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Echocardiography in Translational Research: Of Mice and Men

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Summary

Mice are increasingly used in cardiovascular research, and echocardiography is ideally suited to evaluate their cardiac phenotype. This Review describes current usage of mice echocardiography and focuses on some of its applications in both basic and clinical science.

The use of mice in research

Aristotle (384-322 BC) was amongst the first to perform experiments on animals, giving accurate descriptions of ruminants' fore-stomachs and of the embryological development in the hound shark¹. Since then, animal models have been widely used to better understand the physiology and pathology of human disease. The mid-20th century development of biomedical sciences such as microbiology, pharmacology, toxicology, and immunology caused a large increase in the use of research animals, with as many as 100 million vertebrates used for research each year. Of these, more than 75% are rodents, with most being mice².

Usage of the mouse in research has been increasing sharply (more than 23% per year), mainly due to the expanding availability of genetically-modified mice. An understanding of the roles of genes and biological pathways is crucial to gain insights into the pathophysiology of human diseases and in the development of novel therapies. Mice are ideally suited to genetic modifications; however, the limitations of mice models of diseases must be recognized (Table 1).

Two types of genetically-modified animals are described: transgenic animals, which express or overexpress a "transgene" (a gene external to their genome), and knockout animals, in which a gene has been partially or totally deleted. The technique to obtain a knockout animal is more challenging than that used to create a transgenic animal. Until Zhan *et al.* developed knockout rats in 2003, this technique was only feasible in mice³. For a Review on the techniques used to create transgenic or knockout animals, please consult⁴, and⁵. The phenotype of genetically-modified animals is then compared to that of well genetically-defined (but unmodified), "wild-type" animals. This comparison allows the investigators to tease out the role of the gene of interest.

In order to fully characterize the phenotype of transgenic mice, it is necessary to use a technique that permits the serial evaluation of cardiac structure, function, and response to interventions

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during growth and development. Repeated hemodynamic invasive studies are impractical without damaging the fragile model, while nuclear studies necessitate an intravenous injection. Magnetic resonance imaging is a promising technique, but involves deep anesthesia. Therefore, echocardiography, which can easily be performed on awake or lightly sedated animals and has a high spatial resolution and rapid sampling rate, has become the procedure of choice for most studies of the mouse heart. The increased popularity of the mouse echocardiography is underlined by the more than 800 papers retrieved on PubMed.

Cautionary tales of mice echocardiography

Although the acquisition of mice echocardiograms is relatively simple, echocardiographic studies are challenging because of the mouse's response to stress, as well as the small size, rapid contraction, and orientation of the mouse heart.

Mice are very fragile, and their hemodynamics can be altered significantly by interventions such as anesthesia, ventilation, and thoracotomy. Thus, the proper choice of anesthesia is crucial in mice studies. Cardiac function results obtained at heart rates averaging 300/minute are not rare in the literature, and features of cardiac physiology are greatly modified at such slow heart rates. Similarly, systolic blood pressure in healthy animals should be 100 to 115 mmHg⁶. Ideally, echocardiograms should be performed on awake animals. This is feasible after training the mice by repeating the echocardiograms daily for several days⁷. If anesthesia is necessary, a short duration of anesthesia using ketamine at low doses (50 mg/kg intraperitoneally)⁸, inhaled isoflurane or metoxyflurane⁹ have been recommended.

The average left ventricular end-diastolic diameter in a healthy adult mouse has been measured between 2.5 and 3 mm. In order to detect a 20% increase of the left ventricular end-diastolic diameter (an increase of 50 to 60 mm in humans), the spatial resolution of the echocardiograph has to be inferior to 0.5 mm. As shown in Figure 1, technical advances have markedly facilitated the quality of mice echocardiographic images. Using high frequency probes (above 8MHz), commercial echocardiographs have an axial resolution of about 0.2 mm and a lateral resolution of about 0.3 mm when the image is zoomed at a depth of 1 cm. Most of the probes developed recently are linear probes, which avoid near field artifacts. Recently, very high frequency (30–50 MHz) mechanical probes have been developed, allowing an axial resolution of approximately 50 μ m at a depth of 5–12 mm.

