Magnetic resonance imaging and spectroscopy of the murine cardiovascular system

Ashwin Akki,* Ashish Gupta,* and Robert G. Weiss

Division of Cardiology, Department of Medicine, and Division of Magnetic Resonance Research, Department of Radiology, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

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Akki A, Gupta A, Weiss RG. Magnetic resonance imaging and spectroscopy of the murine cardiovascular system. Am J Physiol Heart Circ Physiol 304: H633–H648, 2013. First published January 4, 2013; doi:10.1152/ajpheart.00771.2011.—Magnetic resonance imaging (MRI) has emerged as a powerful and reliable tool to noninvasively study the cardiovascular system in clinical practice. Because transgenic mouse models have assumed a critical role in cardiovascular research, technological advances in MRI have been extended to mice over the last decade. These have provided critical insights into cardiac and vascular morphology, function, and physiology/pathophysiology in many murine models of heart disease. Furthermore, magnetic resonance spectroscopy (MRS) has allowed the nondestructive study of myocardial metabolism in both isolated hearts and in intact mice. This article reviews the current techniques and important pathophysiological insights from the application of MRI/MRS technology to murine models of cardiovascular disease.

magnetic resonance imaging; magnetic resonance spectroscopy; cardiovascular; ventricle

Introduction

Magnetic Resonance Imaging (MRI) has emerged as a powerful and reliable tool to noninvasively study the cardiovascular system in clinical practice. The technique can also provide critical insights into cardiac and vascular morphology, function, and physiology/pathophysiology in animal models. Transgenic mouse models have assumed a central role in cardiovascular research because they allow investigators to probe very specific basic molecular mechanisms of heart disease. Thus the ability of MRI to nondestructively characterize the phenotype of the whole organ and whole intact transgenic mouse over time plays a significant role toward that end (30, 54). Two-dimensionally directed echocardiography (2-D Echo) is widely used to noninvasively study the murine cardiovascular system, owing to its relatively lower costs, portable nature, and rapid phenotypic analysis (55). However, MRI offers several advantages of high soft-tissue contrast and intrinsic three-dimensional (3-D) image acquisition along image-defined projections whose analysis does not rely on geometrical assumptions for determination of left ventricular (LV) volumes, shape, or mass. In addition, MRI has the ability to study many pathophysiological parameters in a single session, including energy metabolism (142).

Magnetic resonance spectroscopy (MRS) is the sibling technology to MRI that allows the nondestructive study of metabolism in tissues, perfused organs, and intact animals. MRS is performed on the same magnetic resonance (MR) systems used for MRI, but often with additional hardware and software modifications that allow acquisition of both MRI and MRS data in the same study. MRS reveals biochemical information about metabolites that contain atoms with nuclear spin, as detailed in Table 1. This review will focus on the application of MRI/MRS for the study of murine cardiovascular pathophysiology. Detailed explanations of the physics principles underlying these approaches are beyond the scope of this review; however, technical references are provided.

Imaging the Murine Cardiovascular System

A number of murine cardiovascular parameters have been studied in vivo using MRI, including ventricular size, shape, global function, and wall thickening with cine-MRI; local myocardial function with myocardial tagging; myocardial perfusion with arterial spin labeling; infarct quantification with contrast-enhanced MR; and vascular lesion characterization with MR microscopy (30). Here we summarize some of these key applications of MRI for the study of the murine cardiovascular system.

Technical considerations for murine cardiac MRI. During MRI, mice are routinely anesthetized using inhalational anesthetics such as isoflurane (2–4% for induction, 1.0–1.5% for maintenance) to facilitate short induction and recovery times while minimizing negative inotropic and chronotropic effects (113). The mouse heart is small (∼100 mg) and fast (400–600 beats/min), making murine cardiac MRI very challenging. Higher field strengths provide better signal-to-noise ratio that can be used to improve the spatial resolution for the smaller hearts or reduce the scan time (30). Consequently, the majority of murine cardiac MRI studies have been performed at field strengths of 4.7–11.7 T (7, 90, 118, 155).

