Method of Transverse Aortic Constriction in Mice with Reduced Variability of the Aortic Peak Pressure Gradient

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Abstract #0026

Transverse aortic constriction (TAC), a common chronic pressure overload model, is a useful tool to study the effectiveness of emerging drug therapy against the progression of cardiac dysfunction. A 27G needle is usually selected to induce TAC; however, variability in the generated pressure gradient and, consequently, disease progression is frequently a problem. This study assessed whether measuring the aorta diameter at the time of surgery and applying 65-70% constriction would decrease model variability and provide a gradual time course in the development of heart failure. C57BL/6NCrl mice (23.7-26.1 g) underwent TAC (n=6-7) or Sham (n=4) surgery. The transverse aorta diameter was determined at the time of surgery using a divider/caliper. For TAC animals, a ligature was tightly tied around the transverse aorta against hypodermic tubing. TAC animals were divided into 2 groups: TAC using 27G tubing (Gr1) or TAC using a tubing diameter selected to induce 65%-70% constriction (Gr2). Echocardiography was performed at 1-, 4-, 6- and 8-weeks post-surgery. Hearts and lungs were harvested at 8-weeks post-surgery and weighed. Aortic constriction was confirmed by a significant increase in the pressure gradient for Gr1 (39 to 119 mmHg; p=0.0005) and Gr2 (51 to 73 mmHg; p=0.0005) vs Sham (2.3 to 3.6 mmHg). Variance in Gr1 pressure gradient was significantly greater than in Gr2 (F=12.9; p=0.007). Morphological changes in both TAC groups were associated with concentric remodeling at Week 1 (LV mass [mg]: 138±37 in Gr1 [p=0.09] and 125±34 [p=0.11] in Gr2 vs 78±15 in Sham; RWT: 0.57±0.04 in Gr1 [p=0.002] and 0.42±0.05 in Gr2 [p=0.04] vs 0.31±0.05 in Sham) that progressed to eccentric hypertrophy by Week 8 (LV mass [mg]: 302±134 in Gr1 [p=0.04] and 252±105 in Gr2 [p=0.07]) vs 95±17 in Sham; RWT: 0.43±0.02 in Gr1 [p=0.42] and 0.43±0.07 in Gr2 [p=0.23] vs 0.36±0.05 in Sham). Systolic dysfunction was also observed at 8-weeks post-TAC as indicated by decreased LVEF (%; 20±10 in Gr1 [p=0.02] and 26±11 [p=0.03] in Gr2 vs 45±7 in Sham). These results showed that when the degree of constriction is determined based on aorta diameter obtained during surgery, variability of the pressure gradient is reduced post-TAC.

Introduction

Transverse aortic constriction (TAC) in mice is a common chronic left ventricular pressure overload model that results in a gradual progression to heart failure. In this model, concentric left ventricle (LV) hypertrophy with preserved systolic function is generally observed. Further remodeling will occur with chronic pressure overload in the left ventricle, and a progressive loss of function then occurs leading to heart failure with reduced ejection fraction. In end-stage failure, LV and atrial dilation can be observed.

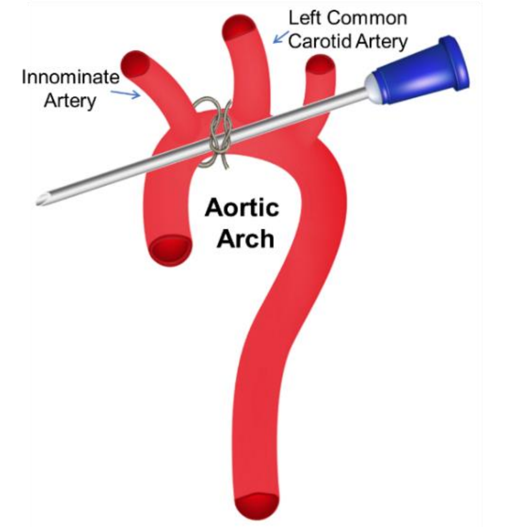


*Figure 1. **TAC Surgery.** Illustration of the aortic arch showing the position of the nylon suture in the transverse aorta.*

TAC is a useful tool to study the effectiveness of emerging drug therapy against the progression of cardiac dysfunction and failure. Thus, it is crucial for the TAC model to show a consistent and gradual time course in the development of heart failure.