The frame rate available on the echocardiograph is another crucial element of the imaging, especially when cardiac function is to be evaluated on two-dimensional images. The resting heart rate of the mouse is between 500 and 700 beats per minute¹⁰. If an acceptable number of frames to accurately detect end-diastole and end-systole is 15/cardiac cycle, a frame rate of 150 Hz will be necessary with a heart rate of 600/minute. Of note, 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 a physiological heart rate.

The mouse heart is more vertically oriented than the human heart. Parasternal long and short axis views may be easily obtained. However, the heart's orientation and the small size of the rib cage cause the acquisition of apical views to be difficult and poorly reproducible. The lack of easily obtained apical view prevents a complete analysis of diastolic function and of the right ventricle. Furthermore, because of its shallow location, small size and complex shape, the right ventricle is not well-visualized on transthoracic echocardiography. Knowing these limitations, echocardiography remains an essential tool to assess myocardial function and perfusion.

Murine models of cardiovascular diseases

Genetic modifications may induce pathological conditions encountered in humans such as dyslipidemia, hypertension, and congenital defects. Also, genetically-modified mice and their wild-type counterparts may be subjected to a variety of stresses that mimic human pathologies. The most frequently used include myocardial infarction or ischemia-reperfusion by ligation of the left anterior descending artery¹¹, and LV pressure-overload produced by aortic constriction or infusion of vasoconstrictors (for a Review of the surgical techniques, see¹²). Of note, new experimental models are constantly being developed to gain insights to the pathways involved in clinical situations, and include such diverse pathologies as chemotherapy-induced cardiotoxicity, transplantation rejection, sepsis, and myocarditis.

The Mouse cardiac analysis using echocardiography

Left Ventricular Size and Systolic Function

Murine echocardiography has mostly been used in a variety of models and genetically-modified mice for the assessment of LV size and function. The modality most commonly used by investigators is the M Mode, as its temporal resolution (more than 1000 Hz) is especially adapted to the fast mouse heart rate. Shown on Figure 1 is a typical M Mode obtained in a healthy adult mouse. Values reported for left ventricular dimensions in healthy adult mice are given in Table 2. Cardiac dimensions vary between mice strains, gender and age, and with heart rate; it is therefore important to verify that groups of mice are matched for these parameters.

Values of left ventricular end-diastolic diameter greater than 4.0 mm in healthy adult mice have been reported. These values, often associated with slow hearts rates and decreased shortening fraction, may be due to the left ventricular dilatation and depressed cardiac systolic function associated with anesthesia.

The M Mode has also been used to measure LV wall thickness and calculate left ventricular mass^{13–17}. 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 variable⁷.

Measurements of LV size and function obtained from 2-dimensional images (Figure 2) are more technically challenging to acquire and measure in a reproducible manner than the M Mode, and necessitate a high frame rate. However, these measurements are of great value, in particular for ventricles of heterogeneous geometry and/or function. Measurement of LV mass was shown to be closer to that measured at necropsy using 2D measurements on a parasternal long axis view than using the M Mode¹⁸. In a model of LV remodeling after myocardial infarction, a greater increase in LV volumes was detected between mice deficient in nitric oxide synthase 3 than in wild-type mice using 2D images, but not M Mode¹⁹. Three-dimensional reconstructions of LV volumes can be acquired and may be facilitated by the use of a clamp to manipulate the echo probe^{20, 21}.

As in humans, load-independent indices of LV function are useful to evaluate myocardial intrinsic contractility. M Mode measurements have been used to determine ventricular elastance (Ees). The end-systolic pressure-dimension relationship can be studied by recording simultaneously LV M Mode and LV pressures obtained by a Millar catheter^{22, 23}. 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.

More recently, measurements of the mitral annular velocities²⁴ and of myocardial velocities and strain rate (Figure 3) using tissue Doppler imaging²⁵ have been validated; the latter have proven to be sensitive parameters of LV systolic function. Peak systolic strain rate measured after myocardial infarction is closely correlated to the transmural extent of the infarct, a strong predictor of viability and prognosis²⁶. In a model of chronic anthracycline-induced cardiotoxicity, endocardial velocities and strain rate measured immediately after treatment were able to predict the later decline of LV ejection fraction and the survival²⁷. Hence, the use of a mouse model allowed the validation of a concept that may be further studied in humans.