Address for reprint requests and other correspondence: R. G. Weiss, Carnegie 584, The Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21287-6568 (e-mail: rweiss@jhmi.edu).
Physiological motion from cardiac contraction and respiratory motion diminishes MR image quality unless properly accounted for in segmented or averaged acquisitions. ECG and respiratory gating are routinely used to synchronize acquisitions and minimize motion artifacts for the collection of high-quality time-resolved cardiac images in mice (22, 54). Specifically, MR images are formed from a number of sequence repetitions, each triggered during the same phase of the cardiac cycle to minimize motion artifacts (73). Gating can be achieved by a number of motion detection methods that sense cardiac and respiratory motion in mice, including the ECG signal (14, 104) and pneumographic (89), infrared (75), fiberoptic (10), or lever-coil (33) sensors.

Furthermore, signal-to-noise ratio can be improved by acquiring a greater number of acquisitions, but not without prolonging the scan time, a setback that has been overcome, in part, by using faster imaging sequences such as fast low-angle shot (FLASH) (49, 113, 131, 148) or fast imaging with steady-state precession (FISP) (90, 99, 155). Briefly, the FLASH imaging sequence takes advantage of excitation pulses with small flip angles, eliminating the need for waiting long periods between successive excitations (49, 54), resulting in a manifold reduction in acquisition time without sacrificing spatial resolution. The technique has been successfully used to quantify murine LV mass and function (113, 148, 149). FISP is another fast imaging sequence with small flip angles but based on the principle of steady-state magnetization after radio frequency excitation (99). Using this technique, investigators have accurately quantified murine ventricular mass, wall thickness, volumes, and contractile parameters (90, 155). The advantage of FISP over FLASH is that it provides much higher signal-to-noise and contrast-to-noise ratios (90).

**LV mass.** High-resolution cine-MRI has been successfully applied to accurately and reliably characterize LV morphology (mass and wall thickness) in mice (37, 113, 122, 123). Ruff and colleagues (113, 149) performed one of the first cardiac cine-MRI studies on mice using an ECG triggered respiratory-gated FLASH sequence on a high-field magnet (7.05 T) to quantify LV mass. Those investigators acquired six to eight contiguous ventricular short-axis slices (each 1 mm in thickness) from the apex to the base of the heart. LV mass and volumes were calculated by contouring the cardiac borders and combining data using Simpson’s rule. These experiments demonstrated that the MRI-measured cardiac masses were in excellent agreement with those determined at autopsy. In addition, intravascular and interobserver variability of in vivo MR measurements of LV mass was low (5%) in this study. In contrast, 2-D Echo measures LV wall thickness at the midventricular level without taking into account variations in LV chamber and wall dimensions at other anatomical levels, which mandates the use of certain geometric assumptions to estimate LV mass. Siri et al. (122) measured LV mass using 2-D Echo and MRI on the same mice (1 day apart) and demonstrated that MR-measured LV mass was in excellent agreement with that determined at necropsy whereas 2-D Echo underestimated LV mass in these same mice. This was most likely due to an underestimation of short-axis LV dimensions with 2-D Echo. Furthermore, these authors went on to show that MR measurements of LV mass accurately quantified both moderate and severe LV hypertrophy in aortic-constricted mice that was confirmed at autopsy (122). A similar increase in murine LV mass subsequent to chronic isoproterenol infusion was correctly measured using MRI by Slawson et al. (123). Another early study demonstrated a close correlation \( r = 0.95 \) between MRI and autopsy-determined LV mass in newborn, juvenile, and adult mice (149), highlighting the ability of cine-MRI to accurately record developmental changes in the murine cardiac phenotype. Subsequently, owing to its high temporal and spatial resolution, cine-MRI was also used to assess the presence and severity of LV remodeling in a number of mouse models including myocardial infarction (MI) (92, 95), tumor necrosis factor-α overexpression (38, 71), guanylyl-cyclase A knockout (37), and murine Chagas’ disease (67).

