Published in final edited form as:
Curr Protoc Mouse Biol. 2011 March 1; 1: 71–83. doi:10.1002/9780470942390.mo100130.

Echocardiography in Mice

Shumin Gao, **David Ho**, **Dorothy E. Vatner**, and **Stephen F. Vatner**
Department of Cell Biology & Molecular Medicine and The Cardiovascular Research Institute at the University of Medicine & Dentistry of New Jersey, New Jersey Medical School, 185 South Orange Avenue, MSB G609, Newark, NJ 07103, USA

Abstract

Murine models have been utilized with increasing frequency mainly due to availability of genetically engineered models. With advancement in high spatial and temporal resolution, echocardiography is used extensively for the evaluation of cardiovascular function in murine models of cardiovascular disease. This review summarizes the general applications and methods involved in echocardiography used to study mouse models for cardiovascular research, based on 20 years of experience in our laboratory. The goal of this article is to provide a practical guide to the use of echo techniques in mice to evaluate cardiac systolic and diastolic function.

Keywords

echocardiography; systolic function; diastolic function; mouse

INTRODUCTION

Murine models for cardiovascular disease have been utilized with increasing frequency mainly because of the expanding availability of genetic models. Echocardiogaphy (echo) is a useful non-invasive method to visualize the cardiovascular structures and evaluate cardiac function in mice. Improved echocardiography instrumentation enhances the spatial and temporal resolution for imaging, resulting in more accurate assessment of left ventricular systolic, diastolic, regional and vascular function. Echocardiography is an extremely versatile tool for cardiovascular research allowing the evaluation of left ventricular (LV) systolic function and diastolic function in mouse cardiomyopathy models (Asai et al., 1999; Iwase et al., 1996; Iwase et al., 1997), myocardial ischemia model (Odashima et al., 2007), and chronic pressure overload induced by transverse aortic constriction (TAC) (Depre et al., 2006; Gelpi et al., 2009; Guellich et al., 2010). Also coronary reserve in mice can be measured by echocardiography (Gao et al., 2008a, b). The applications and the advances of echocardiography in mice have been summarized recently (Rottman et al., 2007; Scherrer-Crosbie and Thibault, 2008; Stypmann, 2007). In this article, we will focus on how to apply echocardiography for research in normal mice, genetically altered mice and models of cardiovascular disease using examples from the general echocardiography protocols used in our laboratory.

Address correspondence to: Stephen F. Vatner, M.D., Department of Cell Biology & Molecular Medicine, University of Medicine & Dentistry of New Jersey-New Jersey Medical School, 185 South Orange Avenue, MSB G609, Newark, NJ 07103, Tel: (973) 972-8920, Fax: (973) 972-7489, vatnersf@umdnj.edu.

ECHOCARDIOGRAPHY IN CONSCIOUS MICE

Anesthesia depresses contraction, heart rate and autonomic reflex control (Vatner et al., 2002; Vatner and Braunwald, 1975). Therefore, it is a generally held view that cardiovascular experiments performed in the conscious state are preferable to those obtained under anesthesia. This holds for experiments in mice as well as large animal models. However, there are also serious limitations to performing echocardiography in conscious mice; most notably excitement in the animals can result in enhanced sympathetic tone and heart rate. Accordingly training of mice for studies in the conscious state is essential.

Echocardiography method for conscious mice

1. Training: Before echocardiography can be performed effectively in conscious mice, the mice need to be trained for two to three sessions over a period of 3 days by holding the nape of the neck with the tail held tightly by the last two fingers, and putting the ultrasound transducer on the chest for probe contact training. After a few days of training, the mouse will remain calm in this position. Just prior to the acquisition of echo images, the chest hair is removed by shaving or by applying hair removal cream. To prevent excessive heat loss, as small an area of hair as possible should be removed. Further details of echocardiography methods are described in the section ‘Author’s mouse echo protocol’.
2. For echocardiography, the mouse is picked up in the palm of one hand. Pre-warmed echo transmission gel is applied to the hairless chest. While holding the mouse with the back of the mouse towards the palm, the hand is turned so the chest of the mouse faces the floor. The transducer is applied from under the mouse to avoid reflex effects induced by the pressure of the transducer on the chest.
3. Parasternal long-axis view, short-axis view at the papillary muscle level and 2-D guided M-mode images are recorded as described in the mouse echo protocol detailed below.

Baseline values for heart rate (HR) and fractional shortening (FS) in conscious and anesthetized mice are summarized and displayed in Table 1, showing much higher values in conscious mice than those under anesthesia. The heart rate in conscious mice is usually 600–700 beats/min (Yang et al., 1999).

The disadvantages of conscious echocardiography include: 1) mice have to be trained for a few days to minimize the excitement induced by manipulating the mice (Rottman et al., 2007; Yang et al., 1999), 2) a second person is needed to operate the ultrasound machine, and 3) the faster heart rates in conscious mice complicate Doppler recording, e.g., the Doppler waveforms fuse together because of the short diastolic time.

ANESTHESIA FOR MOUSE ECHOCARDIOGRAPHY

Because of the limitations of echocardiography in conscious mice (see above), anesthesia is frequently used in murine echocardiography. The regimens of anesthesia, e.g., continuously delivered gas inhalation agent isoflurane (1–3%) (Hartley et al., 2008; Roth et al., 2002; Wikstrom et al., 2008), Avertin (tribromoethanol, 250–400 mg/kg, injected intraperitoneally, IP) (Gardin et al., 1995; Hart et al., 2001; Luo et al., 2007; Roth et al., 2002; Schmidt et al., 2002; Tan et al., 2003), pentobarbital (50 mg/kg, IP) (Rottman et al., 2003; Tan et al., 2003; Yang et al., 1999), ketamine (80–150 mg/kg, IP) mixed with xylazine (5–20 mg/kg, IP) (Hart et al., 2001; Schaefer et al., 2005; Tan et al., 2003; Tanaka et al., 1996; Yang et al., 1999) are employed in many labs. According to Roth et al., 2002, both isoflurane and

Avertin cause less depression of cardiac function and heart rate, are easy to administer, provide reproducible results and are rapid in onset and recovery.