Diastolic Function

Diastolic function has been studied using pulsed Doppler and color M Mode²⁸, and abnormalities have been reported in genetically-modified animals²⁹. The *tei* index, reflecting both systolic and diastolic function has been calculated and was reported to be decreased in a model of myocardial infarction³⁰. The main difficulty in studying mitral flow in murine models is that in order to differentiate the E and A waves, the heart rate needs to be slowed down significantly; the physiological relevance of diastolic findings is unclear.

The LV filling pressure is an important parameter in the assessment of diastolic function. In rats, E/Ea ratio has been shown to correlate with LV end-diastolic pressure³¹. More recently, Parlakian et al. reported that E/Ea increased in a mouse model of cardiomyopathy and was associated with a decrease in LV ejection fraction³².

Right ventricular assessment

To better understand the pathogenesis of pulmonary hypertension, mice models have been developed using genetically-modified mice^{33, 34} and interventions, such as pulmonary artery banding¹² or hypoxic exposure³⁵. Although the right ventricle is not well visualized with transthoracic echocardiography, it may be assessed by transesophageal echocardiography using an intravascular ultrasound catheter³⁶. This method allowed Steudel *et al.* to show that nitric oxide synthase 3 limited the development of pulmonary hypertension and right ventricular remodeling/failure after chronic hypoxic exposure³⁵. However, this technique remains challenging and limited by its invasiveness and the low frame rate of the probe. The spatial resolution of the novel very high frequency probes (30–40 MHz) allow better visualization of the right ventricle using transthoracic echocardiography, and may lead to easier assessment.

Flows

Commercially available pulsed-wave Doppler has been used to measure aortic and mitral velocities. A common problem is misalignment with the flow, due to the small size of the vessels and the lack of a reproducible four chamber view. A better alignment may be obtained by measuring the flow in the descending thoracic aorta from the suprasternal notch¹⁵ or by measuring the pulmonary flow in a parasternal short axis view obtained at the aortic root level (Figure 5). To overcome the misalignment problem, Hartley et al³⁷ developed a 0.5 mm diameter 20 MHz transducer mounted at the tip of a 20-gauge needle to measure the ascending aortic blood velocity in mice. Aortic and mitral flows and regurgitation may be seen with color Doppler³⁸ (which is limited by its frame rate), but a reproducible model of aortic or mitral regurgitation has not yet been developed.

Embryonic mice

Murine fetal echocardiography is a technically challenging technique that presents multiple difficulties. Mice measure between 15–20 mm at birth. The reported heart rate of the embryos varies with the duration of gestation and is greatly influenced by technical issues (temperature,

anesthesia of the dam etc...). Overall, the heart rate appears to be slower than in adult mice (between 180–260 bpm after day E14.5). Good orientation of the fetus *in utero* may be very difficult to achieve, precluding M Mode measurements. To remediate to the incorrect orientation, investigators frequently exteriorize the uterus^{39, 40}; however, the physiological repercussions of this procedure are unknown. At very high frequencies, the blood appears echogenic due to the nucleation of fetal red blood cells. The echogenicity of the blood peaks at E13.5 (Figure 6, panel B)⁴¹, making the differentiation of blood and myocardial walls difficult before E15.5.

Although technically challenging, evaluation of the cardiac dimensions and function of mice embryos is an invaluable tool to assess the role of genes in the early development of cardiac function. Until recently, imaging embryonic mice hearts involved invasive methods. The embryo had to be sacrificed for magnetic resonance microscopy and electron microscopy^{42, 43} or surgically delivered for transillumination microscopy^{44, 45}.

Using commercially available ultrasound machines, investigators have described measurements of fetal hearts from day E14.5 onward⁴⁶. Color Doppler has been particularly useful to identify valvular regurgitation and abnormal communications in embryos^{47, 48}. Although investigators have reported abnormal flows in genetically-modified embryos^{40, 49, 50}, absolute flow measurements are challenging, mainly because of the size of the probes and the embryos. The very high frequency probes that have been developed recently provide excellent spatial resolution, a major advantage for imaging the embryonic heart (Figure 6). Using these techniques, investigators could identify cardiac contraction in some 8.5 days old embryos (corresponding to 3–6 weeks of human pregnancies)⁵¹. To better understand the role of the nuclear factor of activated T cells c1 gene (NFAT) in cardiac development, Phoon *et al.* used very high frequency probes in the functional cardiac assessment of mice deficient in NFAT. Using two-dimensional imaging and pulsed Doppler, these investigators were able to show that the NFAT-deficient mice had reduced cardiac output, aortic regurgitation and suggestion of a non-compliant ventricle⁴⁰.