**Global LV function.** Cine-MRI can be used to reliably measure murine global LV function. The study by Ruff et al. (113), described earlier, was one of the first to make such measurements. After manually tracing the cardiac borders for each slice as described above, these authors calculated stroke volume (SV) as the difference between end-diastolic volume (EDV) and end-systolic volume ( ESV), and ejection fraction (EF) as the ratio of SV to EDV. Cardiac output (CO) was then calculated as the product of heart rate and SV. The authors obtained the following values for mean EDV (45.2 ± 9.3 μl), ESV (14.6 ± 5.5 μl), SV (30.5 ± 4.6 μl), EF (68.6 ± 6.6%), and CO (11.2 ± 2.4 ml/min) in the healthy murine myocardium (113). A number of subsequent MR studies documented similar LV functional parameters with variations depending on the age of the mice studied (15, 37, 148, 149). Indeed, Weismann et al. (149) showed a progressive increase in CO (range, 1.1 to 14.3 ml/min) and SV (range, 3.2 to 40.2 μl) with age from newborn to adult mice (149). However, EF remained unchanged (60–70%) with aging. The values for global EF reported for healthy adult mice (60–75%) in these early studies are similar to those reported in humans (6, 29), suggesting that mice could serve, at least at this level, as a model of human cardiovascular physiology. Once again, Siri et al. (122) compared outer LV dimensions measured using in vivo MRI and 2-D Echo and demonstrated that the MRI-measured average LV outer dimension was in excellent agreement with that quantified postmortem with an optical micrometer, whereas the 2-D Echo-measured value was significantly lower. Furthermore, Franco et al. (38) confirmed that MRI-measured LV function in mice correlated well with that measured invasively (38), emphasizing that MRI rightly quantifies murine LV dimensions and hence function. A subsequent study that directly compared MRI-measured LV function in mice with that quantified invasively using pressure-volume loops demonstrated that absolute ventricular volumes were strikingly underestimated by conductance catheter measurements (63). Furthermore, LV volumes measured using conductance catheter
Regional LV function. Although traditional LV function assessment by conductance catheters versus MRI, the former can provide rapid measures including functional measures that are independent of loading conditions, whereas the latter measures are truly noninvasive, can be repeated in the same animal over time as pathology evolves, and critically provide robust measures of LV volume that are not dependent on meticulous calibration.

Since the early 2000s, MRI has been used to assess murine LV function in many disease models. In mice with heart failure due to chronic MI, cine-MRI revealed gross LV dilatation and a significantly decreased EF (29 vs. 58% in sham-operated mice) (148). In that study, the authors also used a MR micro-imaging system with rapid gradient performance to visualize the ventricular filling process, which meant acquiring a greater number of frames (20–30 per cardiac cycle vs. the usual 12–18) that helped demonstrate lower ventricular ejection rates (MI, 0.17 ± 0.02 vs. sham, 0.37 ± 0.02 μl/ms; P < 0.001) and filling rates (MI, 0.28 ± 0.03 vs. sham, 0.43 ± 0.04 μl/ms; P < 0.01) in infarcted mouse hearts (148). Furthermore, by measuring LV systolic and diastolic dynamics before and after inotropic (dobutamine) challenge, the investigators in this study uncovered the existence of LV filling abnormalities in hearts of β1-adrenoceptor-overexpressing mice, thereby underscoring the inherent ability of MRI to detect latent LV dysfunction. Subsequently, cine-MRI was used to detect reduced EF in a number of murine disease models, including mice overexpressing tumor necrosis factor-α (11, 38, 71), following dobutamine challenge in vascular endothelial growth factor knockout (VEGF KO) mice (151) and phosphocreatine (PCr)-deficient guanidinoacetate-N-methyltransferase knock-out (GAMT KO) mice (134). More recently, Schneider and colleagues (119) validated the use of an eight-channel volume-phased array coil for cardiac MRI and quantified global LV function in mice where they demonstrated a threefold acceleration in data acquisition without compromising accuracy, thereby reducing scan times (119).