We have found 2.5% Avertin, 0.012 ml/g body weight (300 mg/kg), IP allows for the most predictable and reproducible level of cardiac suppression and maintenance of heart rate leading to consistency in the quality of echo measurements. Using this anesthesia, the heart rates of normal mice are generally 400–500 beats/min and typically LV FS is approximately 35%. These values are comparable to the measurements with isoflurane (2%) anesthetized mice (Stypmann, 2007). The effects of different anesthesia on FS and HR are shown in Table 1. Conversely, ketamine/xylazine mixture resulted in the greatest depression of heart rate. One potential adverse effect of too low a heart rate induced by anesthesia is cardiac dilatation with consequent functional valvular regurgitation. This has been observed during rat echo (Droogmans, 2008), and most likely will also occur in mice.

ECHO MACHINES AND TRANSDUCERS

ECHO transducers with frequency higher than 10 MHz are generally selected for mouse echocardiography. This is necessary because of the small size of the heart and its rapid rate of contraction. In our core echo lab, we have one Siemens Sequoia C256 with 13 MHz linear transducer, one GE Vivid7 with i13L probe (14 MHz) and one VisualSonics Vevo770 with 30 and 40 MHz probes for mouse cardiac and vascular examination.

Authors' mouse echo protocol

1. The mouse is injected intraperitoneally with 2.5% Avertin, 0.012 ml/g body weight (300 mg/kg). Heart rates are monitored and generally maintained at 400–500 beats per minute.
2. When using Siemens Sequoia C256 or GE Vivid7, the chest hair is shaved. EKG needle leads are connected to the limbs for electrocardiogram gating. The mouse is then placed on a warm pad to keep the body temperature around 37°C. A rectal thermometer is inserted for monitoring the body temperature.
3. Warmed echo gel is placed on the shaved chest. The mouse heart is imaged with a 13 MHz linear transducer (Siemens Sequoia C256) or 14 MHz probe (GE Vivid7) while the mouse lies on the warm pad at a shallow left-side position.
4. When using VisualSonics Vevo 770, due to the much higher probe frequency and interference from incompletely removed hair, hair remover lotion is applied to the chest to help facilitate the complete removal of hair. The platform temperature of the equipment is set at 40–42 °C, which is higher than optimal animal core temperature in order to help maintain the mouse core temperature at 37 °C. The mouse is placed onto the warm plate in the supine position. The limbs are taped onto the metal EKG leads. For cardiac imaging, the 30 MHz transducer is used, while the 40 MHz transducer is utilized for vascular imaging.
5. By placing the transducer along the long-axis of LV, and directing to the right side of the neck of the mouse, two-dimensional LV long-axis is obtained. Then the transducer is rotated clockwise by 90°, and the LV short-axis view is visualized. The diagrams showing the positions and directions of the transducer for basic mouse echo views are demonstrated in Figure 1. 2D-guided LV M-mode at the papillary muscle level is recorded from either the short-axis view and/or the long-axis view. Transmitral inflow Doppler spectra are recorded in an apical 4-chamber view by placing the sample volume at the tip of the mitral valves. Angle correction can be used for accurate flow velocity measurements. Doppler waveforms from other regions of the heart can be recorded as needed.

6. After the scanning is finished, the residual echo gel is removed, and the mouse is returned to the cage for recovery.

7. Echo images are downloaded and analyzed offline using Scion images software or echo work station. At least three beats need to be measured and averaged for the interpretation of any given measurement. From the authors' experience, at least 5 mice are normally needed per experimental group to show statistically significant relevance.

CONSIDERATIONS IN MURINE ECHOCARDIOGRAPHY

To obtain consistent, reproducible echocardiographic data, aside from obtaining good images, the condition of the animals also needs to be controlled. Therefore, the following should be kept in mind during the echo scanning.

1. The mouse body temperature should be carefully monitored and maintained at 37 °C during the entire procedure.

2. Since cardiac function is closely related to heart rate, the heart rate should be controlled at a similar level within each strain of mice. Therefore, the choice of anesthetic agent, dose and dosing interval should be carefully reproduced and considered. From the authors' experience, the variation of HR within 100 bpm for a strain/set of experiments should be acceptable.

3. Echo measurement time should be similar after anesthesia to minimize the effects of changes in anesthetic levels with time on echocardiography parameters.

ECHO MEASUREMENTS

LV systolic function

LV interventricular septal thicknesses (IVS), LV internal dimensions (LVID) and posterior wall thicknesses (PW) at diastole and systole (IVSd, LVIDd, PWd and IVSs, LVIDs, PWs, respectively) are measured from M-mode images at the level of the papillary muscles. An example of LV M-mode in mice is displayed in Figure 2A. LV ejection fraction (EF), LV fractional shortening (FS), and LV posterior wall thickening (PWT) are calculated by using the following formulas (Gardin et al., 1995; Syed et al., 2005; Tanaka et al., 1996; Tsujita et al., 2005):

$$EF(\%)=100\times[(LVIDd^3-LVIDs)^3)/LVIDd^3]$$
$$FS(\%)=100\times[(LVIDd-LVIDs)/LVIDd]$$
$$PWT(\%)=100^*[(PWs-PWd)/PWd]$$

LV ejection fraction (EF) and LV fractional shortening (FS) are measured for evaluation of LV global systolic function. When the LV contracts without regional wall motion abnormalities, EF and FS are related. However, in ischemia or myocardial infarction models, because of the changes of LV geometry, EF calculated by the simple cubic assumption of LV volume may not be accurate and the calculated LVEF could be different from the actual LV ejection fraction. In these cases, FS is preferable to express LV global function.

Serial echocardiography performed in FVB and C57BL/6J mice at baseline, 1, 2, and 3 weeks after chronic pressure overloading induced by transverse aortic constriction (TAC) are illustrated in Figure 3. Interestingly, the effects of this stress on the heart differ in different mouse strains. Echo techniques are useful to detect the differences. For example, echocardiography detected systolic dysfunction, i.e., reduced LVEF in C57BL/6J mice after

1 week of TAC, whereas LVEF was still maintained at baseline levels even 2 weeks after TAC in FVB mice, demonstrating a major mouse strain difference in response to chronic pressure overload. Furthermore, echo is useful for detecting and monitoring the progression of cardiac dysfunction such as in the case of cardiomyopathy; a sample of M-mode echo for cardiomyopathy is shown in Figure 4C. The progression of cardiomyopathy in transgenic mice (Tg) overexpressing beta 1 adrenergic receptors (β_1-AR) illustrated in Figure 4E, is seen as an initial increase in LV fractional shortening (LV FS) in the young Tg mice (gray bar) with a decrease in LV FS in the older Tg mice (black bar) compared to wild type (WT) mice (white bar) (Peter et al., 2007).

