DOI: 10.1111/j.1540-8175.2006.00356.x

**IMAGING IN BASIC SCIENCE. MOUSE-ECHO: FROM BEDSIDE TO BENCH: PART II**

# Echocardiographic Evaluation of Ventricular Function in Mice

Jeffrey N. Rottman, M.D.*†‡ Gemin Ni, M.D.,*‡ and Michael Brown, B.A.‡§

*Division of Cardiovascular Medicine, Department of Internal Medicine, †Department of Pharmacology, and ‡Mouse Metabolic Phenotyping Center, Vanderbilt University School of Medicine, Nashville, Tennessee; and §Meharry Medical College, Nashville, Tennessee

*Ventricular dysfunction remains a hallmark of most cardiac disease. The mouse has become an essential model system for cardiovascular biology, and echocardiography an established tool in the study of normal and genetically altered mice. This review describes the measurement of ventricular function, most often left ventricular function, by echocardiographic methods in mice. Technical limitations related to the small size and rapid heart rate in the mouse initially argued for the performance of echocardiography under anesthesia. More recently, higher frame rates and smaller probes operating at higher frequencies have facilitated imaging of conscious mice in some, but not all, experimental protocols and conditions. Ventricular function may be qualitatively and quantitatively evaluated under both conditions. Particular detail is provided for measurement under conscious conditions, and measurement under conscious and sedated or anesthestized conditions are contrasted. Normal values for echocardiographic indices for the common C57BL/6 strain are provided. Diastolic dysfunction is a critical pathophysiologic component of many disease states, and progress in the echocardiographic evaluation of diastolic function is discussed. Finally, echocardiography exists among several competing imaging technologies, and these alternatives are compared. (ECHOCARDIOGRAPHY, Volume 24, January 2007)*

*ventricular function*, *murine echocardiography*, *anesthesia effects*

## Echocardiographic Evaluation of Ventricular Function in Mice

The mouse has become an essential model system for cardiovascular biology, providing a nexus between experimental biology and a technology for germline alteration of the mammalian genome. With this, echocardiography has become an essential and established tool in the study of normal and genetically altered mice.[1,2] One compelling advantage of echocardiography is that the technology as well as the conceptual framework transparently translate from the human to the mouse. Technical limitations related to the small size and rapid heart rate (HR) in the mouse initially argued for the performance of echocardiography under anesthesia,[3,4] and data from this experience is reflected in several excellent recent reviews.[5–7] More recently, higher frame rates, and smaller probes operating at higher frequencies, have facilitated imaging of conscious mice in some, but not all, experimental protocols and conditions.[8–10] This review describes the measurement of ventricular function, most often left ventricular function, by echocardiographic methods in mice. Particular detail is provided for measurement under conscious conditions, and measurement under conscious and sedated or anesthestized conditions are contrasted. Normal values for echocardiographic indices for the common C57BL/6 strain are provided. Diastolic dysfunction is a critical pathophysiologic component of many disease states, and progress in the echocardiographic evaluation of diastolic function is discussed. Finally, echocardiography exists among several competing imaging

Address for correspondence and reprint requests: Jeffrey N. Rottman, M.D., Vanderbilt University School of Medicine, Division of Cardiovascular Medicine, 371 PRB, 2220 Pierce Ave., Nashville, TN 37232—6300. Fax: 615-322-7919; E-mail: jeff.rottman@vanderbilt.edu

technologies, and these alternatives are compared.