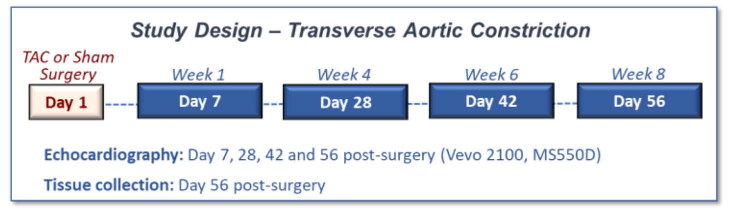
The surgical approach to induce LV pressure overload was first described by Rockman et al. in 1991. Since then, several techniques have been validated for permanent placement of the constriction in the aortic arch to limit LV outflow and create pressure overload in the LV. Most commonly, a blunt needle is placed parallel to the aortic arch with a suture placed between the right innominate artery and the left common carotid artery. The suture is tied around the vessel and needle (Figure 1), and the needle is subsequently removed to create the constriction. Smaller diameter needles produce tight constriction, and larger diameter needles produce loose constriction. The degree of constriction applied in the aortic arch affects the progression of the cardiac dysfunction.

A 27G needle is widely used to induce TAC; however, variability in the generated pressure gradient and, consequently, disease progression is frequently a problem. The variability in disease progression occurs because the same degree of constriction is applied regardless of aortic diameter. To minimize this variability, a narrow range of animal body weight is usually used; however, studies have shown no correlation between aortic diameter and body weight. Therefore, the aim of this study was to determine the aorta diameter at the time of surgery and apply 65-70% constriction by using different diameters of hypodermic tubing.

Objectives

Assess whether measuring the aortic diameter at the time of surgery and applying 65-70% constriction will decrease variability in the TAC model and provide a gradual time course in the development of heart failure.

Methods



C57BL/6N male mice (Charles River Labs, 23.7−26.1 g on the day of surgery) were allocated into 3 groups: Sham operated, TAC using 27G hypodermic tubing (TAC 410 µm) and TAC using a hypodermic tubing diameter selected to induce 65%-70% constriction (TAC 310-460 µm).

Transverse Aortic Constriction Surgery: On the day before surgery, mice received 2 mg/kg Meloxicam (SC) and Lactated Ringer's solution (20 mL/kg, SC). On the day of surgery, anesthesia was induced with isoflurane at 3% driven by 100% oxygen followed by administration of 10 mg/kg of etomidate (IP). Mice were then intubated for mechanical ventilation and anesthesia maintained at 1-2% isoflurane and the remainder 100% oxygen at 1 L/min.

The skin from the neckline to the diaphragm was shaved and disinfected. Bupivacaine block at a dose of 1 mg/kg was infiltrated intradermally approximately 10 minutes prior to incision at the intended site. A sternotomy was performed from the manubrium through the second or third rib in order to expose the aorta in the thoracic cavity. The transverse aorta was cleaned of surrounding tissue and a 6-0 nylon suture was placed around the transverse aorta between the right innominate artery and the left common carotid artery (Figure 1). In the TAC 310-460 µm group, the transverse aorta diameter was determined using a divider/caliper and stainless-steel hypodermic tubing was selected to induce 65%-70% constriction. In the TAC 410 µm group, 27G stainless-steel hypodermic tubing was used. Hypodermic tubing was placed against the transverse aorta and the suture was tightly tied. Immediately after placing the ligature, the hypodermic tubing was removed to produce a constriction. For the Sham group, the transverse aorta was cleaned of surrounding tissue, but no suture was placed around the transverse aorta. The intercostal muscle, the greater pectoral muscle and skin were closed with a 6-0 nylon suture in a simple interrupted pattern. Animals were kept on ventilation until commencing voluntary breathing after which the endotracheal tube was removed.

