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Ventricular remodeling and function: insights using murine echocardiography

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Summary

Extracellular matrix disturbances play an important role in the development of ventricular remodeling and failure. Genetically modified mice with abnormalities in the synthesis and degradation of extracellular matrix have been generated, in particular mice with deletion or overexpression of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs). Echocardiography is ideally suited to serially evaluate left ventricular (LV) size and function, thus defining the progression of LV remodeling and failure. This Review describes the echocardiographic parameters that may provide insights into the development of ventricular remodeling and heart failure. The application of echocardiography to study LV remodeling and function after myocardial infarction and LV pressure-overload in wild-type mice and mice deficient or overexpressing MMPs or TIMPs is then detailed. Finally, using the example of mice deficient in nitric oxide synthase 3, a cautionary example is given illustrating discrepancies between the cardiac echocardiographic phenotype and modifications of the extracellular matrix.

An increasing body of evidence associates alterations in the extracellular matrix and the development of diastolic and systolic heart failure. Perivascular and interstitial fibrosis were noted in the failing myocardium in the 1940s and linked with the presence of abnormal diastolic function. Additionally, therapies that decrease fibrosis ameliorate diastolic function [1]. Alterations in extracellular matrix can also directly affect cardiomyocyte contractile function by modifying cellular signaling [2]. Last, changes in extra-cellular matrix also impact the changes in cardiac size and shape (i.e. cardiac remodeling) associated with the progression of heart failure. Matrix metalloproteinases (MMPs), a family of enzymes that catabolize collagen, elastin, and gelatin, and their tissue inhibitors (TIMPs) play an important role in cardiac remodeling. It has been demonstrated that several MMPs become activated in heart failure [3]. Recognition of the involvement of MMPs and TIMPs in the development of heart failure has been considerably accelerated by the emergence of genetically-modified mice, deficient or overexpressing MMPs and TIMPs (for a Review, see [4]). Deletion of MMPs have been accompanied by reduced left ventricular (LV) dilation and inflammation after myocardial infarction [5–7]. Conversely, deletion of TIMPs has been accompanied by increased LV

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dilation after myocardial infarction (MI) [8] and a progressive dilated cardiomyopathy with aging [9].

Elucidating the cardiac phenotype of mice necessitates techniques that will allow the characterization of the cardiac structure and function of mice in a serial manner. Mice are very fragile, and their hemodynamic state and cardiac function can be altered significantly by anesthesia [10]. The mouse heart rate approximates 600 beats per minute in awake animals [11], necessitating a sampling rate superior to 120–150Hz in order to best identify end-systole and end-diastole. The average LV end-diastolic diameter in a healthy adult mouse measures 2.5–3 mm, requiring a spatial resolution of the images inferior to 500µm in order to detect a 20% change in LV size.

Hemodynamic studies using a conductance catheter allow the recording of ventricular pressures, relatively load-independent indices of ventricular function such as ventricular elastance, LV volumes and cardiac output. These studies however are invasive and cannot be serially repeated. Nuclear studies of LV function have been proposed however they necessitate an intravenous injection of isotope. The mouse cardiac size and function are well defined using magnetic resonance imaging (MRI). The disadvantage of this technique is that its acquisition time is long and involves deep anesthesia. Echocardiography can be rapidly performed on awake or lightly sedated animals and has a high spatial resolution and rapid imaging rate, making it a procedure of choice for most studies of the mouse heart.

In this Review, we will provide an overview of how mouse echocardiography may help elucidate the role of extra-cellular matrix in heart failure. We will describe the parameters that echocardiography provides to the investigators regarding LV remodeling, systolic and diastolic function, myocardial perfusion, and tissue characterization of the mouse myocardium. We will report an application of mouse echocardiography in the study of a model of increased myocardial fibrosis.