Maximal systolic contraction velocity, a measure of LV function, was assessed in creatine kinase (CK) knock-out mice using phase-contrast MRI (93, 94). These studies showed that the maximal systolic contraction velocity was markedly reduced (Fig. 1) in CK knock-out mice (vs. wild-type mice) despite preserved EF. This highlights the sensitivity of phase-contrast MRI in detecting myocardial functional abnormalities in the presence of preserved EF.
demonstrated by MR tagging in neuronal nitric oxide (NO) synthase knockout mice (vs. wild-type mice), despite normal baseline contractile function (138). These observations demonstrate the sensitivity of MR tagging techniques for detecting regional differences in myocardial mechanics before the onset of global ventricular dysfunction.

Another MR technique that has been widely used to ascertain regional LV motion is phase-contrast MRI. Indeed, motion-encoded phase-contrast MRI provides pixel-wise measurement of myocardial velocities at the same spatial resolution as the magnitude image. This is in contrast to MR tagging where the spatial resolution of ventricular strain is essentially determined by the tag spacing rather than image pixel size. Consequently, phase-contrast MRI has been used to analyze murine LV wall motion in vivo using both 2-D (131) and 3-D (53) approaches. Those studies not only characterized normal LV wall velocities in healthy mice (53) but also demonstrated markedly reduced LV wall velocities in murine myocardium post-MI (131). Finally, displacement-encoded MRI has been used to quantify systolic myocardial displacement, 2-D strain, and twist and torsion in healthy adult mice as well as those subjected to MI (43, 44, 70). When compared with other MR techniques (viz. MR tagging and velocity-encoded phase-contrast MRI), displacement-encoded MRI provided higher spatial resolution of the strain data and demonstrated reduced displacement and strain in the infarcted murine myocardium (43, 44). Together, these studies highlight the wealth of information that MRI can provide with respect to regional LV function.

Myocardial perfusion. Myocardial perfusion is critical for normal ventricular function and viability. Although information pertaining to murine myocardial blood flow can be obtained using contrast echocardiography (110, 111), those methods have the limitations of echocardiography and contrast administration. Spin-labeling perfusion MRI does not require exogenous contrast administration, is performed by acquiring two successive (nonselective and slice selective) inversion recovery images, and offers a relatively high spatial resolution for the quantification of regional blood flow in murine hearts (132). With the use of this technique, a significant reduction in perfusion was demonstrated in infarcted mouse myocardium (94, 132) as well as in the hypertrophied hearts of CK-deficient mice (94). More recently, Makowski and colleagues (83) adapted dynamic first-pass contrast-enhanced MRI, previously used in humans, to measure perfusion in the murine myocardium following infarction. This technique accelerated data acquisition by reducing the scan times and demonstrated the expected reduced myocardial blood flow in mice subjected to MI (1.2 ± 0.8 ml·g⁻¹·min⁻¹) compared with that in control mice (7.3 ± 1.5 ml·g⁻¹·min⁻¹) (83).