## Basic Measurements of Left Ventricular Function

The hallmark of many cardiac diseases in man is systolic dysfunction of the ventricle, and the same is true for models of these conditions in mice. In the absence of regional wall motion abnormalities, fractional shortening (FS%) and ejection fraction (EF) are predictably related. In the mouse, the extent of typical cavity obliteration is far greater than in man, so that the normal EF is near 0.90. It is therefore much more common to use FS% as the basic measure of ventricular function; this is by far the most commonly requested method to evaluate LV function in our murine echocardiographic core. To accomplish this measurement, wall thickness and chamber dimension are determined from M-mode tracings Figure 1. LV wall thickness is evaluated in the interventricular septum (IVS) and the posterior wall (LVPW). End-diastolic measurements (IVSd, LVPWd, and left ventricular internal dimension, diastolic [LVIDd]) are obtained at the point of maximal LV diastolic dimension. LV end-systolic dimensions (IVSs, LVPWs, and LVIDs) are obtained at the time of most anterior systolic excursion of the LVPW associated with minimal chamber dimension. All LV dimensions are presented as the average of measurements using the leading-edge technique of 3 to 5 consecutive selected sinus beats by two experienced readers.[11] FS% is then calculated from M-mode-derived LV dimensions using the formula (LVIDd – LVIDs) /LVIDd × 100%. Concurrently, HR is determined from the cardiac cycles recorded on the M-mode tracing, using at least 3 consecutive beats. When regional wall motion abnormalities are present, alternative approach is required; this is explored in another article in this series.

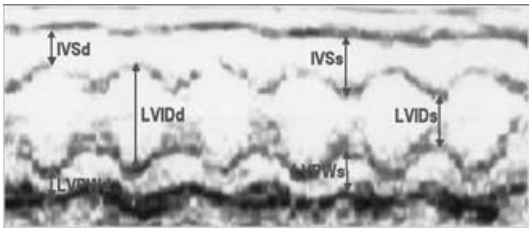


**Figure 1.** *Echocardiographic measurements for determination of left ventricular function from M-mode measurements obtained with conscious transthoracic echocardiography in mice.*

## Technique for Conscious Echocardiographic Recording

The majority of our echocardiographic studies are performed under conscious conditions.[12] Transthoracic echocardiography is performed using a Sonos 5500 (Agilent, Andover, MA, USA) with a 15 MHz high frequency linear transducer at a frame rate of 100 frames/sec, with images acquired at a depth setting of 20 mm. Prior to initiation of the study, the mice are trained on two separate occasions over 1–2 days. Training includes holding the mice in the position required for echocardiographic imaging for at least 5 minutes, and touching the chest in simulation of probe contact. Chest contact is performed first with a plastic probe, and then, to acclimate the mice to the sensation of the gel, with a metallic probe at 34 °C.

In the conscious mice, we perform echocardiography by picking up the mouse by the nape of the neck and holding it in one hand in the prone position, with the tail held between the last two fingers. The prone position appears to be an essential element in minimizing the vagal/bradycardic response to echocardiography. Typically, the mouse chest area is not shaved; ultrasound-coupling gel heated to 34 °C is applied to the precordium, with the ultrasound probe beneath the animal. The same positioning and approach can be used in anesthetized mice, but a second operator is more often required to manipulate the probe and record images while the animal was fully supported. Alternatively, a carved support can be used to maintain the mouse in a decubitus position for prolonged echocardiographic acquisitions under anesthetized conditions. Other laboratories routinely shave the mice prior to echocardiography; this has not been necessary for adequate image quality in our hands, but this may depend on mouse strain and equipment characteristics. Under both conscious and anesthetized conditions, attention must be paid to maintenance of body temperature, and this is even more critical when anesthesia, long protocols, or shaving is used. Optimal parasternal long- and short-axis views are obtained by adjusting gain settings for visualization of endocardial and epicardial walls. Two-dimensional targeted M-mode echocardiographic images are obtained at the level of the papillary muscles from the

**Table I**

Normal Echocardiographic Parameters for Conscious C57BL/6 Mice

| Category | Name | Units | Mean | Standard Deviation |
|---|---|---|---|---|
| | IVSd | cm | 0.092 | 0.014 |
| Diastolic dimensions | LVIDd | cm | 0.302 | 0.032 |
| | LVPWd | cm | 0.093 | 0.023 |
| | IVSs | cm | 0.168 | 0.025 |
| Systolic dimensions | LVIDs | cm | 0.124 | 0.019 |
| | LVPWs | cm | 0.138 | 0.025 |
| Contractile function | FS% | | 59 | 4.7 |
| Heart rate | HR | bpm | 683 | 63 |

parasternal short-axis view and recorded at a speed of 150 cm/sec (maximal temporal resolution) for measurement of HR. All other measurements are made on screen using the digitally recorded signals, but recorded as well for documentation and archival purposes.