Analysis of mouse echocardiography

Although mouse echocardiography may appear relatively easy to obtain, caution must be applied to several aspects of the technique in order to provide meaningful data. As mentioned, the mouse hemodynamic state is easily impaired by anesthesia. When reporting LV function, investigators should aim for heart rates between 500–700 beats per minute and systolic blood pressures of 100 to 115 mmHg. Lower heart rates due to the effect of anesthesia are accompanied by LV dilation and a decrease in cardiac function [10]. Ideally, echocardiograms should be performed in awake animals. This is feasible after training the mice by repeating the echocardiograms daily for several days [11]. If anesthesia is necessary, a short duration of anesthesia using ketamine at low doses (50 mg/kg intraperitoneally) [12], inhaled isoflurane or metoxyflurane [13] have been recommended. When using the M Mode, investigators also have to be cautious to obtain an image that is perpendicular to the long axis of the heart in order not to overestimate the diameter of the short axis of the LV. When using 2D-dimensional images, the highest frame rate available must be obtained by narrowing the window of interest.

Left Ventricular Size and Systolic Function

The major application of mouse echocardiography is to assess LV size and function. M Mode is the echocardiographic modality that is most commonly used as its temporal resolution is more than 1000Hz, allowing precise definition of all temporal events. Left ventricular end-diastolic and end-systolic diameters (LV_{EDD} , LV_{ESD}), fractional shortening ($(LV_{EDD}-LV_{ESD})/LV_{EDD}$) and LV wall thickness at end-diastole are obtained on an M Mode image at midpapillary muscle level (Figure 1). Using the M Mode, LV volumes and LV mass

can be approximated using the cubed diameter algorithm [14]. Of note, these approximations may underestimate the volumes in dilated ventricles.

Cardiac dimensions vary between mice strains, gender and age, and with heart rate; in an awake or lightly anesthetized 2 month old adult mice, LV end-diastolic diameter varies between 2.5 and 3.3 mm and shortening fraction between 50 and 60% [11]. Left ventricular wall thickness measures approximately 0.7–0.9 mm and LV mass 70–80mg. Intra-observer and inter-observer variability are low for diameter measurements, allowing detection of changes of less than 20%; measurements of wall thickness are slightly more variable [11].

Left ventricular size and function can also be assessed using 2-dimensional images. Left ventricular volumes can be calculated from the parasternal long axis view using the area-length method [15] or from several short axis views using a modified Simpson's rule [16]. Left ventricular ejection fraction is then derived from the volumes. Although 2-dimensional measurements are more challenging to obtain reproducibly than M Mode, these measurements are of great value for LV mass calculations [17] and for ventricles of heterogeneous function as found in ischemic models [16]. Three-dimensional reconstructions of LV volumes from multiple consecutive short axis views can also be acquired [18,19].

Investigators have also explored less load-dependent indices of LV contractility. The end-systolic pressure-dimension relationship reflects the pressure-volume relationship and can be studied by recording simultaneously LV M Mode and LV pressures obtained by a Millar catheter [20,21]. The limitation of the pressure-dimension relationship, as compared with the pressure-volume relationship, is that the former cannot be used in models of regional dysfunction.

In recent years, myocardial velocities, deformation (strain) and deformation rate (strain rate) have been measured using echocardiography in humans and large animals. These indices have been validated in mice using a technique of tissue Doppler imaging [22]. Peak myocardial systolic radial strain rate correlates closely with the end-systolic pressure-volume relationship [23], and both myocardial velocities and strain rate are sensitive parameters of LV systolic function [22,24]. In a model of chronic anthracycline-induced cardiotoxicity, peak systolic radial myocardial velocities and strain rate measured immediately after treatment were able to predict the later decline of LV ejection fraction and the survival [24]. Peak radial strain rate obtained from tissue Doppler imaging early after myocardial infarction has been reported to predict later LV remodeling [25]. Measuring strain and strain rate from 2 dimensional images has been validated in large animals but is challenging in mice due to the relatively low frame rate of the echocardiogram when compared to the heart rate. Peak systolic circumferential strain, obtained from 2-dimensional images has nonetheless been correlated closely with MRI measurements using a technique of ECG-gating [26]; its prognostic significance is unknown.