Myocardial perfusion assessment

Genetically-modified mice and mouse models of heart disease have been used to better understand the role of coronary circulation and its responses to vasodilators or stressors in the development of human pathology. Two techniques have been proposed to assess myocardial perfusion: coronary Doppler, and myocardial contrast echocardiography.

Coronary Doppler

Zhou *et al.* reported that Doppler at the level of the proximal left coronary artery was able to detect coronary flow⁵². More recently, the same investigators demonstrated the feasibility of using high frequency ultrasound imaging to evaluate heterotopic cardiac grafts in mice and suggested that Doppler coronary flow parameters predicted acute rejection in mice⁵³.

Mouse coronary velocity reserve was measured using the same technique either before and after adenosine infusion, moderate hypoxia^{54, 55}, or more recently using the vasodilatation induced by inhalation of isoflurane⁵⁶. Although these techniques have not been validated versus a reference method, they appear to be useful to better understand the role of coronary microcirculation dysfunction in the development of heart diseases. For example, Saraste, Kytö *et al.* described that a decreased coronary velocity reserve is a determinant of poor outcome in murine viral myocarditis. Using isoflurane-induced vasodilation, Hartley *et al.*⁵⁷ recently reported that coronary velocity reserve could be measured serially in mice after aortic banding, and concluded that impairment of velocity reserve precedes heart failure.

Myocardial contrast echocardiography

Myocardial contrast echocardiography (MCE) has proven its usefulness in humans and large animals for the assessment of myocardial perfusion and coronary reserve ^{58, 59}.

Applying MCE to mice presents some challenges. The resonant frequency used to detect the harmonic signal of MCE depends on bubble size, and is 3 MHz for a 3- μ m-diameter bubble; the high-frequency probe necessary to permit sufficient spatial resolution in mice precludes the efficient use of harmonic imaging. However, bubble destruction at a high probe frequency is minimal and the development of new contrast imaging modalities, which can detect the nonlinear energy that exists at the fundamental frequencies, now allows the identification of low concentrations of microbubbles within the myocardium, even at relatively high frequency ⁶⁰. Interestingly, the size of mice pulmonary capillaries is only marginally smaller than that of humans, allowing the use of common ultrasonic microbubbles.

Bolus injections of contrast agents provide good opacification of both the cavity and the myocardial walls ⁸. MCE using bolus injections allows the measurement of perfusion defects in models of ischemia and infarction ^{8, 61}. The contrast defect area can be measured on one ⁸ or several short axis views ⁶¹. The ratio of the perfusion defect area over the total myocardial area has been shown to be closely correlated with the area at risk measured by post-mortem techniques ^{8, 61}.

Whereas a bolus injection is sufficient to visualize perfusion defects, the assessment of myocardial blood flow requires a continuous injection. MCE can be acquired using a real-time or a triggered mode ^{58, 62}.

A recent study showed that MCE is feasible in mice and can reproducibly provide an accurate index of myocardial blood perfusion ⁶³. MCE-estimated myocardial blood flow correlated closely with microsphere-derived myocardial blood flow and could be used to measure mouse coronary reserve and coronary responses to infused vasodilators (Figure 7). In this study, Raher, Thibault *et al.* demonstrated that an enzyme synthesizing nitric oxide, the endothelial nitric oxide synthase or NOS3, is indispensable to acetylcholine but not to adenosine vasodilator response in the coronary arteries. In conclusion, MCE is a potent tool to study genetically modified mice and further our understanding of resting and stimulated myocardial blood flow responses.

Stress echocardiograms

Stressing the heart is an important technique to detect subtle abnormalities of the left ventricular function which may not be detected at rest, or hypo- or hyper-responsiveness to adrenergic stimulation. Several methods can be used. Hoit *et al.*⁶⁴, used 2 μ g/g of body weight of isoproterenol injected intraperitoneally. The effect was noted as soon as 1–2 minutes after injection, with an increase in heart rate from 252 to 424 bpm and of shortening fraction from 42 to 62%. Dobutamine has been used intravenously, at rates from 0.5–2 μ g/kg/min, and intraperitoneally ^{16, 65} at a dose of 1–4 μ g/g body weight. At the highest dose, the shortening fraction was reported to increase from 50 to 75% ⁶⁵. Atropine can also be used at a dose of 0.5 μ g/g IP.