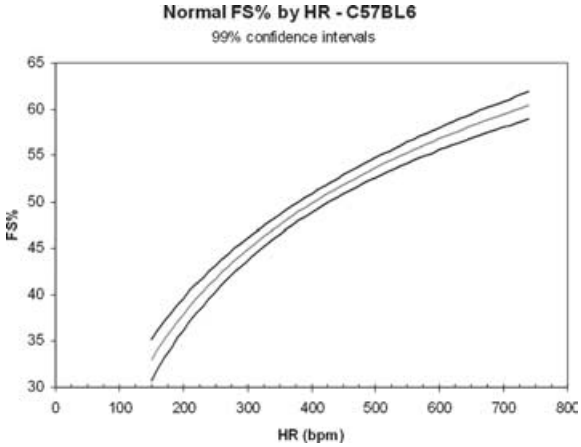


**Figure 2.** *Relationship between heart rate (HR) and fractional shortening (FS%), conscious conditions, and with anesthetic agents suppressing HR. Aggregate least-squares log-linear relationships between mean HR (beats/min, bpm) and FS% with confidence intervals illustrated.*

## Normal Values and Effects of Anesthesia

This C57BL/6 strain represents the predominant strain basis for mouse metabolic physiology, in part due to its susceptibility to diet-induced obesity and insulin resistance,[13] and represents the background for a broad range of transgenic and germline-altered models (see http://www.informatics.jax.org/external/testing/mouse/STRAINS.shtml). Normal echocardiographic values for adult C57BL/6 mice from our laboratory are presented in Table I. We evaluated the influence of sex, age, and body weight (BW) on echocardiographic measurements by regression analysis. Multivariate analysis demonstrated that body mass predicted echocardiographic LV mass, but did not alter LV function.[12] HRs associated with echocardiographic measurement decreased slightly with advancing age. The regression analysis of echocardiographic dimensions with the same covariates demonstrated that the increase in heart mass was a consequence of increased wall thickness, rather than an increase in chamber dimension. There is a high degree of consistency on these measurements during both sequential and repeated measurements, arguing strongly for the representative and robust nature of these echocardiographic normal values.

A remarkably consistent set of data shows marked changes in echocardiographic variables in mice as a consequence of anesthesia.[8,10,12,14–17] Common regimens for echocardiographic evaluation include ketamine-xylazine,[4,10,18] ketamine-acepromazine,[19] pentobarbitol,[10,20] and inhalation agents. These agents differ with respect to duration and depth of anesthesia; however, while anesthesia depresses HR, the HR:FS% relationship assessed by regression analysis appears roughly independent of the presence or choice of anesthesia, thus allowing prediction of an overall relationship (Fig. 2). A logarithmic relationship explained the greatest fraction of the variance (66%): $FS\% = 17.17\ ln(HR) - 53$. Thus, when the experimental protocol requires repeated measurements but does not otherwise impose a requirement for anesthesia, sequential measurements under conscious conditions are likely to be possible, can provide consistent values, and may be preferable. In the conscious mouse, the echocardiogram also allows assessment of the ventricular (heart) rate, an important physiological parameter. When anesthesia is required, appropriate choice of an anesthetic agent, a narrow time window for performance, and concurrent measurement of HR with the echocardiographic dimensions are advisable, and should decrease experimental variability.