Mice received 1 mg/kg Buprenorphine SR-LAB (SC) and were transferred to a warm recovery cage for at least 2 hours or until alert and ambulatory. Mice were then placed in a clean cage in the recovery room overnight. Mice received Lactated Ringer's solution (20 mL/kg, SC) in the morning after surgery and were returned to general housing. DietGel® 31M was provided until 5 days post-surgery.

Echocardiography: Echocardiographic measurements were performed at 7-, 28-, 42- and 56-days post-TAC or Sham surgery under sedation with isoflurane (1-2%) using Vevo 2100 (Visual Sonics, Toronto, ON, Canada) equipped with a 40-MHz solid state transducer (MS550). Hearts were imaged in B-mode in the parasternal long axis and short axis and M-mode images were obtained from the short axis B-mode by placing the M-mode sample gate perpendicular to the LV walls at the level of the papillary muscle and from the long axis by placing the M-mode sample gate perpendicular to the to the aortic valve. The apical four-chamber view also was obtained to assess the mitral valve flow patterns by PW doppler.

The success of surgery was assessed with pulse wave doppler and color doppler of the transverse aorta. The aortic arch, major arterial branches and the constriction site were visualized in B-mode and the waveforms of the aortic flow obtained by PW Doppler/Color Doppler. The 21 MHz solid state transducer (MS250S) was used for the TAC animals, and the 40-MHz (MS550) was used for the Sham animals. In the TAC animals, the sample volume was placed distal to the constriction site, and the peak velocity measured. The pressure gradient across the constriction site was determined using the modified Bernoulli's equation (Pressure Gradient = $4xV_{max}^2$).

Terminal Procedures: Animals were euthanized via CO_2 asphyxiation after final ECG measurements were obtained. Hearts and lungs were harvested and weighed. The atria was removed, and the right and left ventricles were weighed separately. The left tibia was also removed and separated from the soft tissue; longitudinal measurements were obtained with a digital micrometer.

Statistics: Parameters are presented as means ± SD. All statistical analyses were conducted with SAS® version 9.4. Comparisons were considered significant at the .05 level. Comparisons between groups were conducted with two-way ANOVA and Tukey post-hoc tests.. Student´s t-test was used to compare tissue weight means. Variance in peak pressure gradient for TAC 410 µM vs TAC 310-460 µM was compared using an F-test for equality of variances (Snedecor and Cochran, 1983).

Results

- TAC mice with a pressure gradient below 40 mmHg were excluded from the study. One mouse was excluded from each TAC group.
- Aortic constriction was confirmed by a significant increase in the pressure gradient in TAC mice as compared to Sham mice (Figure 2).
- Variance in the pressure gradient was significantly greater when the constriction was induced by using a 27G needle (TAC 410 µm Group) as compared to applying a constriction proportional to the aorta diameter (TAC 310-460 µm Group) (Figure 2B).
- Morphological changes in both TAC groups were associated with concentric remodeling at 1-week post-surgery as indicated by increases in relative wall thickness (RWT) as compared to Sham mice but no change in LV mass was observed (Figure 3).
- Progressive hypertrophic remodeling occurred in both TAC groups and by 8-weeks post-surgery eccentric hypertrophy was observed as indicated by an increase in LV mass, and a tendency to increase LV internal diameter during diastole (LVIDd) and LV volume during diastole (EDV).
- M-Mode analysis in the parasternal short axis detected a significant, progressive decline in systolic function post-TAC surgery. LV ejection fraction (LVEF) and fractional shortening (FS) were reduced in both TAC groups at 6- and 8-weeks post-surgery as compared to the Sham group (Figure 4).
- Cardiac tissue mass at terminal harvest (8 weeks) supported the echocardiographic data and showed an increase in cardiac tissue weight in both TAC groups as compared to the Sham group (Figure 5).
- Mice in the TAC 410 µm group showed a significant increase in lung weight as compared to mice in the Sham group, indicating progression to heart failure (Figure 5). There was a tendency to increased lung weight for mice in the TAC 310-460 µm group as compared to mice in the Sham group.