Infarct imaging. Infarct size is a key determinant of acute LV dysfunction as well as long-term remodeling of the surviving myocardium (141). Although cine-MRI has excellent soft tissue contrast, the use of contrast agents enhance the visualization and delineation of infarcted myocardium (22, 30). One such contrast-enhanced MRI technique widely used to study infarcted myocardium in mice is late gadolinium (Gd) enhancement (LGE). Tissue characterization with LGE results from the relative differences in the volume of distribution of Gd between normal and abnormal myocardium (1, 112). Gd contrast-enhanced MRI protocols for infarct characterization in mouse hearts have been recently reviewed (22, 30). A number of LGE cardiovascular MRI studies that quantify MI size and LV remodeling post-MI in mice merit additional comment (7, 31, 153). The study by Yang et al. (153) showed a strong correlation (r = 0.96) between infarct sizes determined in vivo using Gd-enhanced MRI and those measured postmortem with triphenyl tetrazolium chloride staining together with a marked reduction in EF. More recently, Bohl and colleagues (7) validated an improved 3-D MRI method to noninvasively quantify infarct size in mice with high spatial resolution and tissue contrast. Again, there was an excellent correlation between infarct sizes derived from 3-D LGE and histology (r = 0.91). Collectively, these studies provide compelling evidence that...
cardiac MR techniques provide the most robust noninvasive means for quantifying infarct size in intact mice. Related LGE techniques may also be useful for identifying diffuse fibrosis in nonischemic cardiomyopathy. Unlike infarct settings where a remote zone without enhancement can be analyzed and used for comparison, the identification of diffuse fibrosis with LGE MRI requires a means to quantify T1 in absolute terms after Gd contrast administration (T1 mapping). Such T1-mapping LGE approaches have been used to study patients with nonischemic cardiomyopathy (58) or aortic disease (126), and the findings have correlated with indexes of fibrosis. Similar techniques have been adapted to rodents (21, 76, 88). In addition, a modified T2-mapping approach that is not reliant on Gd contrast agent administration produces a signal that correlates with diffuse fibrosis in a murine heart failure model (12).

Imaging myocardial fiber structure. Cardiac muscle architecture influences the mechanical and electrical properties of the heart. The spatial organization of the ventricular fibers can be remodeled in some important cardiac pathologies but fiber remodeling is difficult to detect and/or quantify. Diffusion tensor imaging (DTI) is an evolving MRI technique that can be used to nondestructively measure such complex fiber orientation at high spatial resolution (51). Only recently the technique has been used to quantify the arrangement of murine myocardial fiber structure in health and disease (50, 133), albeit in nonbeating hearts. One such study demonstrated that the apparent diffusion coefficient and fractional anisotropy are significantly altered in the infarcted and healing murine myocardium (133). Indeed, the apparent diffusion coefficient was lower in infarcted regions than in remote regions and increased as a function of time after infarction, whereas fractional anisotropy was higher in the infarcted region peaking at 28 days postinfarction (133). Furthermore, substantial myofiber disarray was observed in the infarcted regions. Histo logical analysis correlated well with the DTI parameters at different stages of the healing process (133). In vivo DTI of the beating mouse heart is challenging because of the bulk tissue motion that occurs during the evolution period when the diffusion measurements are made (22). However, Huang (57) has recently shown that the beating mouse heart can be imaged using DTI tractography, suggesting that technological advancements have pushed the boundaries of murine myocardial DTI. The technical considerations and various schemes/protocols used for murine DTI have been extensively reviewed elsewhere (51, 125).

Imaging myocardial calcium uptake. Calcium plays a critical role in excitation-contraction coupling necessary for normal myocardial contraction. Although fluorescent techniques can be used to assess intracellular calcium in isolated cardiac myocytes or perfused hearts (81), in vivo studies using these techniques are challenging. Manganese-enhanced MRI has been successfully used to noninvasively assess intracellular calcium as well as myocyte calcium uptake in murine hearts, since Mn$^{2+}$ is known to enter cardiac myocytes via voltage-gated calcium channels and since the rate and extent of Mn$^{2+}$ enhancement is proportional to changes in calcium influx (56, 82, 139). Using this technique, Hu et al. (56) simultaneously measured global cardiac function and calcium influx to evaluate in vivo responses to inotropic challenge (dobutamine) and calcium channel blockade (diltiazem) in mice. Subsequently, MacGowan and colleagues (82) demonstrated a decline in vivo calcium influx in the hearts of transgenic mice expressing troponin I lacking protein kinase C phosphorylation sites using manganese-enhanced MRI (82), a finding that was hitherto demonstrated only in isolated perfused hearts using fluorescent dyes (81). Manganese-enhanced MRI has been used in a number of studies to assess calcium cycling in vivo in the murine myocardium, highlighting the potential of the technique for noninvasive measurements.

