 (2015).
19. Brittain, E., Penner, N. L., West, J., & Hemnes, A. Echocardiographic assessment of the right heart in mice. *J Vis Exp.* (2013).
20. Cheng, H. W. *et al.* Assessment of right ventricular structure and function in mouse model of pulmonary artery constriction by transthoracic echocardiography. *J Vis Exp.*, e51041 (2014).


 