Transverse Aorta Pulse-Wave Doppler in Mice Post-TAC

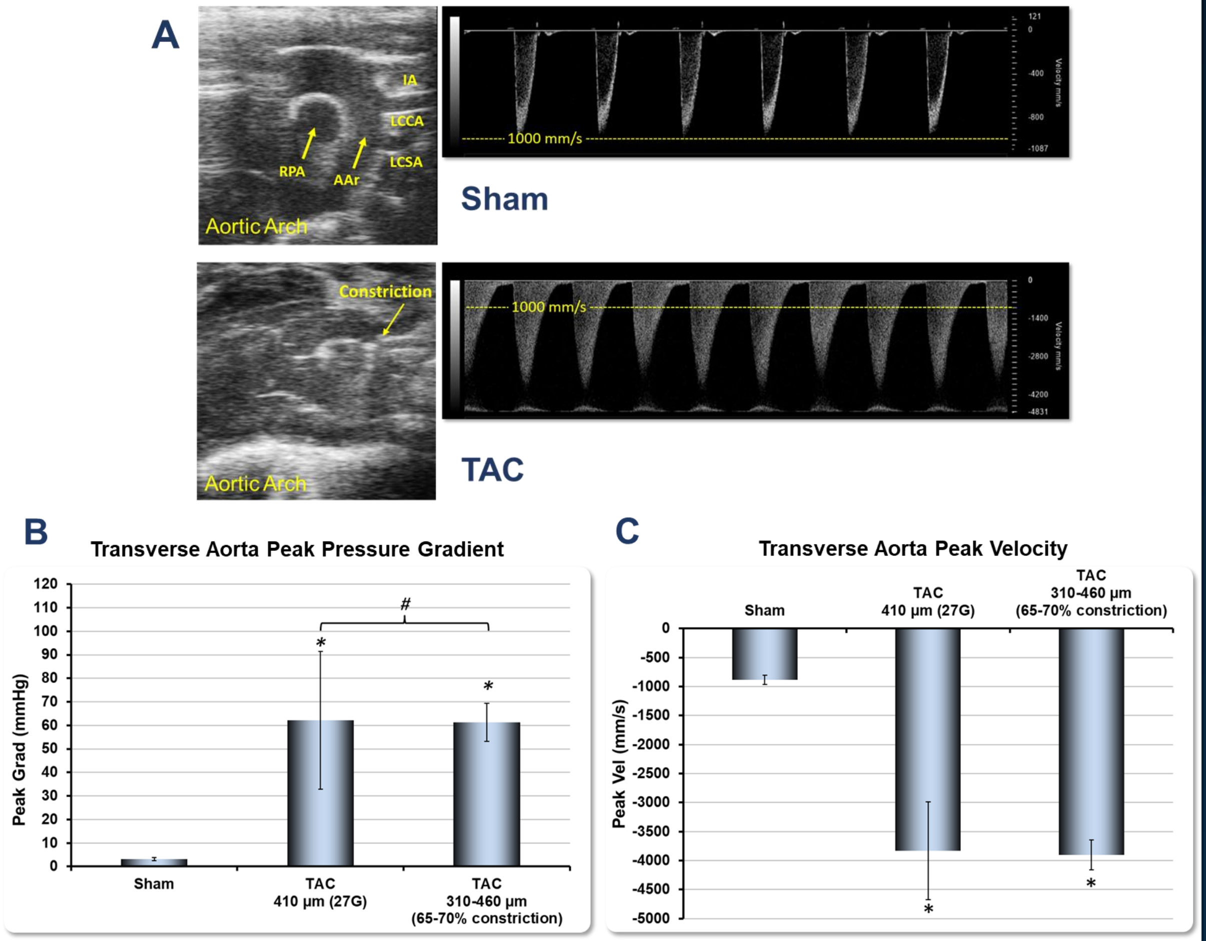


*Figure 2. **Confirmation of Transverse Aorta Ligation using Pulsed-Wave Doppler.** (A) Representative two-dimensional and pulse-wave doppler images from the transverse aorta of Sham and TAC mice post-surgery. (B) Peak pressure gradients of the transverse aorta calculated from Doppler velocities using the Bernoulli equation. (C) Peak flow velocity at the transverse aorta. Values are expressed as mean ± SD. *p<.05 vs Sham, two-way ANOVA with Tukey's multiple comparison test. # p=.007 variance TAC 410 µm vs TAC 310-460 µm (F=12.9, F-test for equality of variances); n=4-7 per group. AAR: Aortic Arch; RPA: Right Pulmonary Artery; IA: Innominate Artery; LCCA: Left Common Carotid Artery; LSCA: Left Subclavian Artery.*

