# Angiotensin-Converting Enzyme Inhibition in a Mouse Model of Transverse Aortic Constriction with Reduced Variability of the Aortic Peak Pressure Gradient

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## 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 is observed with chronic pressure overload in the left ventricle and a progressive loss of function that occurs lead to heart failure with reduced ejection fraction. In end-stage failure, LV and atrial dilation is observed.

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 failure.

A 27G needle is widely used to induce TAC; however, variability in disease progression occurs most likely because the same constriction is applied regardless of aortic diameter. Recently our lab has shown reduced variability of the aortic peak pressure gradient in the TAC model when the degree of constriction was based on aorta diameter obtained during surgery rather than using the standard 27G needle. In this study we utilized this new approach to access the therapeutic benefit of enalapril, an angiotensin-converting enzyme inhibitor, in the progression of cardiac dysfunction and failure.

## Methods

C57BL/6N male mice (Charles River Labs, 23.8 to 26.1 g on the day of surgery) were allocated into 3 groups: Sham-Vehicle (0.5% methylcellulose/1% tween 80, n = 15), TAC-Vehicle (0.5% methylcellulose/1% tween 80, n=17) and TAC-Enalapril (*ad libitum* access to water containing 200 mg/L enalapril, n=17). Treatment started on Day 1 and continued through Day 56.

**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 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. The diameter of the transverse aorta was determined using a divider/caliper and stainless-steel hypodermic tubing was selected to induce 65%-70% constriction. 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, and animals were kept on ventilation until voluntary breathing commenced after which the endotracheal tube was removed.

Mice received 1 mg/kg buprenorphine SR-LAB (SC) and were transferred to a warm recovery cage with oxygen 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 4-, 6- and 8-weeks post-TAC or Sham surgery under sedation with isoflurane (1-2%) using a Vevo 2100 (Visual Sonics, Toronto, ON, Canada) equipped with a 40-MHz solid state transducer (MS550). Body temperature was monitored and kept at 37±0.5°C throughout the procedure. Heart rate was also monitored throughout the procedure and maintained above 450 bpm when possible. Hearts were imaged in B-mode in the parasternal long axis and anatomical M-Mode images of the left ventricle (LV) aortic root and left atrium (LA) were taken to determine the left atria dimension. Anatomical M-Mode images of the LV in the parasternal short axis view were obtained by placing the M-mode sample gate perpendicular to the LV walls at the level of the papillary muscle and used to measure the LV internal dimension, anterior and posterior wall thicknesses and systolic function. The apical four-chamber view also was obtained to assess the mitral valve flow patterns by PW doppler and obtain the peak velocities of flow in the early phase of diastole (E wave) and after LA contraction (A wave) and deceleration time (DT). Both the mitral inflow and LV outflow were also simultaneously recorded to measure the isovolumic relaxation time (IVRT). Additional information about systolic and diastolic function was obtained with tissue Doppler imaging. Peak diastolic myocardial velocities in the early phase of diastole (E') and after LA contraction (A') and systolic velocity (S') were obtained with the sample volume at the septal side of the mitral annulus in the four-chamber view.

The success of the TAC 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 = $4xV_{max}^2$).

VevoStrain software was also used to analyze the data (Chowdhury SAK et. al. 2020). Four to five consecutive cardiac cycles were selected for the analysis, and semiautomated tracing of the endocardial and epicardial borders was used. The tracing was manually corrected and longitudinal (LS), circumferential (CS) and radial strain (RS) and strain rates were calculated.

All measurements and calculations were averaged from 3 consecutive cycles and performed according to the American Society of Echocardiography guidelines. Data analysis was performed off-line with the VevoLab Software (version 3.2.0).

**Terminal Procedures:** Animals were euthanized after final echocardiographic measurements were obtained. Hearts and lungs were harvested and weighed.

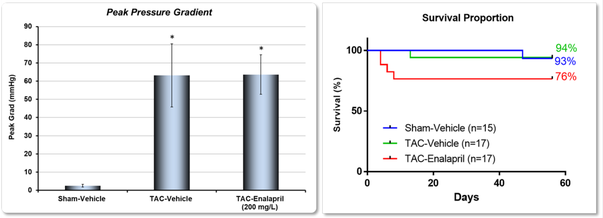
**Statistics:** Parameters are presented as mean ± SD. All statistical analyses were conducted with SAS® version 9.4. Comparisons were considered significant at the .05 level. Comparisons between groups were conducted with two-way ANOVA and Tukey's multiple comparison test.