The velocity of circumferential fiber shortening (Vcf) is a pre-load independent measurement for LV systolic function, which is calculated by Vcf = FS/ET. ET is the ejection time of the LV, which can be measured by PW Doppler. Since ET is heart rate dependent, correcting ET by dividing it by the square root of the R-R interval can make the corrected Vcf heart rate independent (Odley et al., 2004; Syed et al., 2005). This correction is relevant in both conscious and anaesthetised mice.

LV wall thickening is another method for the assessment of global LV systolic function in the absence of abnormal wall motion. However, when abnormal wall motion exists, the wall thickening represents only regional LV systolic function. Furthermore, LV mass (in diastole) can be obtained from M-Mode measurements by the cubed formula:

$$\text{LV mass} = 1.05 \times [(\text{IVSd} + \text{LVIDd} + \text{PWd})^3 - \text{LVIDd}^3]$$

LV diastolic function

Pulse wave Doppler—Transmitral inflow Doppler obtained in apical 4-chamber view or LV long-axis view is used for evaluation of LV diastolic function in mice (Du et al., 2008; Schaefer et al., 2003; Schmidt et al., 2002; Semeniuk et al., 2003). A Doppler example of transmitral flow is displayed in Figure 2B. The Doppler indexes include the ratio of peak velocity of early to late filling of mitral inflow (E/A), deceleration time (DT) of early filling of mitral inflow, isovolumetric relaxation time (IVRT) and isovolumetric contraction time (IVCT). There are four basic Doppler patterns of transmitral inflow and these four patterns represent the progression from normal to severe diastolic dysfunction (Du et al., 2008; Ohno et al., 1994): (i) Normal LV filling E>A; (ii) Abnormal LV relaxation E<A; (iii) Pseudonormal filling E>A; and (iv) Restrictive filling E≫A. Since diastolic dysfunction progresses rapidly in mice, multiple different Doppler patterns may exist in the same group of surgically modeled or genetically altered mice and this may lead to misinterpretation of the stage of diastolic dysfunction. Thus, confirming Doppler measurements by other methods such as tissue Doppler, color M-mode Doppler or pressure measurements is essential. The Doppler parameters at baseline and two weeks after TAC in FVB mice were measured, diastolic dysfunction was evident in the echocardiogram, as reflected by decreased A wave velocity, increased E/A ratio and an increased index (IVRT+IVCT)/ET, implying increased stiffness of LV after TAC as seen in Table 2. However, systolic function in these same FVB mice was maintained two weeks after TAC (Figure 3).

Tissue Doppler Imaging—Tissue Doppler imaging (TDI) is tissue motion velocity obtained from the mitral annulus or LV posterior wall from the myocardium, which normally consists of three basic waveforms: two in early and late diastole (Ea and Aa respectively), and one in systole (Sa). Decreased Ea/Aa ratio indicates diastolic dysfunction. Importantly, these values are influenced to a lesser extent by loading conditions (Schaefer et al., 2003). A TDI example is demonstrated in Figure 2C.

Color M-Mode Doppler—Color M-Mode Doppler flow propagation of transmitral inflow (Vp) is obtained by placing the M-mode cursor through the center of mitral inflow, which is guided by color Doppler. Decreased Vp implies impaired LV relaxation, as correlated to pulse wave Doppler parameters (Schmidt et al., 2002; Tsujita et al., 2005).

Myocardial performance index—Pulse wave Doppler or tissue Doppler derived myocardial performance index (MPI), is a useful index for assessing cardiac systolic and diastolic function in mice. It can be calculated by using the ratio of isovolumetric contraction and relaxation time to ejection time (IVRT+IVCT)/ET. Increased MPI indicates diastolic dysfunction. Since this index is based on the ratio of several portions within the same cardiac cycle, MPI is independent from heart rate and LV shape (Broberg et al., 2003; Schaefer et al., 2005).

LV regional function

LV wall thickening—As mentioned above, LV wall thickening is measured from several regions of the LV wall and is a basic index for evaluation of LV regional systolic function (Thibault et al., 2007). This can be a critical measurement in a heart with dysynchronous contraction, for example, after myocardial infarction, where one LV wall might exhibit enhanced function, while the other wall may not contract at all, or even paradoxically.

Tissue Doppler Imaging and strain rate—Systolic waveform (Sa) is a measurement of regional LV wall systolic motion velocity as obtained by tissue Doppler and represents regional wall contraction. Strain rate (SR) is the relative change of length of myocardial tissue over time, and as such it can be measured using TDI. TDI and SR have been demonstrated to be sensitive methods for the detection of LV regional wall contractile changes associated with aging, exercise, cardiac toxic drugs or myocardial ischemia (Derumeaux et al., 2008; Jassal et al., 2009; Sebag et al., 2005; Thibault et al., 2007).

Two-dimensional speckle tracking echocardiography—Two-dimensional (2-D) speckle tracking echocardiography (STE), also known as real-time strain rate, is a novel method for the assessment of LV segmental function by tracking the speckle motion in a 2-D echocardiography imaging. Briefly, LV short axis view is acquired at a high frame rate, e.g., over 200 frames/sec, and specific software is needed to measure the radial and circumferential strain and strain rate for each segment of the LV wall. The feasibility of 2D-STE in mice has been tested (Peng et al., 2009). Compared to the strain rate derived from TDI which is Doppler angle dependent and can only be obtained in the anterior and posterior LV segments; 2-D speckle tracking has the ability to assess all segments in radial and circumferential strain components. However, due to the thin LV wall thickness and very high heart rate in mice, the application of the (2-D) speckle tracking technique in mice needs to be improved.

VASCULAR ULTRASOUND IN MICE

Coronary flow reserve in mice

Coronary reserve (CR) is the ratio of maximal coronary flow under hyperemia to baseline coronary flow. Therefore, monitoring the coronary flow is essential for the measurement of CR. High resolution echocardiography machines make it possible for the measurement of coronary reserve in mice (Hartley et al., 2008; Saraste et al., 2006; Wikstrom et al., 2005; Wikstrom et al., 2008). Since CR derived from coronary flow velocity (CFVR) correlates very well with the CR derived from volumetric coronary flow (CFR) in mice, it is acceptable to simply use CFVR for determination of CR (Wikstrom et al., 2008).