Considerable variability in HRs among mouse strains and with modest differences in level of activity (e.g., quietly resting vs. feeding/grooming) has been noted,[21–23] as well as phenotypic variation within a strain.[16] This

includes variation in anesthetic response and echocardiographic measurements of HR and FS% in animals obtained from different vendors.[16] Genetic background can also modulate the cardiovascular manifestations of germline genetic alterations, with recent progress in even identifying responsible genetic loci.[24] Normal values should therefore be considered strain specific. Since there are inevitable differences in genetic background even on backcrossed animals, ideally mice should be compared with littermate controls.

## Diastolic Function and Inotropic Stimulation

Diastolic dysfunction is more difficult to define, and more difficult to measure, than systolic dysfunction. Transmitral filling can be evaluated echocardiographically, and alterations in the magnitude of the A-wave, or the E/A ratio, have been used to define abnormal diastolic function in a variety of genetically or environmentally altered mice.[25–28] However, significant variations in these measurements, and the dependence of this index on loading, age, and HR, make it more useful for direct comparison among groups than for comparison with nominal normal values. One important limitation is that at physiologic HRs, the E- and A-waves are typically fused, and thus slowing of the HR, usually with anesthesia, is required for these measurements, with the consequent physiologic perturbations.[28–31] Color imaging can be used to provide correlative data supporting changes in inflow pattern as evidence of diastolic dysfunction.[26] Other indices such as isovolumetric relaxation times or the Tei index[32] and Doppler tissue imaging[33] have also been evaluated for measurement of diastolic dysfunction.

Adrenergic stimulation can be used to increase HR and ventricular contractility in the mouse.[34,35] Imaging under these stimulated conditions can reveal differences in systolic or diastolic function not apparent on basal measurements.[36] We have found isoproterenol at a dose of 2$\mu$g/g BW to be effective and well-tolerated for this purpose in conjunction with conscious echocardiographic imaging. A variety of arrhythmias, including paradoxical bradyarrhythmias, can be observed, so simultaneous assessment of heart rhythm and rate and well as ventricular function is required. Even in rats, direct measurement of cardiac output using Doppler is technically challenging.[37] An indirect measure, based on computed stroke volume and HR, may, however, be sufficient for comparative purposes in many experimental protocols. When invasive measurements are not available, a tail-cuff system can be reliably applied in mice[38] to provide an index of afterload in conjunction with echocardiographic estimates of ventricular morphology and function.

## Alternative and Complementary Approaches to Evaluating LV Function

Catheterization can be performed to provide a measure of inotropic state using $+dP/dt_{max}$; this measure is preload dependent, and may be regarded as a less useful measure of basal contractile state than LV FS%.[6,39] Measurement of the diastolic properties of the left ventricle can be estimated from indices such as the maximal rate of pressure decay ($-dP/dt_{max}$), or relaxation half time ($RT_{1/2}$), but like many echocardiographic measurements these values can depend on loading, HR, and a variety of other factors.[40] Retrograde catheterization of the left ventricle directly is technically demanding and requires significant sedation, and in the small mouse heart even microcatheters represent a significant fraction of cavity space and aortic valve area. Direct transduction of central arterial pressure is possible in a conscious chronically instrumented state, and can even be combined with exercise or metabolic protocols.[21,41] However, these measurements represent a combined index of ventricular and vascular function, rather than a measure of LV function per se. A dual-frequency conductance catheter can be used to directly measure ventricular volume in the mouse, and thus, stroke volume and cardiac output, or even pressure–volume relationships.[42] This requires, however, both complex technology and a very specific surgical setup.

## Nuclear Imaging

In man, specific isotopes can be used to evaluate ventricular function and perfusion, and to delineate further aspects of myocardial metabolism and function. Similar studies can be performed in the mouse, but technical factors still limit the versatility of this approach. For example, on static images microPET can delineate changes in relative glucose and fatty acid tracer uptake,[43] and by tissue analysis, relative uptake can be assessed in both exercise and basal conditions.[44] Imaging of myocardial perfusion using $^{13}N$ ammonia using positron emission tomography (PET) has been recently

reported.[45] In the rat, regional perfusion defects can be defined with a high-resolution microPET system,[46] and techniques using a specific tracer have allowed in vivo imaging of exogenous gene expression in the heart.[47] Thus nuclear imaging can provide essential information about these nonmechanical aspects of ventricular function in mice. However, the most mundane sort of cardiac nuclear measurement in man, the simple measure of EF under conscious conditions, is still beyond current nuclear or CT technical capabilities.

### Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) in the mouse represents a remarkably powerful and rapidly evolving technology. The technical challenges for cardiac MR imaging in the mouse, common to many of the modalities described, relate to the small size and rapid HR. The advantages of MR include the potentially high spatial and temporal resolution, the fundamentally tomographic nature of the technology, and the capability to couple physiologic and anatomic studies (reviewed RG Weiss[48]). Progress in MRI imaging depends on both improvements in software and imaging protocols as well increasing magnetic field strength. By 2000, using a 4.7 Tesla magnet, superb pictures gated throughout the cardiac cycle were available at HRs approaching physiologic (about 600 bpm), although total acquisition times of 2–8 minutes were required.[49] These anatomic imaging studies were coupled with $^{31}$P magnetic resonance spectroscopic studies of high energy phosphates (phosphocreatine-to-adenosine triphosphate ratio along a line transecting the left ventricle). A study 1 year later using a 7 Tesla magnet was able to image under basal and dobutamine-stress conditions, with images obtained at HRs up to 750 bpm; these data were able to define differences in adrenergically stimulated systolic and diastolic function in mice with heart failure due to chronic myocardial infarction, or in mice with cardiac-specific overexpression of the beta-1 adrenergic receptor.[36] Importantly, while baseline LV function differed among normal and chronically infarcted mouse hearts, the difference in LV function between the normal and transgenic mouse hearts was only revealed under stress conditions. More recent studies have evaluated the relationship between contractile function in infarcted and adjacent areas using gadolinium contrast enhancement,[50] used myocardial tagging in the mouse heart,[51] mapped myocardial blood volume using iron oxide nanoparticles,[52] and reported imaging using field strengths up to 11.7 Tesla.[53] MRI imaging is particularly well suited to analysis of right ventricular function and morphology, because no inherent geometric assumptions are required[54]; the right ventricle is not well visualized on transthoracic echo because of its anterior location and complex shape, and echocardiographic analysis in mice has typically relied on transesophageal imaging performed using an IVUS catheter (Sonicath cv, Mansfield Boston Scientific, Watertown, MA, USA).[55] MR inherently trade-offs between spatial and temporal resolution. The asymptotic capabilities are illustrated in images of embryonic mouse hearts allowing detailed 3D reconstruction of 1-mm embryonic mouse hearts at resolutions approaching 20 $\mu$m; however, 9-hour acquisitions on fixed specimens were required, thus defining LV function only in the most anatomic sense![56]

### Summary

Echocardiography is an inexpensive, easily applied, and readily interpreted tool in evaluating ventricular function in mice, using techniques and indices familiar from human echocardiography. Technical advances make echocardiographic measurement of ventricular dimension and function under conscious conditions practical in many experimental protocols. Increasing attention is now directly at measurement of diastolic function and evaluating response to stress. Echocardiographic data may be augmented by direct or indirect hemodynamic measurements, or metabolic measurements, including nuclear imaging. In many respects, MRI already rivals or surpasses echo in its capabilities for studies of ventricular form and function. However, it represents a far less accessible and more expensive and complex technology. The times required for acquisition and the necessity for sedation during the period also limit its potential experimental applications, and diminish its utility for primary evaluations or screens of the cardiac function. Echocardiography and MRI are likely to have complementary roles in the evaluation of ventricular function in mice, depending on experimental, physiologic, and logistic considerations.

*Acknowledgments:* This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant U24-DK-59637, Mouse Metabolic Phenotyping Center (JR), and other grants from the Veterans Administration and American Heart Association (JR).

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