## Objectives

Assess the therapeutic benefit of enalapril, an angiotensin-converting enzyme inhibitor, in the TAC model with reduced variability of the aortic peak pressure gradient.

## Results

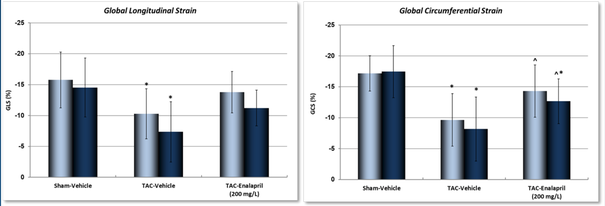
### Figure 1: Aortic constriction was confirmed by increases in the peak pressure gradient post-TAC surgery




- Peak pressure gradient was significantly increased in TAC-Vehicle and TAC-Enalapril groups as compared to Sham-Vehicle. TAC mice with a pressure gradient below 40 mmHg were excluded from the study (TAC-Enalapril group: n = 1 excluded). The transverse aorta diameter obtained during surgery ranged from 1.21 mm to 1.40 mm and the constriction applied to the aorta during TAC surgery ranged from 67–70%.
- Kaplan-Meier survival curves for Sham-Vehicle, TAC-Vehicle and TAC-Enalapril Group; p = 0.1803 log-rank test (Mantel-Cox test). Eleven mice died either during TAC surgery or within 48 hours of TAC surgery (18% death rate) and hence were not included in the survival analysis.

*Values are expressed as average ± SD of measurements obtained at 4- and 6-weeks. *p<.05 vs Sham-Vehicle, two-way ANOVA with Tukey's multiple comparison test. n = 13-16 per group.*

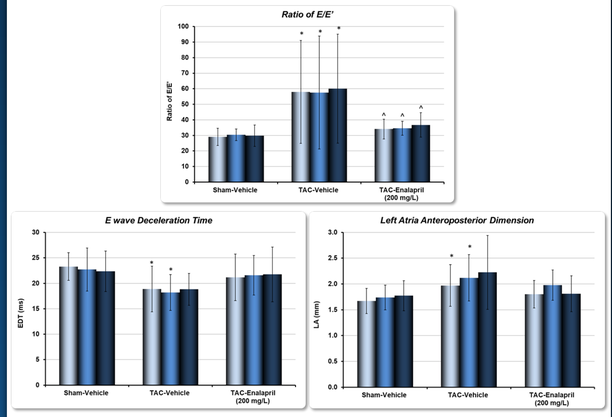
### Figure 2: Enalapril treatment improves systolic function as shown by speckle-tracking analysis post-TAC in C57BL/6N male mice




- LV speckle-tracking analysis revealed LV dysfunction as indicated by a decrease in the global longitudinal (GLS) and circumferential (GCS) strain rate at 4- and 8-weeks post-TAC surgery as compared to Sham-Vehicle group.
- Enalapril treatment blunted the decrease in GCS at 4- and 8-weeks post-TAC surgery as compared to TAC-Vehicle group.

*Values are expressed as average ± SD. *p<.05 vs Sham-Vehicle within each week; ^p<.05 vs TAC-Vehicle within each week; two-way ANOVA with Tukey's multiple comparison test. n = 12-16 per group.*

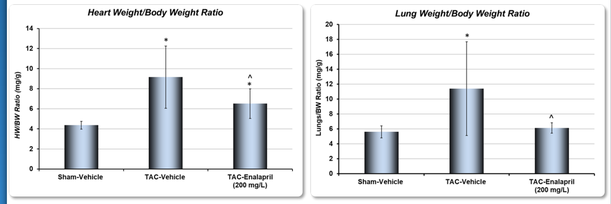
### Figure 3: Enalapril treatment resulted in a decrease in filling pressures and improved myocardial relaxation following TAC in C57BL/6N male mice




- Diastolic dysfunction in TAC-Vehicle group was observed by an increase in E/E' ratio at 4-, 6- and 8-weeks post-TAC surgery as compared to Sham-Vehicle group. E wave deceleration time (E DT) was decreased, and left atria was dilated at 4- and 6-weeks post TAC surgery as compared to Sham-Vehicle, which is consistent with increased LV stiffness and diastolic dysfunction.
- Enalapril treatment abolished TAC induced increases in the E/E' ratio that occurred at 4-, 6- and 8-weeks as compared to the TAC-Vehicle group. LV stiffness also was minimized as E DT tend to increase following enalapril treatment. Left atrial dilation tend to diminish in TAC-Enalapril group as compared to the TAC-Vehicle group, further supporting an improved diastolic function with enalapril treatment.