In the authors' lab, the high resolution ultrasound machine-VisualSonics Vevo770, with probe frequency of 30 MHz or 40 MHz is used for this measurement. The proximal left coronary artery (LCA) is visualized in a modified parasternal LV long-axis view, and Doppler spectrum of LCA is recorded at baseline, and under hyperemic conditions induced by infusing adenosine (160 μg/kg/min) for at least 3 minutes. From the Doppler spectrum of the left coronary artery, mean diastolic velocity and peak diastolic velocity are measured at baseline ($CFV_{baseline}$) and following maximal coronary vasodilation induced by adenosine infusion ($CFV_{hyperemia}$). Coronary reserve based on coronary flow velocity is calculated using the following formula: $CFVR= CFV_{hyperemia}/CFV_{baseline}$. Simultaneously, left main coronary artery diameter (d) is measured in the modified LV short-axis view. Cross-sectional area (A) of LCA is calculated as $A= \pi \times d^2/4$. Velocity time integral (VTI) of LCA is obtained from Doppler. Blood flow of LCA (CF) $=VTI \times A \times HR$. HR is heart rate. Coronary reserve from blood flow $CFR= CF_{hyperemia}/CF_{baseline}$. CR measured in normal 129SVJ mice in the authors' lab by maximum velocity and by volumetric blood flow are 2.28 ± 0.1 and 2.68 ± 0.15 respectively (Gao et al., 2008a, b). These are similar to those from previously reported studies (Wikstrom et al., 2005; Wikstrom et al., 2008).

Other vessels in mice

With the high frequency probe (30–40 MHz), mouse carotid arterial lumen, length, and Doppler waveform can also be studied. Williams et al. (Williams et al., 2007) verified the feasibility by measuring the pulse wave velocity in mouse carotid artery. Using the same technique, the aortic arch and abdominal aorta can also be visualized in mice (Feintuch et al., 2007; Luo et al., 2007). In TAC mice, measuring the flow velocity through the banded site can help assess the pressure gradient between LV and aorta non-invasively. In general, the following steps can be used to scan the vessels: first, place the probe along the course of the vessel of interest to obtain the long-axis images for lumen, length and wall thickness measurements, and then tilt the probe to direct the ultrasound beam along the direction of blood flow to record the Doppler signals.

MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY

Myocardial contrast echocardiography (MCE) is performed with the aid of intravenously injected contrast agents (micro bubbles) to enhance the myocardial image for evaluation of myocardial perfusion and the perfusion defect in myocardial ischemia experiments. The feasibility of MCE in mice has been demonstrated by several groups (French et al., 2006; Kaufmann et al., 2007; Mor-Avi et al., 1999; Raher et al., 2007; Scherrer-Crosbie et al., 1999). The high resolution of the VisualSonics echocardiography machine makes it easier to perform this function in mice. One might predict that it will be more difficult with echo machines of lower resolution.

STRESS ECHOCARDIOGRAPHY IN MICE

In mice, stress echocardiography is generally performed with administration of pharmacologic agents under anesthesia. In the authors' echo lab, we often perform echocardiography for the purpose of monitoring cardiac response to sympathomimetic amines, e.g., isoproterenol or dobutamine. For example, the protocol for isoproterenol in the authors' lab is as follows:

1. A jugular vein catheter is inserted in advance for drug infusion.
2. A Harvard infusion/withdrawal pump is used for drug infusion and set to deliver isoproterenol at 0.01, 0.02, 0.04 μg/kg/min.

3. The isoproterenol solution is prepared to deliver a final concentration of 0.01 μg/kg/min using an infusion speed of 2 μl/min. When preparing the solutions, it is important to take into account the body weight for each mouse.
4. The mouse is anesthetized using 2.5% Avertin, as described above, and LV 2-D and M-mode images are obtained at baseline.
5. The catheter is connected to a 100 μl syringe prefilled with the isoproterenol solution. The syringe diameter in the infusion pump is input and the infusion speed is set at 2 μl/min. The first dose is infused at 0.01 μg/kg/min for 5 min. The echo images are recorded at 5 minutes of infusion.
6. Switch to the next dose at 0.02 μg/kg/min by adjusting the infusion speed to 4 μl/min, and increase again to 0.04 μg/kg/min by increasing the infusion speed to 8 μl/min. Echo is recorded after 5 min of infusion for each of these dosages.
7. After completing all of the doses, the echo data are analyzed offline.

LV M-mode images at baseline and after isoproterenol infusion are compared in Figure 4A-B. LV fractional shortening is increased with increasing isoproterenol dose in FVB mice as shown in Figure 4D.

COMMENTARY

Multiple methods for cardiac imaging have been developed over the years for the visualization and assessment of cardiac function. Among these cardiac echocardiography, micro CT (Nahrendorf et al., 2007), PET scan (Kreissl et al., 2006), and contrast enhanced cardiac MRI are included (Slawson et al., 1998; Wiesmann et al., 2001; Yang et al., 2004). However, due to the cost and frequent need for contrast material in cardiac MRI and CT and the ease of echocardiography, echo remains the most frequently used modality for the routine evaluation of cardiac function in mice. In performing echocardiography in mice, care must be taken to control the heart rate, body temperature and the level of anesthesia. Once the animal has been properly prepared and good images are obtained, both systolic and diastolic cardiac function can be accurately measured and compared for the monitoring of cardiac pathophysiology, as well as the effectiveness of any intervention.

Acknowledgments

Supported in part by grants HL033107, HL069020, AG027211, HL101420, HL093481, HL059139, HL095888, DK083826, and HL102472.

Literature Cited

Asai K, Yang GP, Geng YJ, Takagi G, Bishop S, Ishikawa Y, Shannon RP, Wagner TE, Vatner DE, Homcy CJ, et al. Beta-adrenergic receptor blockade arrests myocyte damage and preserves cardiac function in the transgenic G(salpha) mouse. The Journal of clinical investigation. 1999; 104:551–558. [PubMed: 10487769]

Broberg CS, Pantely GA, Barber BJ, Mack GK, Lee K, Thigpen T, Davis LE, Sahn D, Hohimer AR. Validation of the myocardial performance index by echocardiography in mice: a noninvasive measure of left ventricular function. J Am Soc Echocardiogr. 2003; 16:814–823. [PubMed: 12878990]

Depre C, Wang Q, Yan L, Hedhli N, Peter P, Chen L, Hong C, Hittinger L, Ghaleb B, Sadoshima J, et al. Activation of the cardiac proteasome during pressure overload promotes ventricular hypertrophy. Circulation. 2006; 114:1821–1828. [PubMed: 17043166]

Derumeaux G, Ichinose F, Raher MJ, Morgan JG, Coman T, Lee C, Cuesta JM, Thibault H, Bloch KD, Picard MH, et al. Myocardial alterations in senescent mice and effect of exercise training: a strain rate imaging study. Circ Cardiovasc Imaging. 2008; 1:227–234. [PubMed: 19808547]