Morphological Changes in Mice Post-TAC

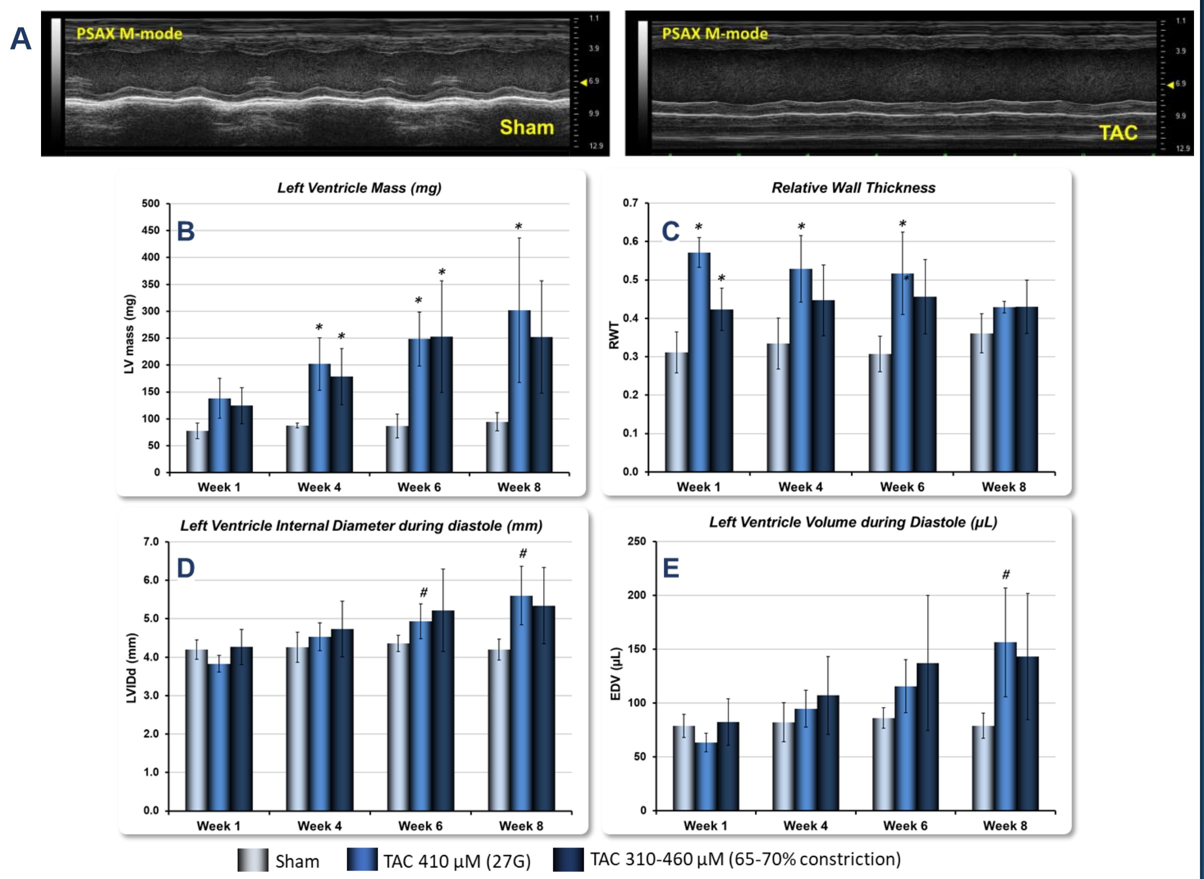


*Figure 3. **Left Ventricle morphological changes in Sham and TAC mice at 1-, 4-, 6- and 8-weeks post-surgery**. (A) Representative images of m-Mode echocardiography after 8-weeks post-surgery showing enlarged LV chamber in TAC mice. (B) LV mass: Left ventricular mass. (C) RWT: Relative wall thickness. (D) LVIDd: Left ventricle internal diameter during diastole. (E) EDV: Left ventricle volume during diastole. Values are