How murine echocardiography helps us to gain insights into cardiovascular diseases; an example

Echocardiographic analysis of the mouse phenotype has led to important insights in the mechanisms underlying cardiovascular diseases, including hypertension, heart failure and remodeling, and atherosclerosis. Given the rapidly expanding domain of cardiovascular genetic

modifications (more than 1000 modified strains are available commercially), the purpose of this review is not to give an exhaustive list of genetically modified mice but rather to discuss some possible applications of murine echocardiography. In the following paragraphs, we will discuss the application of murine echocardiography to gain a better understanding of the role of nitric oxide and nitric oxide synthases in cardiovascular pathologies.

Nitric oxide is a soluble gas constitutively present in most cells, and has a variety of cardiovascular effects. 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^{66–68}. All of these effects may play a role in cardiovascular disease.

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)⁶⁹. The use of genetically-modified mice, either as knock-out or overexpressors of the different NOSs, has allowed us to better understand and differentiate the role of each of these enzymes in cardiovascular diseases, leading to some potential therapeutic directions.

Numerous models of chronic global left ventricular dysfunction have been followed echocardiographically. Acute left ventricular dysfunction can also be documented, such as that encountered in septic shock. We studied the effect of inducible nitric oxide synthase (NOS2) in a model of endotoxemic shock²³. NOS2, which is induced in sepsis, produces large quantities of nitric oxide that may contribute to the myocardial dysfunction. Using echocardiography, wild-type mice were found to significantly enlarge their left ventricle and decrease their shortening fraction after endotoxin injection whereas mice lacking the NOS2 gene were partially protected. Myocardial contractility, as assessed by the end-systolic pressure-dimension relationship, was also better preserved in NOS2-deficient mice, therefore proving the deleterious role of NOS2 on myocardial function in endotoxemic shock. Selective inhibitors of NOS2 were then tested and were protective of sepsis-induced LV dysfunction in a murine model of sepsis⁷⁰, leading to potentially novel therapeutic directions.

The impact of NO donor compounds on myocardial ischemia-reperfusion (I/R) injury has been studied extensively in animal models with varying effects on MI size and left ventricular (LV) function (for a review, see⁷¹). In humans, the therapeutic use of NO-donor compounds after myocardial infarction has been hindered by their systemic vasodilator effects, which potentially lead to hypotension and further compromise of myocardial perfusion. Inhalation of NO has proven valuable over the last decade for the treatment of hypoxic pulmonary hypertension in newborns. Because NO is rapidly bound to hemoglobin *in vivo*, it was initially suggested that the actions of inhaled NO would be limited to the lungs. More recently, however, inhaled NO has been shown to increase coronary artery patency after thrombolysis by decreasing platelet adhesion and aggregation⁷² and improve blood flow and decrease leukocyte adhesion in a feline model of intestinal ischemia-reperfusion⁷³, effects which may favorably impact cardiac injury in myocardial ischemia-reperfusion. Taking advantage of the easy and quick breeding of mice, Hataishi et al. reported that the inhalation of NO before reperfusion in a murine model of myocardial ischemia-reperfusion decreased the size of the myocardial infarction without causing systemic hypotension⁷⁴. Murine echocardiography was used to assess the functional consequences of this decrease. Mice breathing NO had a lesser decrease in left ventricular ejection fraction and a trend towards lesser LV dilation after myocardial ischemia-reperfusion than mice breathing room air. The results of this small animal study were confirmed in a porcine model of ischemia-reperfusion⁷⁵ and are now been applied in humans.

Another example is the insights gained by the use of murine echocardiography into the pathogenesis of LV remodeling after myocardial infarction. Myocardial remodeling is an adaptative process by which the myocardium changes shape, size, and function in response to mechanical, neurohumoral, or genetic factors. It occurs during normal growth or in response to hemodynamic stresses, including those caused by myocardial infarction. Changes in LV mass, size, and geometry, which define LV remodeling, are major prognostic factors in patients after myocardial infarction or with essential hypertension^{76, 77}. As mentioned previously, NO produces a variety of cardiovascular effects, many of them potentially involved in the pathophysiology of LV remodeling.