Right ventricular mass and function. The intricate geometry of the right ventricle (RV), its relatively coarse trabeculae, and the complexity of RV contraction make it challenging to assess RV mass and function with conventional imaging techniques (97). Nonetheless, cine-MRI has been successfully used to characterize RV structure and function (123, 147). MR-measured RV SVs were in excellent agreement with the internal standard of LV SVs in healthy mice (147). Furthermore, RV dysfunction and remodeling were evaluated in a mouse model of infarction-induced LV failure (147), whereas RV wall thickness was correctly determined in caveolin-1/3 knockout mice (26). Together, these studies underline the ability of MRI to noninvasively assess murine RV morphology and function.

Vascular imaging. The high soft tissue contrast and spatial resolution offered by MRI have been used to also visualize lesions in the aorta and coronary arteries. Fayad et al. (32) described a MR microscopy technique to visualize atherosclerotic plaques in the aortic wall of apolipoprotein-E knockout mice in vivo (32). There was a good agreement between MR-measured wall area and that determined by histopathology in this study. Subsequent studies quantified aortic atherosclerosis over a wider range of lesion severity (17), recorded the evolution of atherosclerotic lesions using serial MR microscopy (18), and identified aortic root lesions (61, 150). These studies used a “black blood” spin-echo sequence to suppress the signal from flowing blood in the vessels, thereby giving a clear view of the vascular lumen and atherosclerotic plaque size. Furthermore, Ruff and colleagues (114) were able to image murine coronary arteries and cardiac valves in great detail using a segmented 3-D multiple thin-slab FLASH MR sequence.

Murine Cardiac Magnetic Resonance Spectroscopy

In a magnetic resonance spectrum, the peak position is determined by chemical bonds of that particular nucleus and the area under each peak is determined by the number of nuclei in that chemical compound (Fig. 3). Most cardiac MRS studies have exploited the phosphorus (31P) MR signal because it uniquely enables the noninvasive quantification of the high-energy phosphates in the beating heart, including ATP (γ-, α-, β-phosphates) and PCr (Fig. 3) (40). The 31P MR spectrum can be used to quantify the cardiac PCR-to-ATP ratio, absolute high-energy phosphate metabolite concentrations, and intracellular pH in a nondestructive manner (42). 1H nuclei have the highest MR sensitivity and are used in MRI where the signals primarily arise from water and lipid protons. 1H cardiac MRS allows the quantification of various metabolites including creatine, lactate, and carnitine as well as myocardial lipids. 13C is a stable, nonradioactive form of carbon that is 1% abundant in nature. 13C MRS experiments can detect naturally abundant cardiac lipids but most cardiac 13C MRS studies are combined with the administration of 13C-enriched compounds such as glucose, fatty acids, or ketone bodies so that the fate of the
labeled $^{13}$C nuclei in specific exogenous substrates can be followed through various intracellular metabolic pathways, including glycolysis, β-oxidation, and the citric acid cycle (146). $^{23}$Na has a very high natural abundance, short relaxation times, and relatively high MR sensitivity (9.3%). It has been used in various perfused heart studies to study the intra- and extracellular cation pools (66, 105).

We will present a review of the MRS techniques used in murine isolated heart and in vivo studies as well as the pathophysiological insights generated from selected studies.

