ONLINE SUPPLEMENT

MODELS AND MOLECULAR MECHANISMS OF WHO GROUP 2 – 4 PULMONARY HYPERTENSION

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Materials & Methods

The experimental protocol has been approved by Queen's University Animal Care Committee and the University Research and Ethic Board. All animals are raised in the Queen's Animal care facility.

Supracoronary aortic banding (SAB) surgery:

Male Sprague Dawley rats (Charles River, Montreal, QC) between 175-225g underwent supra-coronary aortic banding surgery. The rat was intubated and anesthetized with isoflurane (1-2%). A 2-cm thoracotomy incision was made in the second left intercostal space. A deeper 1.5 cm incision was made through the intercostal muscles layer between the second and third ribs. The ribs and the thymus were retracted and the ascending portion of the aorta was visualized. The ascending aorta was isolated from the pulmonary artery by blunt dissection, and a small Weck® Hemoclip® clip (Catalog# 523835, Teleflex, Markham, ON) was placed around the ascending aorta with a Hemoclip® applier (Catalog# 523150, Teleflex, Markham, ON). The clip constricted the aorta to ~50% of the original diameter. Following the aortic constriction, the second and third ribs was approximated with 3-0 vicryl suture (polyglactin) in an interrupted pattern. Simultaneously, the ventilator was paused for 2-4 seconds to re-inflate the lungs. The muscle layers were opposed with 3-0 vicryl (polyglactin) sutures and the skin incision closed with 4-0 silk sutures in an interrupted suture pattern. The animal was given time to recover on the heating pad while weaning off anesthesia. After the surgery, the rats were returned to their cages, positioned on their sides and observed until they were awake and in stable condition. Standard post-operative care was provided to minimize pain and risk of wound infection.

Nω-Nitro-L-arginine methyl ester (L-NAME) feeding

After a week of recovery, the SAB rats were fed with drinking water containing the nitric oxide synthase inhibitor L-NAME (1.85mM, Sigma-Aldrich, Canada) or unadulterated tap water (sham control). Echocardiography was performed at 4- and 8-week under isoflurane anesthesia. Catheterization and tissue collection were performed at 8-week post SAB.

Echocardiography:

Estimation of pulmonary arterial pressure (PAP) was done using Doppler ultrasound. Serial 2-dimensional, M-mode and pulsed Doppler ultrasound recordings were performed under light anesthesia, suing isoflurane (1.6–2.0%) mixed with humidified medical air delivered at a stroke volume of 1.0 mL via a cone inhaler. A Vevo® 2100 (FUJIFILM VisualSonics Inc, Toronto, ON) phased-array color-Doppler ultrasound system with a 37.5 MHz transducer and frame rates up to 1000/s was used. After 1-week, ultrasound studies were performed weekly to measure tricuspid annular plane systolic

excursion (TAPSE), right ventricle wall thickness, pulmonary artery acceleration time (PAAT), left ventricle wall thickness, and early filling velocity to atrial filling velocity (E/A) ratio. Mean PAP was estimated from PAAT.

Catheterization:

Rats were anesthetized with isoflurane (1.6 – 2.0%). Right atrial pressure (RAP), right ventricular systolic pressure (RVSP), and right ventricle pressure-volume loop were measured in closed-chest rats with a 1.9-F rat pressure volume catheter (Scisense Inc. London, ON) via the right jugular vein. Systemic blood pressure (SBP), left ventricular end-diastolic pressure (LVEDP), and left ventricle pressure-volume loop were measured via the right common carotid artery using the same catheter. The rat was euthanized by exsanguination while deeply anesthetized. No rat experienced pain or discomfort using these methods.

Tissue Collection:

Tissues were harvested immediately following the hemodynamic measurement. The heart was washed and dissected in phosphate buffered saline (PBS) solution at 0^0C and the right ventricle (RV) was separated from the left ventricle plus septum (LV+S) and weighed. The RV and LV+S were cut into 3-4 small pieces, cryo-fixed in liquid nitrogen and then stored in the -80 °C freezer. Samples of both ventricles were also fixed with formalin for histology. Hematoxylin and eosin (H&E) stain was done to look for cardiomyocyte morphology changes in treated animals vs. control. Picrosirius red stain was done to look for fibrosis. The lung was processed similarly. The left lower lobe was inflated and fixed with formalin and later sent for histology tissue processing.