Diastolic Function

Investigators have applied echocardiographic indices of diastolic function used in humans and large animals to the mouse. The first abnormalities to be reported in genetically-modified mice were alterations of the mitral diastolic flow (E and A waves) using pulsed Doppler [27]. More recently, the tei index, reflecting both systolic and diastolic function was reported to be decreased in a model of myocardial infarction [28].

The LV filling pressure is an important parameter in the assessment of diastolic function, as it augments with increasing diastolic dysfunction. Measurement of the mitral annular diastolic motion (E' and A' waves) by pulsed Doppler allows the calculation of the E/E' ratio, which is correlated to LV end-diastolic pressure in humans and large animals. A similar correlation has been demonstrated in rats [29]. More recently, Parlakian et al. reported that E/E' increased in

a mouse model of cardiomyopathy and was associated with a decrease in LV ejection fraction [30]. Du *et al.* serially evaluated the diastolic function of mice expressing a mutation of cardiac troponin I using both the mitral flow and the mitral annular motion and reported a progressive alteration in the diastolic function of these mice with aging [31].

Words of caution must be given as to the feasibility of diastolic assessment in mice. Apical views of the heart are not easily reproducible in mice, thus the mitral flow and mitral annular motion are not parallel to the ultrasound beam. The lack of alignment forces the investigator to use angle correction algorithms that may not be reliable. More importantly, the rapid heart rate of the mouse is accompanied by a fusion of the mitral E and A waves, preventing the detailed analysis of mitral flow at physiological heart rates. In order to differentiate the E and A waves, the heart rate needs to be slowed down significantly; the physiological relevance of findings at a low heart rate is unclear.

Myocardial perfusion assessment

Impaired myocardial perfusion may participate in the development of LV remodeling [32]. Conversely, alterations in the extracellular matrix influence angiogenesis [33,34]. Two echocardiographic techniques have been developed to assess myocardial perfusion in mice *in vivo*.

Pulsed Doppler at the level of the proximal left coronary artery has been reported to measure flow velocity in mice [35,36]. Recently, Hartley *et al.* [36] reported that coronary velocity reserve could be measured serially in mice after aortic banding, and that impairment of velocity reserve preceded heart failure.

Myocardial contrast echocardiography (MCE) allows the estimation of myocardial blood flow using the intravenous continuous infusion of echogenic microbubbles and has proven its usefulness in humans and large animals [37]. A recent study showed that MCE is feasible in mice and can reproducibly provide an accurate index of myocardial blood perfusion [38]. Using MCE, myocardial blood flow and its augmentation after dobutamine were estimated in standard diet-fed mice and insulin resistant high fat-fed mice after pressure-overload. The coronary response to dobutamine was impaired in high fat fed mice and this impairment correlated with a decreased functional response to dobutamine [39], suggesting a relationship between perfusion and functional alterations.

Tissue characterization

Tissue characterization using echocardiography relies on information derived from the interaction of ultrasound waves with tissue. This interaction can be estimated by measuring the backscattered ultrasonic energy [40]. Particular interest has been given to the variations of the backscattered signal throughout the cardiac cycle. It has been reported that reduction in the cardiac cycle-dependent variation of the integrated backscatter signal (IBS-CV) reflects myocardial collagen deposition in hypertensive hearts [41]. One paper reported the feasibility of measuring integrated backscatter and its variations in mice [42]. It has to be noted however that integrated backscatter depends on a variety of factors, including depth and ultrasonic gain [43], contractile function [44] and myocardial fiber orientation [45], decreasing its specificity to estimate myocardial fibrosis.

Murine echocardiography and ventricular remodeling

Myocardial remodeling is an adaptive process by which the myocardium changes shape, size, and function in response to mechanical, neurohumoral, or genetic factors [46]. It occurs during normal growth or in response to hemodynamic stresses, including those caused by myocardial

infarction or chronic load increase. LV remodeling plays a major role in the subsequent development of heart failure [47,48].

Mouse echocardiography has been extensively used in the serial assessment of LV remodeling (more than 220 Pubmed references). We will limit this Review to the studies of ventricular remodeling in wild-type mice, and examples of the studies of ventricular remodeling in mice with known alterations in extra-cellular matrix.