**Isolated, perfused heart MRS.** Ex vivo $^{31}$P MRS studies performed in isolated hearts perfused in the Langendorff mode were first described more than 30 years ago in rat hearts (41, 62) and more than 15 years ago in mouse hearts (116, 136, 137). The specific details of the surgical heart isolation and perfusion procedure have been described before (19, 115) and typically involve placing the heart and canulated aorta in a glass MR tube in the sensitive volume of a vertical high-field MR spectrometer (116). This approach allows precise control of coronary flow rates, carbon substrate availability, and myocardial temperature, all of which can be useful in studies of ischemia-reperfusion, inotropic stimulation, extracellular Ca$^{2+}$ or other ion manipulations, and exogenous substrate competition. Another advantage of isolated, perfused heart studies is that essentially all of the signals arise from the heart with no signals from blood or surrounding tissues to confound interpretation.

In addition to the repetitive quantification of cardiac high-energy phosphates in the same heart over time, $^{31}$P MRS has been used in isolated hearts to quantify ATP synthesis rates and intracellular pH and Mg$^{2+}$. ATP synthesis rates can be quantified with magnetization transfer MRS approaches that use chemically selective pulses to irradiate one of the $^{31}$P-containing metabolites while the effect on other chemically exchanging moieties is observed (115). This will be described later. Intracellular pH can be determined by the chemical shift position of the inorganic phosphate (Pi) peak relative to that of the PCr resonance (34, 91). Intracellular free Mg$^{2+}$ content can be estimated from the chemical shift difference between α- and β-phosphate resonances of ATP (136).

**ENERGETIC ASSESSMENT BY $^{31}$P MRS OF CK-DEFICIENT PERFUSED HEARTS.** The first ex vivo-perfused mouse heart $^{31}$P MRS studies were likely performed in late 1990s with bioengineered mice lacking muscle-specific isoenzyme of CK (CK-M) (116, 136, 137). These experiments demonstrated, remarkably that the PCr-to-ATP ratio, the absolute PCr and ATP concentrations, and phosphorylation potential are normal in CK-M knockout mice. Even with increased pacing frequency, all $^{31}$P-MRS-derived metabolites were roughly similar in CK-M knockout and wild-type mouse hearts (116, 136). However, at higher workloads, the levels of cytosolic free ADP were similar or a bit higher (116, 136) and ATP flux through CK increased less in CK-M knockout compared with wild-type mice (137). These studies suggested that the residual mitochondrial CK (CK-mito) is able to preserve most of the CK function in the heart and may play a more important role than CK-M (137). Mouse hearts lacking sarcomeric mitochondrial CK (ScCKmt$^{-/-}$) have normal contractile function but a ~30% reduction in total CK activity and in cardiac PCr/ATP with an increase in cytosolic [ADP] and ΔG$^{-\text{-ATP}}$ compared with wild-type animals (127). It was concluded that bidirectional high-energy phosphate transfer is preserved even if CK is absent in the mitochondrial intermembrane compartment (127), similar to skeletal muscle lacking ScCKmt (60). In double CK knockout mice, those lacking both the muscle and mitochondrial isoforms (CK-M/ScCKmt$^{-/-}$), reduced PCr, and PCr/ATP were observed while ATP content was preserved (116). Although PCr appears to be metabolically inert in skeletal muscle of CK double-knockout mice, myocardial PCr can be hydrolyzed in these mice, probably because of residual CK-B (59, 116). In hearts lacking both CK-M and CK-mito, the rate of ATP synthesis via CK was only 9% of the rate of ATP synthesis from oxidative phosphorylation (115). Taken together, these $^{31}$P MRS studies in CK-deficient hearts suggest...
that CK does not play an obligate intracellular transport role in the normal mouse heart and that its presence is not critical for baseline contractile function in normal hearts. However, it is important to point out that significant cytoarchitectural modifications occur with constitutive CK deletion that enhance energetic cross talk and minimize the consequences of CK deficiency (69). Nevertheless, the combination of 31P MRS technology and targeted transgenic manipulations of specific isoforms of CK have provided unique and powerful insights into myocardial high-energy phosphate metabolism and the rates of ATP synthesis under resting and increased workload conditions in the intact heart that would never have been possible with conventional “freeze and grind” biochemical techniques.