Several studies suggest that NOS3-derived NO limits the development and progression of LV remodeling and failure after myocardial infarction (MI)^{19, 78, 79}. Scherrer-Crosbie *et al.* reported that LV remodeling after occlusion of the left anterior descending artery was exacerbated in NOS3-deficient mice as compared to wild-type mice¹⁹. Jones *et al.* demonstrated that overexpression of NOS3 within the systemic and pulmonary vascular endothelium of mice prevented cardiac and pulmonary dysfunction in a murine model of heart failure due to MI⁷⁹. Janssens and colleagues further demonstrated that murine cardiomyocyte-restricted overexpression of NOS3 (NOS3^{TG}) limited the degree of LV dysfunction and ventricular remodeling after MI⁷⁸. In all these studies, murine echocardiography was used to document the effect of the deletion or overexpression of the NOS3 gene on LV size and function after myocardial infarction. Recently, using myocardial contrast echocardiography, Raheer, Thibault *et al.*⁶³ reported that NOS3-deficient mice had a decreased coronary artery vasodilatory response to acetylcholine, a possible mechanism explaining their worse remodeling after a hemodynamic stress.

Interestingly, the effect of NOS2 on ventricular remodeling after myocardial infarction is opposite, with a better LV function and survival in mice deficient in NOS2^{80, 81}. These findings are important as they preclude the development of a therapy activating all NOSs; a selective activator of NOS3 would be more appropriate.

The importance of understanding the role of NOS3 in LV remodeling and heart failure has been illustrated recently by the African-American Heart Failure Trial⁸². In retrospective studies on V-Heft II, the survival benefit of enalapril compared to the association of hydralazine and isosorbide dinitrate was not noted in black patients⁸³. African-Americans have been shown to have altered NOS3-mediated vasodilator responses compared to white^{84, 85}. Because of these differences, Taylor *et al.* hypothesized and showed that African-Americans greatly benefited from the combination of hydralazine and isosorbide dinitrate added to the standard heart failure therapy⁸². A better understanding of the role of the NOSs is therefore crucial in directing and customizing therapies of cardiovascular diseases.

Future directions of murine echocardiography- micro-imaging?

Over the last decade, murine echocardiography has evolved from a pure research tool to a validated mean of exploring the cardiac phenotype of mice, thereby greatly contributing to the understanding of cardiovascular diseases.

With the advent of increasingly higher frequency probes, the anatomy of the mouse can be seen in exquisite details, and assessment of fine structures becomes feasible using echocardiography (Figure 8). Recently, mouse coronary artery morphology has been studied in pathological models of atherosclerosis, and investigators^{55, 86} reported that lumen-narrowing plaques could be visualised and measured using ultrasound biomicroscopy. The spatial resolution of the probes compared to the coronary arteries dimensions (100–200 μ m) still remains a challenge for accurate quantitative morphological measurements, and higher frequency probes may be needed.

Although the precise anatomical assessment of mouse cardiovascular phenotype is exciting, the use of targeted contrast agents opens the very broad field of functional imaging. Microbubbles may be targeted to a variety of molecules expressed in processes such as inflammation, ischemia, or angiogenesis (for a Review see ⁸⁷). In his pioneering work, Weller *et al.* used microbubbles targeted to arginine-arginine-leucine to detect angiogenesis in mice tumors ⁸⁸. The apolipoprotein E-deficient mouse fed a hypercholesterolemic diet develops extensive atherosclerosis. Using this recognized model, Kaufmann *et al.* ⁸⁹ demonstrated that targeted detection of VCAM-1 allows one to monitor early vascular inflammatory changes at the level of the aortic arch. As atherosclerosis often progresses silently, such a technique may potentially be useful for early risk stratification in patients. Using microbubbles targeted to another inflammatory marker, ICAM-1, Weller *et al.* were able to detect acute cardiac transplantation ⁹⁰. Other markers of inflammation, such as P-selectin, can be targeted and may detect post-ischemic myocardium in the absence of necrosis ⁹¹ (Figure 9). Recently, Behm *et al.* ⁹² described that different components of the inflammatory response that participate in vascular development can be separately monitored using targeted imaging.