Myocardial infarction is accompanied by an increase in LV volumes and a decrease in LV ejection fraction. In wild-type mice, Patten *et al.* were able to detect moderate and large myocardial infarctions based on the measurement of LV volumes and fractional shortening [49]. Other investigators [50,51] correlated the changes in LV size and fractional shortening with infarct size and hemodynamic measurements of systolic function. Myocardial remodeling has also been studied using echocardiography after pressure-overload, such as that obtained by transverse aortic banding. Shown on Figure 2 is an example of the parasternal long axis and short axis views of a control mouse (Panel A), a mouse with a myocardial infarction (Panel B) and a mouse after transverse aortic banding (Panel C). As with myocardial infarction, the scale of remodeling greatly depends on the degree of stress produced by the pressure-overload. Rothermel *et al.* [52] illustrated this point by studying two cohorts of mice, subjected either to moderate or to severe transverse aortic banding and followed by echocardiography up to 21 days after banding. Both groups developed LV hypertrophy that could be documented using echocardiography. The mice subjected to moderate aortic banding did not dilate their ventricular cavity or decrease their ejection fraction. The mice subjected to severe aortic banding however decreased their ejection fraction rapidly after transverse aortic banding and progressively increased their LV end-diastolic volume.

Murine echocardiography, ventricular remodeling and extracellular matrix

Changes in extracellular matrix including an increase in both collagen and extracellular matrix protein synthesis and degradation are associated with the process of ventricular remodeling. Matrix metalloproteinases mRNA and protein levels increase rapidly and disproportionately compared to TIMPs levels in the days following a myocardial infarction, when LV remodeling is greatest [53]. Conversely, the level of several matrix metalloproteinases appears to decrease in the early phases of pressure-overload but increases at a later stage. This later increase is associated with LV dilation and dysfunction [54].

The evaluation of genetically modified mice by echocardiography has facilitated our understanding of the role of extra-cellular matrix changes in ventricular remodeling. Mice deficient in MMP9 were serially followed by echocardiography after MI and compared to wild-type mice [5]. Whereas the wild-type mice increased their LV diameter by 20% two weeks after myocardial infarction, the LV diameter of MMP9-deficient mice was unchanged. Collagen accumulation was decreased in the infarct scar in the MMP9-deficient mice. Similarly, Hayashidani *et al* [55] reported that mice deficient in MMP-2 developed attenuated LV remodeling after myocardial infarction with slightly less dilated LV than wild-type mice (4.5 ± 0.1 vs 5.0 ± 0.1 mm) 28 days after left coronary ligation. The role of TIMPs was also studied after myocardial infarction- both mice deficient in TIMP1 and mice deficient in TIMP3 developed large LVs and more impaired LV systolic function than wild-type mice [56,57].

Studies of genetically-modified mice with known alterations in extra-cellular matrix bring considerable insights to the pathogenesis of pressure overload-induced LV remodeling and failure. Targeted deletion of MMP2 was accompanied by decreased LV hypertrophy in a model of moderate transverse aortic constriction without LV failure [58]. Loss of MMP9 significantly decreased LV hypertrophy 2 weeks after transverse aortic banding and LV failure 7 weeks after banding [6]. Both mice deficient in MMP2 and MMP9 developed less interstitial fibrosis

than wild-type mice. Cardiac specific overexpression of MMP1 in mice leads to loss of collagen and development of LV dysfunction at 1 year of age [59]. Interestingly, the same mice develop less LV dilation (3.1mm vs 3.8 mm) dysfunction, and collagen deposition than wild-type mice 5 weeks after aortic banding [60]. Thus, the role of collagen deposition may depend on the amount of collagen and the stimuli to which the heart is subjected.

A cautionary tale- fibrosis, NOS3 and pressure-overload

It appears mechanistically plausible to link increased myocardial fibrosis to decreased LV function and compensatory hypertrophy. The findings below however illustrate how cautious investigators must be before relating LV dysfunction and hypertrophy to an increase in fibrosis.