Droogmans S, Lauwers R, Cosyns B, Roosens B, Franken PR, Weytjens C, Bossuyt A, Lahoutte T, Schoors D, Van Camp G. Impact of anesthesia on valvular function in normal rats during echocardiography. Ultrasound in medicine & biology. 2008; 34:1564–72. [PubMed: 18455290]

Du J, Liu J, Feng HZ, Hossain MM, Gobara N, Zhang C, Li Y, Jean-Charles PY, Jin JP, Huang XP. Impaired relaxation is the main manifestation in transgenic mice expressing a restrictive cardiomyopathy mutation, R193H, in cardiac TnI. Am J Physiol Heart Circ Physiol. 2008; 294:H2604–2613. [PubMed: 18408133]

Feintuch A, Ruengsakulrach P, Lin A, Zhang J, Zhou YQ, Bishop J, Davidson L, Courtman D, Foster FS, Steinman DA, et al. Hemodynamics in the mouse aortic arch as assessed by MRI, ultrasound, and numerical modeling. Am J Physiol Heart Circ Physiol. 2007; 292:H884–892. [PubMed: 17012350]

French BA, Li Y, Klibanov AL, Yang Z, Hossack JA. 3D perfusion mapping in post-infarct mice using myocardial contrast echocardiography. Ultrasound in medicine & biology. 2006; 32:805–815. [PubMed: 16785003]

Gao S, Yan L, Hong C, Zhao X, Chen L, Shen YV, SF, Vatner D. Enhanced Coronary Reserve Mediated by Nitric Oxide in Adenylyl Cyclase Type 5 Knockout. Circulation. 2008a; 118:367.

Gao S, Yan L, Hong C, Zhao X, Chen L, Shen YV, SF, Vatner D. Reduced Coronary Reserve as a Mechanism in Beta-Adrenergic Receptor Mediated Cardiomyopathy and its Rescue by Adenylyl Cyclase Type 5 Knockout. Circulation. 2008b; 118:562.

Gardin JM, Siri FM, Kitsis RN, Edwards JG, Leinwand LA. Echocardiographic assessment of left ventricular mass and systolic function in mice. Circulation research. 1995; 76:907–914. [PubMed: 7729009]

Gelpi RJ, Gao S, Zhai P, Yan L, Hong C, Danridge LM, Ge H, Maejima Y, Donato M, Yokota M, et al. Genetic inhibition of calcineurin induces diastolic dysfunction in mice with chronic pressure overload. Am J Physiol Heart Circ Physiol. 2009; 297:H1814–1819. [PubMed: 19717730]

Guellich A, Gao S, Hong C, Yan L, Wagner TE, Dhar SK, Ghaleb B, Hittinger L, Iwatsubo K, Ishikawa Y, et al. Effects of Cardiac Overexpression of Type 6 Adenylyl Cyclase Affects on the Response to Chronic Pressure Overload. Am J Physiol Heart Circ Physiol. 2010

Hart CY, Burnett JC Jr, Redfield MM. Effects of avertin versus xylazine-ketamine anesthesia on cardiac function in normal mice. Am J Physiol Heart Circ Physiol. 2001; 281:H1938–1945. [PubMed: 11668054]

Hartley CJ, Reddy AK, Madala S, Michael LH, Entman ML, Taffet GE. Doppler estimation of reduced coronary flow reserve in mice with pressure overload cardiac hypertrophy. Ultrasound in medicine & biology. 2008; 34:892–901. [PubMed: 18255218]

Iwase M, Bishop SP, Uechi M, Vatner DE, Shannon RP, Kudej RK, Wight DC, Wagner TE, Ishikawa Y, Homcy CJ, et al. Adverse effects of chronic endogenous sympathetic drive induced by cardiac GS alpha overexpression. Circulation research. 1996; 78:517–524. [PubMed: 8635208]

Iwase M, Uechi M, Vatner DE, Asai K, Shannon RP, Kudej RK, Wagner TE, Wight DC, Patrick TA, Ishikawa Y, et al. Cardiomyopathy induced by cardiac Gs alpha overexpression. The American journal of physiology. 1997; 272:H585–589. [PubMed: 9038982]

Jassal DS, Han SY, Hans C, Sharma A, Fang T, Ahmadie R, Lytwyn M, Walker JR, Bhalla RS, Czarnecki A, et al. Utility of tissue Doppler and strain rate imaging in the early detection of trastuzumab and anthracycline mediated cardiomyopathy. J Am Soc Echocardiogr. 2009; 22:418–424. [PubMed: 19269133]

Kaufmann BA, Lankford M, Behm CZ, French BA, Klibanov AL, Xu Y, Lindner JR. High-resolution myocardial perfusion imaging in mice with high-frequency echocardiographic detection of a depot contrast agent. J Am Soc Echocardiogr. 2007; 20:136–143. [PubMed: 17275698]

Kreissl MC, Wu HM, Stout DB, Ladno W, Schindler TH, Zhang X, Prior JO, Prins ML, Chatziioannou AF, Huang SC, et al. Noninvasive measurement of cardiovascular function in mice with high-temporal-resolution small-animal PET. J Nucl Med. 2006; 47:974–980. [PubMed: 16741307]

Luo J, Fujikura K, Homma S, Konofagou EE. Myocardial elastography at both high temporal and spatial resolution for the detection of infarcts. Ultrasound in medicine & biology. 2007; 33:1206–1223. [PubMed: 17570577]

Mor-Avi V, Korcarz C, Fentzke RC, Lin H, Leiden JM, Lang RM. Quantitative evaluation of left ventricular function in a Transgenic Mouse model of dilated cardiomyopathy with 2-dimensional contrast echocardiography. J Am Soc Echocardiogr. 1999; 12:209–214. [PubMed: 10070185]

Nahrendorf M, Badea C, Hedlund LW, Figueiredo JL, Sosnovik DE, Johnson GA, Weissleder R. High-resolution imaging of murine myocardial infarction with delayed-enhancement cine micro-CT. Am J Physiol Heart Circ Physiol. 2007; 292:H3172–3178. [PubMed: 17322414]

Odashima M, Usui S, Takagi H, Hong C, Liu J, Yokota M, Sadoshima J. Inhibition of endogenous Mst1 prevents apoptosis and cardiac dysfunction without affecting cardiac hypertrophy after myocardial infarction. Circulation research. 2007; 100:1344–1352. [PubMed: 17395874]