It is conceivable that microbubbles will be used in a much broader way to detect cells such as stem cells, as well as in a large array of physiological and pathological processes by targeting enzymes expressed on endothelial cells membranes. In these indications, murine echocardiography will have a premier standing, as mice remain a crucial tool to advance cardiovascular science.

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Figure 1. M-mode tracings obtained on wild-type mice using a 7 MHz transducer (A), a 13 MHz transducer (B) and a 30 MHz transducer (C), respectively.

Figure 2. 2-dimensional images obtained on wild-type mice. Panel A displays a parasternal long axis view of the left ventricle. Panel B displays a short axis view of the left ventricle, at the papillary muscles level.

Figure 3.
Example of strain rate tracing obtained within the posterior wall and within the anterior wall in a wild-type mouse.

Figure 4. Transesophageal echocardiogram in a wild-type mouse. Left, TEE view at midventricular level at end diastole. Right, in the same animal, TEE view at midventricular level at end systole. Each marker corresponds to 1 mm (reprinted from Scherrer-Crosbie; *Circulation* 1998 with permission).

Figure 5.

Pulsed Doppler of the pulmonary flow (panel B) obtained in a parasternal short axis view (panel A), in a wild-type mouse. RA, right atrium, RV, right ventricle, RVOT, right ventricle outflow tract, Ao, aortic valve, RPA, right pulmonary artery, LPA, left pulmonary artery.

Figure 6.

Embryonic echocardiography obtained with a 30 MHz transducer in a wild-type mouse. 2-dimensional images of a short axis view obtained at 18 days (panel A) and a 4 chamber view obtained at 12 days (panel B). Panel C: M-mode tracing of an embryonic heart. LA, left atrium, RA, right atrium, LV, left ventricle, RV, right ventricle.

Figure 7.

Panel A: 2-dimensional image of a parasternal long axis view obtained in a wild-type mouse during myocardial contrast echocardiography (MCE).

Panel B: Replenishment curves measured in real time in the myocardial posterior wall after destruction of microbubbles by a high energy flash. Left panel, curve obtained at rest. Left panel, curve obtained after acetylcholine injection.

Figure 8. Proximal right coronary artery of a wild-type mouse visualized using high frequency ultrasound. RA, right atrium, RVOT, right ventricle outflow tract, Ao, aortic valve, RCA, right coronary artery.

Figure 9.

(A) Myocardial contrast echocardiography performed during ischemia revealed an anterior perfusion defect. (B) Targeted imaging with P-selectin microbubbles after 45 min of reperfusion demonstrated selective enhancement in the post-ischaemic anterior wall. Reprinted from Kaufmann, Eur Heart J 2007 with permission.

Table 1**Mice models of diseases- advantages and limitations**

Advantages	Limitations
Genome completely mapped	
Genome 98% identical to human	Differences with humans : different physiology, species-dependent isoform expression, species-Dependent phenotype consequence of transgene Expression
Genetic modifications protocols well validated –Transgenesis -Gene deletion (Knockout mice)	Transgenesis and gene deletion are not physiological and main be accompanied by Compensatory mechanisms
Surgical and pharmacological models of humans cardiac diseases available	Models may not reflect pathological conditions (i.e. surgical infarction in healthy animal)
Low cost compared to big animals: -inexpensive -easy to house and handle	Small size increases the technical difficulty of interventions, fragile animal
Easy to breed	
-short gestational (19D) and reproductive cycles (10W)	
-large litters (5–10 pups)	

Table 2

M Mode values in healthy mice

	Buys, Raher (AJPhysiol 2007)	Yang (Am J Physiol 1999)
Heart rate (bpm)	607±51	658 ± 9
BW (g)	25–28	23–28
LVID _{ED} (mm)	3.1±0.1	2.52 ± 0.29
LVID _{ES} (mm)	1.3±0.1	1.2 ± 0.24
PWT (mm)	0.8±0.03	0.6 ± 0.05
FS (%)	57±5	51 ± 1

Buys and Raher: C57Bl6 2 months old male mice, sedated with ketamine intraperitoneal 50mg/kg; Yang: 129SvEv/Tac 3 months old male mice, awake. BW: body weight, LVID_{ED} left ventricular diastolic diameter, LVID_{ES} left ventricular systolic diameter, PWT: posterior wall thickness in diastole, FS: fractional shortening.