Nitric oxide, a soluble gas constitutively present in most cells, has a variety of cardiovascular effects that may be involved in the physiopathology of ventricular remodeling. In addition to acting as a potent vasodilator that can alter cardiac loading conditions, NO stimulates angiogenesis, reduces cardiomyocyte hypertrophy, and limits production of extracellular matrix proteins by cardiac fibroblasts [61–63]. Nitric oxide is produced from the conversion of L-arginine to L-citrulline, by three NO synthases (NOS) isoform enzymes: NOS1 (neuronal NOS or nNOS), NOS2 (inducible NOS or iNOS), and NOS3 (endothelial NOS or eNOS) [64].

Several studies suggest that NOS3-derived NO limits the development and progression of LV remodeling and failure after myocardial infarction (MI) [16,65,66]. In a model of transverse aortic constriction, Ichinose *et al.* reported that LV remodeling was exacerbated in NOS3-deficient mice compared to wild-type mice [67]. Left ventricular hypertrophy and decreased LV fractional shortening were detected 7 days after aortic banding in mice deficient in NOS3. This echocardiographic phenotype was accompanied by a significantly increased myocardial fibrosis in NOS3-deficient mice (Figure 3). Twenty-eight days after transverse aortic banding, the LV dilated, LV dysfunction and increased fibrosis persisted in the NOS3-deficient mice. Thus, in NOS3-deficient mice, increased fibrosis was accompanied by adverse LV remodeling. In a further study, Buys *et al.* [68] restored cardiac NOS3 in NOS3-deficient mice by breeding these mice with mice overexpressing NOS3 in their cardiomyocytes. Restoration of NOS3 in NOS3-deficient mice decreased LV hypertrophy after aortic banding and preserved LV fractional shortening. Twenty-eight days after aortic banding, fractional shortening was $47 \pm 9\%$ in wild-type, $33 \pm 3\%$ in NOS3-deficient mice and $54 \pm 5\%$ in NOS3-deficient mice bred with cardiac NOS3 overexpressors. The degree of interstitial fibrosis however was similar in NOS3-deficient mice with or without restoration of cardiac NOS3. Thus, although cardiac fibrosis was a prominent feature of NOS3-deficient mice after pressure overload, this increased collagen deposition did not play a prominent role in the LV hypertrophy and dysfunction observed in NOS3-deficient mice after aortic banding.

Many aspects of the interaction of fibrosis and NOS3 deficiency remain unexplored. Nitric oxide synthase 2 plays a controversial role in ventricular remodeling and fibrosis [69–71] and may participate in the phenotype observed in the NOS3-deficient animals. The explanation of the discrepancy observed between fibrosis and ventricular function after aortic banding when cardiac NOS3 is restored in NOS3-deficient mice warrants further experiments. It is conceivable that restoration of cardiac NOS3 is accompanied by a direct change in cardiomyocyte structure and/or function that may compensate for the development of fibrosis [68]. Finally, the interactions of nitric oxide and fibrosis in other cardiovascular pathologies such as myocardial infarction and ischemia-reperfusion demand further studies. These experiments will further help to elucidate the relationship between nitric oxide, the development of ventricular remodeling and concomitant extra-cellular matrix alterations.

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Figure 1. M-mode tracing obtained on a wild-type mouse. LV_{EDD}: left ventricular end diastolic diameter; LV_{ESD}: left ventricular end systolic diameter; AWT: anterior wall thickness; PWT: posterior wall thickness.

Figure 2. Parasternal long axis (left) and short axis (right) views obtained on a control wild-type mouse (Panel A), a mouse 2 days after myocardial infarction (Panel B) and a mouse 28 days after transverse aortic banding (Panel C). Schematic of views in control mouse (Panel D). LV: left ventricle; LA: left atrium; RV: right ventricle; Ao: aorta.

Figure 3.

Collagen deposition (red staining) in the LV of wild-type (Panel A) and NOS3-deficient (Panel B) mice 28 days after aortic banding. Twenty-eight days after aortic banding, collagen deposition was increased in NOS3-deficient as compared to wild-type mice. Reprinted with permission of the American Journal of Physiology.