Odley A, Hahn HS, Lynch RA, Marreez Y, Osinska H, Robbins J, Dorn GW 2nd. Regulation of cardiac contractility by Rab4-modulated beta2-adrenergic receptor recycling. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:7082–7087. [PubMed: 15105445]

Ohno M, Cheng CP, Little WC. Mechanism of altered patterns of left ventricular filling during the development of congestive heart failure. Circulation. 1994; 89:2241–2250. [PubMed: 8181149]

Peng Y, Popovic ZB, Sopko N, Drinko J, Zhang Z, Thomas JD, Penn MS. Speckle tracking echocardiography in the assessment of mouse models of cardiac dysfunction. Am J Physiol Heart Circ Physiol. 2009; 297:H811–820. [PubMed: 19561310]

Peter PS, Brady JE, Yan L, Chen W, Engelhardt S, Wang Y, Sadoshima J, Vatner SF, Vatner DE. Inhibition of p38 alpha MAPK rescues cardiomyopathy induced by overexpressed beta 2-adrenergic receptor, but not beta 1-adrenergic receptor. The Journal of clinical investigation. 2007; 117:1335–1343. [PubMed: 17446930]

Raher MJ, Thibault H, Poh KK, Liu R, Halpern EF, Derumeaux G, Ichinose F, Zapol WM, Bloch KD, Picard MH, et al. In vivo characterization of murine myocardial perfusion with myocardial contrast echocardiography: validation and application in nitric oxide synthase 3 deficient mice. Circulation. 2007; 116:1250–1257. [PubMed: 17709634]

Roth DM, Swaney JS, Dalton ND, Gilpin EA, Ross J Jr. Impact of anesthesia on cardiac function during echocardiography in mice. Am J Physiol Heart Circ Physiol. 2002; 282:H2134–2140. [PubMed: 12003821]

Rottman JN, Ni G, Brown M. Echocardiographic evaluation of ventricular function in mice. Echocardiography (Mount Kisco, NY). 2007; 24:83–89.

Rottman JN, Ni G, Khoo M, Wang Z, Zhang W, Anderson ME, Madu EC. Temporal changes in ventricular function assessed echocardiographically in conscious and anesthetized mice. J Am Soc Echocardiogr. 2003; 16:1150–1157. [PubMed: 14608286]

Saraste A, Kyto V, Saraste M, Vuorinen T, Hartiala J, Saukko P. Coronary flow reserve and heart failure in experimental coxsackievirus myocarditis. A transthoracic Doppler echocardiography study. Am J Physiol Heart Circ Physiol. 2006; 291:H871–875. [PubMed: 16501009]

Schaefer A, Klein G, Brand B, Lippolt P, Drexler H, Meyer GP. Evaluation of left ventricular diastolic function by pulsed Doppler tissue imaging in mice. J Am Soc Echocardiogr. 2003; 16:1144–1149. [PubMed: 14608285]

Schaefer A, Meyer GP, Hilfiker-Kleiner D, Brand B, Drexler H, Klein G. Evaluation of Tissue Doppler Tei index for global left ventricular function in mice after myocardial infarction: comparison with Pulsed Doppler Tei index. Eur J Echocardiogr. 2005; 6:367–375. [PubMed: 16153558]

Scherrer-Crosbie M, Steudel W, Ullrich R, Hunziker PR, Liel-Cohen N, Newell J, Zaroff J, Zapol WM, Picard MH. Echocardiographic determination of risk area size in a murine model of myocardial ischemia. The American journal of physiology. 1999; 277:H986–992. [PubMed: 10484420]

Scherrer-Crosbie M, Thibault HB. Echocardiography in translational research: of mice and men. J Am Soc Echocardiogr. 2008; 21:1083–1092. [PubMed: 18723318]

Schmidt AG, Gerst M, Zhai J, Carr AN, Pater L, Kranias EG, Hoit BD. Evaluation of left ventricular diastolic function from spectral and color M-mode Doppler in genetically altered mice. J Am Soc Echocardiogr. 2002; 15:1065–1073. [PubMed: 12373248]

Sebag IA, Handschumacher MD, Ichinose F, Morgan JG, Hataishi R, Rodrigues AC, Guerrero JL, Steudel W, Raher MJ, Halpern EF, et al. Quantitative assessment of regional myocardial function in mice by tissue Doppler imaging: comparison with hemodynamics and sonomicrometry. Circulation. 2005; 111:2611–2616. [PubMed: 15897347]

Semeniuk LM, Severson DL, Kryski AJ, Swirp SL, Molkentin JD, Duff HJ. Time-dependent systolic and diastolic function in mice overexpressing calcineurin. Am J Physiol Heart Circ Physiol. 2003; 284:H425–430. [PubMed: 12388248]

Slawson SE, Roman BB, Williams DS, Koretsky AP. Cardiac MRI of the normal and hypertrophied mouse heart. Magn Reson Med. 1998; 39:980–987. [PubMed: 9621922]

Stypmann J. Doppler ultrasound in mice. Echocardiography (Mount Kisco, NY). 2007; 24:97–112.

Syed F, Diwan A, Hahn HS. Murine echocardiography: a practical approach for phenotyping genetically manipulated and surgically modeled mice. J Am Soc Echocardiogr. 2005; 18:982–990. [PubMed: 16153531]

Tan TP, Gao XM, Krawczyszyn M, Feng X, Kiriazis H, Dart AM, Du XJ. Assessment of cardiac function by echocardiography in conscious and anesthetized mice: importance of the autonomic nervous system and disease state. Journal of cardiovascular pharmacology. 2003; 42:182–190. [PubMed: 12883320]

Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, Ross J Jr. Transthoracic echocardiography in models of cardiac disease in the mouse. Circulation. 1996; 94:1109–1117. [PubMed: 8790053]

Thibault H, Gomez L, Donal E, Pontier G, Scherrer-Crosbie M, Ovize M, Derumeaux G. Acute myocardial infarction in mice: assessment of transmurality by strain rate imaging. Am J Physiol Heart Circ Physiol. 2007; 293:H496–502. [PubMed: 17384134]

Tsujita Y, Kato T, Sussman MA. Evaluation of left ventricular function in cardiomyopathic mice by tissue Doppler and color M-mode Doppler echocardiography. Echocardiography (Mount Kisco, NY. 2005; 22:245–253.