expressed as mean ± SD. *p<.05 vs Sham, #p<0.05 vs 1 Week, two-way ANOVA with Tukey's multiple comparison test; n=2-7 per group.*

Changes in Systolic Function in Mice Post-TAC

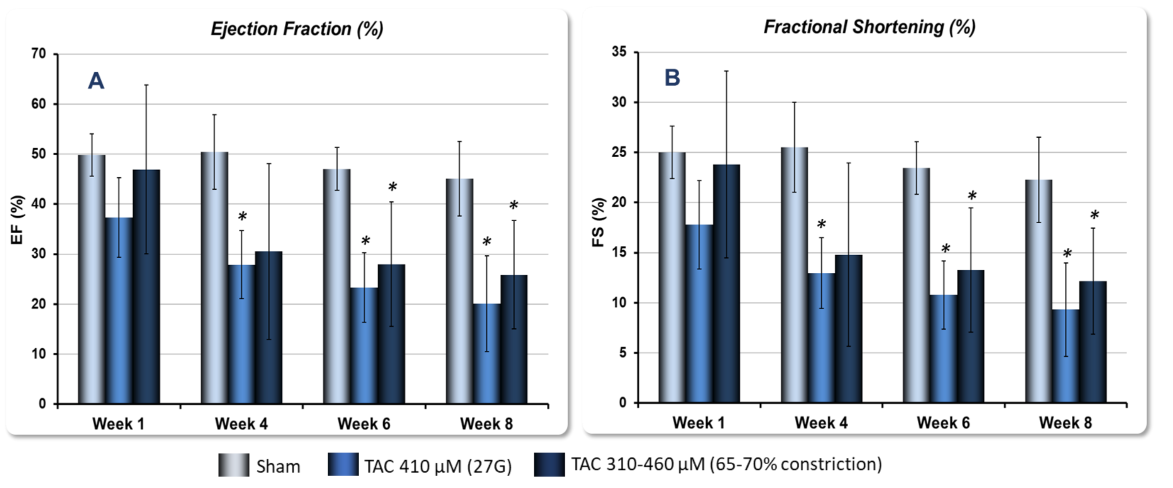


*Figure 4. **Changes in systolic function in Sham and TAC mice at 1-, 4-, 6- and 8-weeks post-surgery.** (A) Left ventricular ejection fraction. (B) Fractional shortening. Values are expressed as mean ± SD. *p<.05 vs Sham, two-way ANOVA with Tukey's multiple comparison test. n=2-7 per group.*