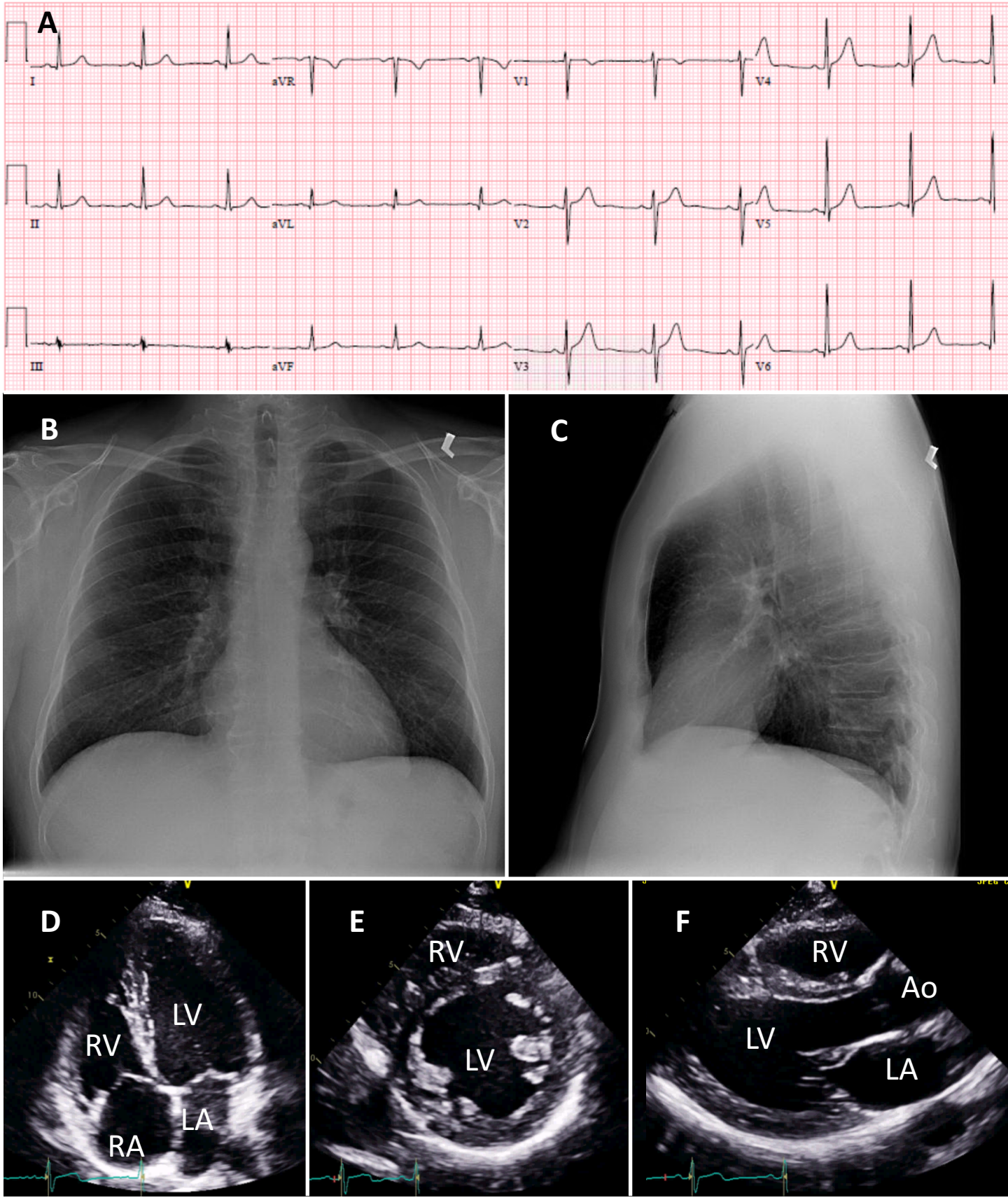

Figure S1. Normal electrocardiogram, chest x-ray, and echocardiogram.

A. ECG showing normal sinus rhythm.
B. Normal chest x-ray (posteroanterior view).
C. Normal chest x-ray (lateral view).
D. Echocardiogram four chamber view showing the right atrium (RA), right ventricle (RV), left ventricle (LV), and left atrium (LA).
E. Echocardiogram parasternal short axis view showing the RV and LV.
F. Echocardiogram parasternal long axis view showing the RV, LV, LA and aorta (Ao).