Vatner, S.; Takagi, G.; Asai, K.; Shannon, RP. Cardiovascular physiology in mice: Conscious measurements and effects of anesthesia. In: Hoit, RWBD., editor. Cardiovascular Physiology in the Genetically Engineered Mouse. Massachusetts: Kluwer Academic Publishers; 2002. p. 257-275.

Vatner SF, Braunwald E. Cardiovascular control mechanisms in the conscious state. The New England journal of medicine. 1975; 293:970–976. [PubMed: 1101063]

Wiesmann F, Ruff J, Engelhardt S, Hein L, Dienesch C, Leupold A, Illinger R, Frydrychowicz A, Hiller KH, Rommel E, et al. Dobutamine-stress magnetic resonance microimaging in mice : acute changes of cardiac geometry and function in normal and failing murine hearts. Circulation research. 2001; 88:563–569. [PubMed: 11282889]

Wikstrom J, Gronros J, Bergstrom G, Gan LM. Functional and morphologic imaging of coronary atherosclerosis in living mice using high-resolution color Doppler echocardiography and ultrasound biomicroscopy. Journal of the American College of Cardiology. 2005; 46:720–727. [PubMed: 16098442]

Wikstrom J, Gronros J, Gan LM. Adenosine induces dilation of epicardial coronary arteries in mice: relationship between coronary flow velocity reserve and coronary flow reserve in vivo using transthoracic echocardiography. Ultrasound in medicine & biology. 2008; 34:1053–1062. [PubMed: 18313201]

Williams R, Needles A, Cherin E, Zhou YQ, Henkelman RM, Adamson SL, Foster FS. Noninvasive ultrasonic measurement of regional and local pulse-wave velocity in mice. Ultrasound in medicine & biology. 2007; 33:1368–1375. [PubMed: 17561330]

Yang XP, Liu YH, Rhaleb NE, Kurihara N, Kim HE, Carretero OA. Echocardiographic assessment of cardiac function in conscious and anesthetized mice. The American journal of physiology. 1999; 277:H1967–1974. [PubMed: 10564153]

Yang Z, Berr SS, Gilson WD, Toufektsian MC, French BA. Simultaneous evaluation of infarct size and cardiac function in intact mice by contrast-enhanced cardiac magnetic resonance imaging reveals contractile dysfunction in noninfarcted regions early after myocardial infarction. Circulation. 2004; 109:1161–1167. [PubMed: 14967719]

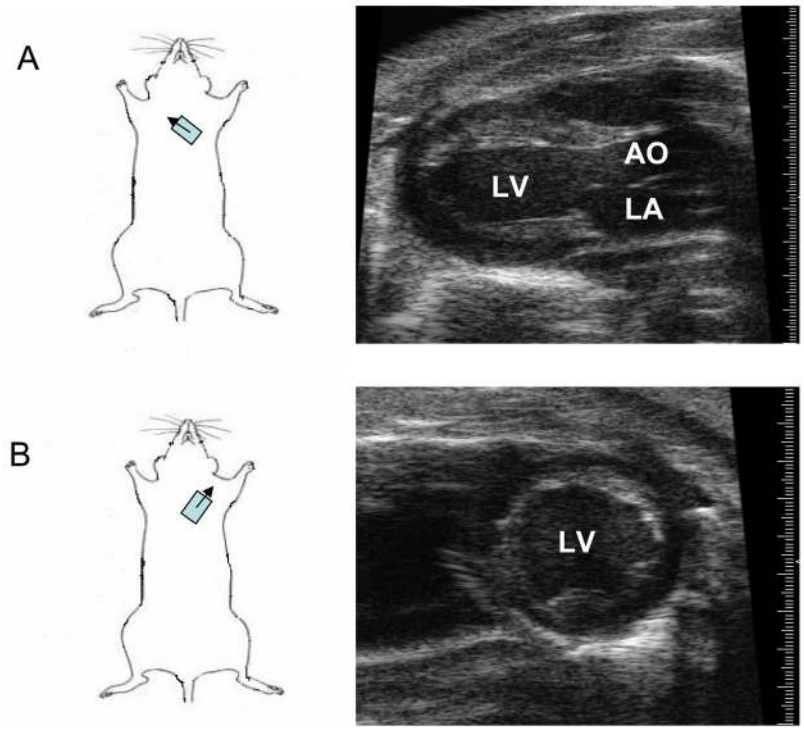

Figure 1.
Diagrams for basic mouse echocardiography views. Panel A shows the position and direction (small arrow) of probe (upper left) for LV long-axis view (upper right). Panel B demonstrates the position and direction (small arrow) of probe (lower left) for LV short-axis view (lower right). LA=left atrium, AO=aorta.

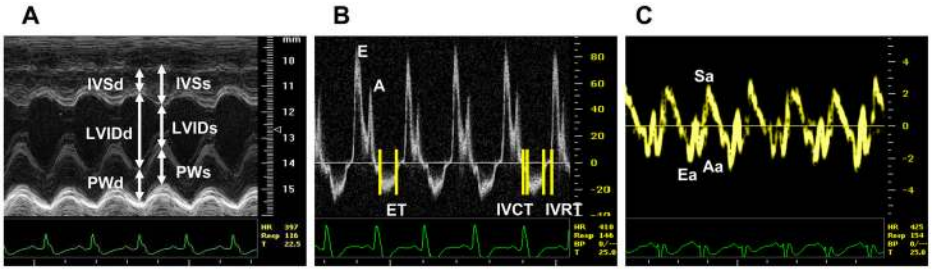

Figure 2.

Images of echocardiographic measurements in mice. (A) LV M-mode, allows for assessment of LV systolic function. (IVSd, LVIDd, PWd and IVSs, LVIDs, PWs are LV interventricular septum thicknesses, LV internal dimensions and LV posterior wall thicknesses at diastole and systole, respectively.) (B) Doppler of transmitral inflow most often used for evaluation of LV diastolic function. (E and A are peak velocities at early and late filling respectively. IVRT and IVCT are isovolumetric relaxation and contraction time. ET is LV ejection time.) (C) Tissue Doppler waveform obtained in LV posterior wall, used for assessing regional wall motion abnormality. (Ea and Aa were two waveforms at early and late diastolic phases. Sa is the peak wall motion velocity in systole.)