Organ Weight in TAC Mice at 8-Weeks Post-Surgery

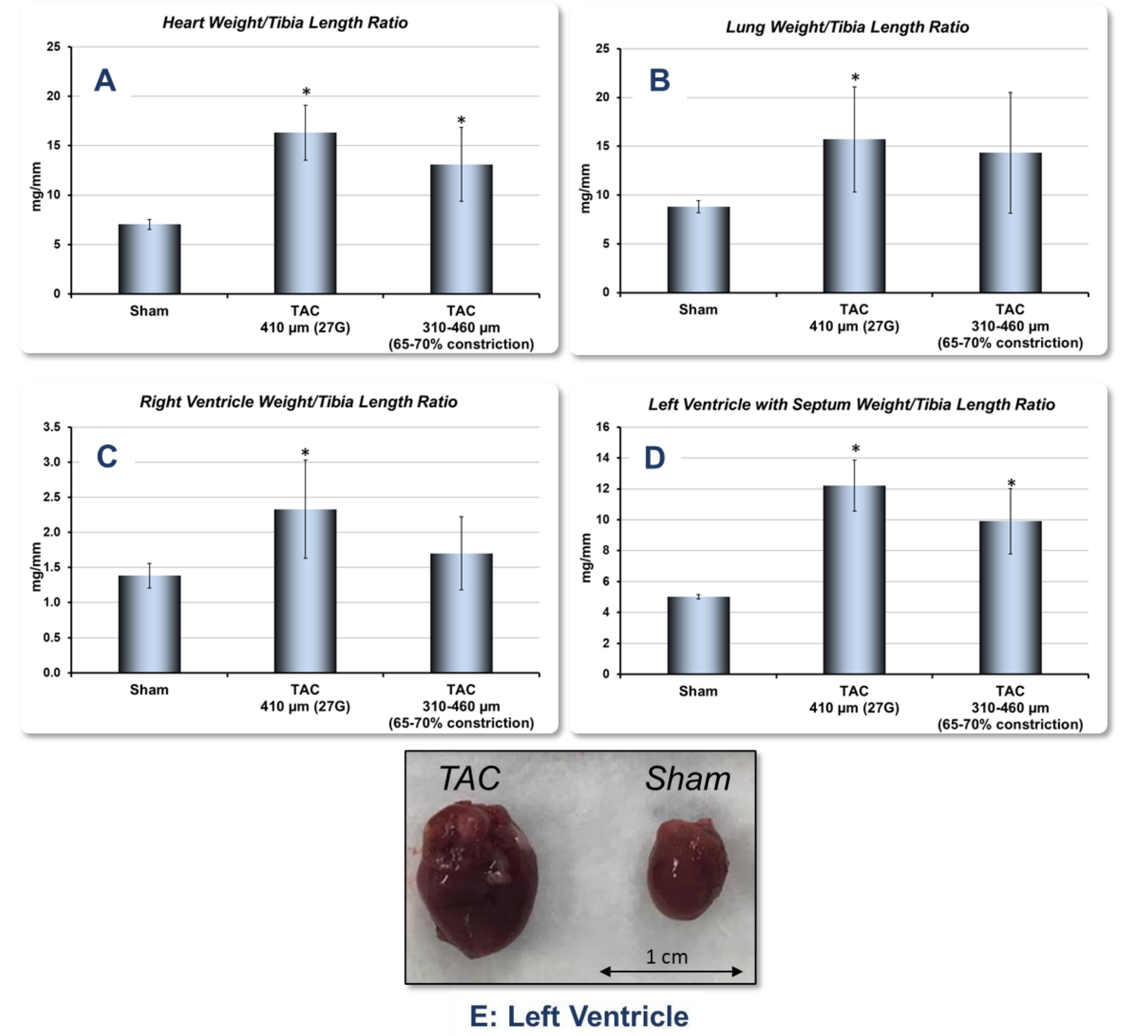


*Figure 5. **Organ weight comparisons between Sham and TAC mice at 8-weeks post-surgery.** Post-mortem (A) whole heart weight, (B) lungs weight, (C) right ventricle weight and (D) left ventricle with septum weight normalized to tibia length at 8-weeks post Sham or TAC surgery. (E) Representative left ventricle with septum image of Sham and TAC mouse. Values are expressed as mean ± SD. *p<.05 vs Sham, Student t-Test; n=3-6 per group.*

Conclusion

These results showed that when the degree of constriction is determined based on aorta diameter obtained during surgery, variability of the pressure gradient post-TAC is reduced. In addition, this study confirms that this model can be very useful in preclinical drug studies seeking to improve cardiac dysfunction and failure.