*Values are expressed as average ± SD. *p<.05 vs Sham-Vehicle within each week. ^p<.05 vs TAC-Vehicle within each week; two-way ANOVA with Tukey's multiple comparison test. n = 13-16 per group.*

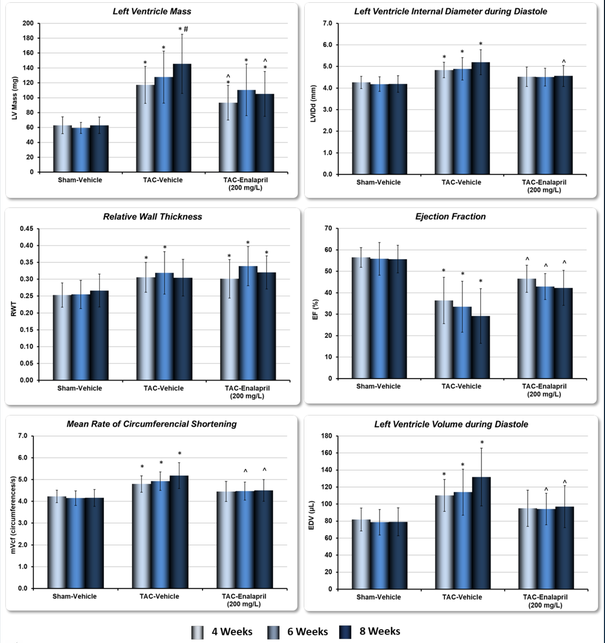
### Figure 4: Enalapril treatment diminished LV hypertrophy and pulmonary congestion following TAC in C57BL/6N male mice




- Cardiac tissue mass at terminal harvest supported LV hypertrophy as noted in the echocardiographic data for TAC-Vehicle group. Normalized heart weight (HW/BW) was increased in TAC-Vehicle group at 8-weeks post-surgery as compared to Sham-Vehicle. Pulmonary congestion was also present at 8-weeks in TAC-Vehicle group as shown by an increased lung weight normalized by body weight.
- Enalapril administration reduced the increase in cardiac tissue mass and lung weight at 8-weeks post TAC surgery as compared to the TAC-Vehicle group.

*Values are expressed as average ± SD. *p<.05 vs Sham-Vehicle. ^p<.05 vs TAC-Vehicle; two-way ANOVA with Tukey's multiple comparison test. n = 13-16 per group.*

### Figure 4: Enalapril blunted TAC-induced cardiac remodeling and systolic dysfunction in C57BL/6N male mice




- Progressive hypertrophic remodeling and decline in systolic function occurred post-TAC surgery, as indicated by a significant increased LV mass, internal diameter during diastole (LVIDd), mean rate of circumferential shortening (mVcf) and a decrease in LV ejection fraction (EF) as compared to Sham-Vehicle group. Relative wall thickness (RWT) was increased at 4- and 6-weeks post-TAC; however, no change in RWT was observed at 8-weeks post-surgery. Dilated LV was also evident in TAC-Vehicle group as shown by a significant increase in LV end-diastolic volume (EDV) as compared to Sham-Vehicle group.
- The hypertrophy noted at 4-, 6- and 8-weeks post-TAC surgery was diminished with enalapril treatment as shown by a decrease in LV mass and LVIDd, with no changes in RWT as compared to TAC-Vehicle group. Enalapril treatment also blunted TAC-induced decreases in EF as well as the increase in mVcf and EDV at 6- and 8-weeks post TAC surgery as compared to TAC-Vehicle group.

*Values are expressed as average ± SD. *p<.05 vs Sham-Vehicle within each week. ^p<.05 vs TAC-Vehicle within each week; two-way ANOVA with Tukey's multiple comparison test. n = 13-16 per group.*

## Conclusion

These results demonstrate that enalapril treatment blunted TAC-induced cardiac remodeling and dysfunction in a TAC model of reduced variability of the aortic peak pressure gradient. In addition, this study confirm that this model can be very useful in preclinical drug studies seeking to improve cardiac dysfunction and failure.