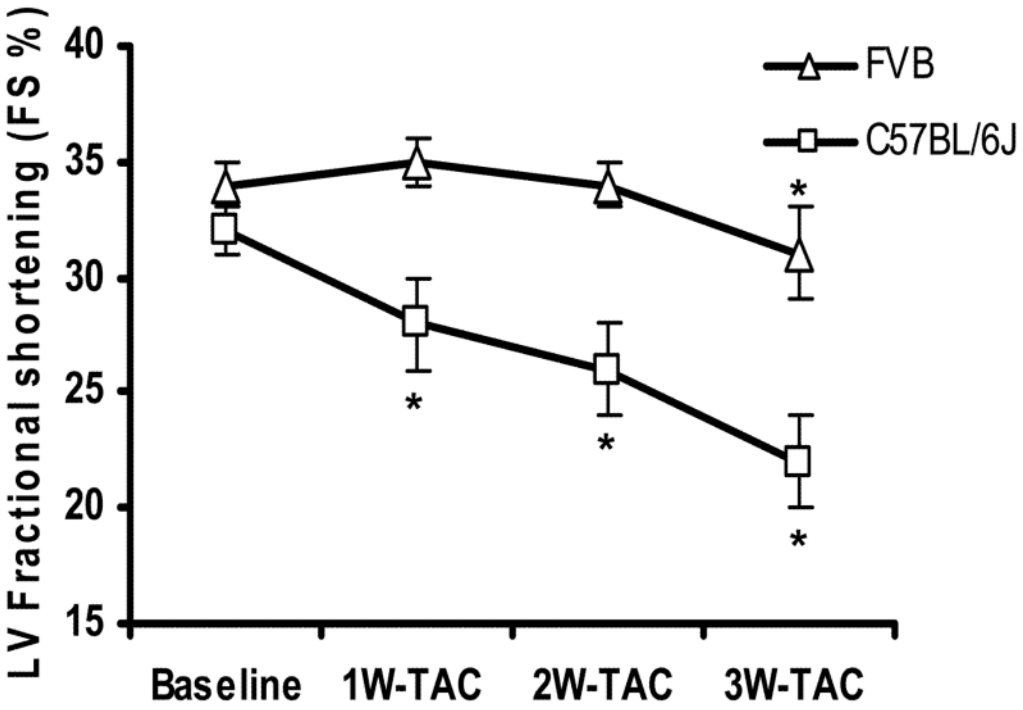

Figure 3.
Comparing fractional shortening (FS) in two strains of mice (FVB, C57BL/6J) before and after 1, 2, and 3 weeks of pressure overload induced by transverse aortic constriction (TAC). FS was significantly decreased in C57BL mice (square) even 1 week after TAC. However, in FVB mice (triangle) FS was maintained at normal levels even after 2 weeks of TAC. *p<0.05 vs baseline. (FS=fractional shortening).

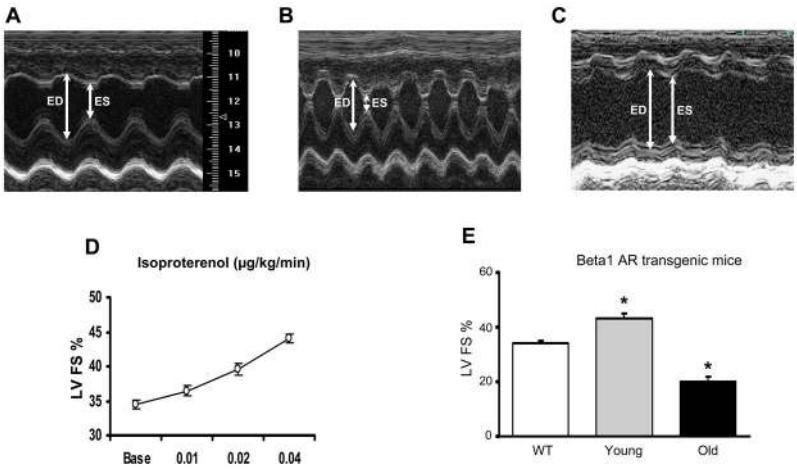

Figure 4.

These are representative images and echocardiography data displaying changes in LV fractional shortening (FS) with isoproterenol and cardiomyopathy. (A) Represents a baseline image (ES=end systole, ED=end diastole). (B) After infusion with isoproterenol 0.04 μg/kg/min, LV contraction was markedly increased. (C) In transgenic mice with cardiomyopathy a clear decrease in LV contraction is observed. (D) LV FS increases with increasing doses of isoproterenol. (E) LV FS is enhanced in young transgenic mice over-expressing β1-adrenergic receptors in the heart (β1-AR Tg, gray bar) as compared to wild type (WT, white bar). However, as the mice develop cardiomyopathy with age (black bar) LV FS is found to be decreased. * $p<0.05$ vs WT. (Peter *et al.* 2007). Figure modified and used with permission.

Table 1

Fractional shortening (FS) and heart rate (HR) in conscious and anesthetized mice

Type of Anesthesia	None (Conscious)	Avertin	pentobarbital	K+X	isoflurane
FS (%)	59±5	35±0.4	33±2	35±1	39±1
HR (bpm)	683±63	411±17	377±11	293±19	457±17
Dose	none	300 mg/kg ip	50 mg/kg ip	150+15 mg/kg, ip	2% inhalation
Data resource	Rottman et al., 2003	Yan et al., 2007	Yang et al., 1999	Yang et al., 1999	Stypmann, 2007

All values are mean ± SE. The heart rate and systolic function in conscious mice were much higher than those seen in anesthetized mice. (K=ketamine, X=xylazine; FS=fractional shortening; HR=heart rate; IP=intraperitoneal injection).

Table 2

LV diastolic function: Transmitral Doppler parameters at baseline and at 2 weeks after TAC in FVB mice

	Baseline	2 week TAC
E wave velocity(cm/s)	54.2±2.7	61.9±3.5
A wave velocity(cm/s)	43.8±2.8	28.0±4.1*
E/A	1.26±0.06	2.5±0.4*
DT(ms)	18.6±1.7	21.9±2.3
IVRT(ms)	17.5±0.4	14.8±0.7*
R-R(ms)	133±3.4	136±5.8
ET of LVOT(ms)	53.3±1.0	48.9±1.3*
(IVRT+IVCT)/ET	0.43±0.03	0.59±0.05*
n	11	10

All the values are mean ± SE.

* $p<0.05$ vs baseline. After 2 weeks of TAC, there was clearly LV diastolic dysfunction as reflected by a decrease in A wave velocity, increase in E/A ratio as well as myocardial performance index (IVRT+IVCT)/ET. (DT=deceleration time; IVRT=isovolumetric relaxation time; R-R=interval between R waves in EKG; ET=LV ejection time; n=number of mice).