ENERGETIC CHANGES ASSOCIATED WITH SARCOMERIC PROTEIN MUTATIONS IN PERFUSED HEARTS. Familial hypertrophic cardiomyopathy (FHC) is the most common inherited cardiac disease, and it is typically associated with varying degrees of hypertrophy, fibrosis, and sudden cardiac death. Although most cases are attributable to mutations in sarcomeric proteins, energetic abnormalities are often present in patients with the disease and in animal models bearing specific mutations (23, 68, 128). In transgenic mice bearing the Arg403Gln mutation in the myosin heavy chain, the first described FHC mutation and one associated with high-mortality rates, 31P MRS studies demonstrated normal cardiac [ATP] levels but reduced [PCr] and higher [Pi] compared with wild-type (128). In these mice, the available free energy of ATP hydrolysis, ΔG\text{ATP}, was less at high rates of energy consumption than in control animals and in the range of the minimal energy requirement of the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase, the ATPase with the highest minimal energy requirement in muscle cells (128). It was speculated that the Arg403Gln hearts have an increased energetic cost of contraction and at high workload reach an energetic state where the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase is unable to maintain the cytoplasm-sarcoplasmic reticulum Ca\textsuperscript{2+} gradients, resulting in diastolic Ca\textsuperscript{2+} overload (128). Hearts of mice bearing the missense mutation R92Q in the tropomyosin-binding domain of cTnT, another clinically severe form of FHC, were studied with 31P MRS and also demonstrated lower [PCr] and ΔG\text{ATP} with preserved [ATP] (64). The findings of an increased energetic cost of contraction in FHC hearts due to different mutations was surprising, given that the mutations were in sarcomeric and not metabolic proteins. Nevertheless, similar findings have been reported in patients with FHC (23).

METABOLIC CHANGES DURING MYOCARDIAL ISCHEMIA-REPERFUSION IN PERFUSED HEARTS. The nondestructive nature of MRS is ideally suited to studies of ischemia-reperfusion whereby high-energy phosphates and carbon metabolites can be repetitively sampled in the same heart as coronary flow is precisely manipulated, as initially described in rat hearts (41, 62, 143) and more recently applied to mouse hearts. During total ischemia there is a rapid decline in PCr and a slower decline in ATP, as the CK reaction acts as a buffer to sustain ATP levels. The repetitive energetic sampling that can be accomplished with 31P MRS can provide some insight into the impact of specific molecular manipulations during ischemia or reperfusion or both (13, 35, 107, 134).

For example, overexpression of the adenosine A\textsubscript{3} receptor lessened ischemic injury as the rate of ATP depletion during ischemia was decreased, and this was associated with improved postischemic recovery of metabolism and function (24). In contrast, the rates of ATP and PCr decline during ischemia were identical in wild-type and endothelial NO synthase knockout (eNOS\textsuperscript{−/−}) mice, but during postischemic reperfusion, eNOS\textsuperscript{−/−} mice exhibited improved recovery of PCr and developed pressure (35). This suggests that endogenously produced NO plays a significant role in ischemia-reperfusion injury but that the mechanism is not via ATP preservation during ischemia per se.

OVEREXPRESSION OF GLUTAMINE TRANSPORTER AND MURINE HEART FAILURE: INSIGHTS FROM COMBINED 31P AND 13C MRS STUDIES IN PERFUSED AND INTACT HEARTS. Studies of glucose transporter function highlight several early and powerful applications of MRS for the study of murine cardiac metabolism. The combined use of 31P and 13C MRS provided important insights into metabolic remodeling in heart failure especially from studies in mice overexpressing glucose transporter 1 (GLUT1), an insulin-independent transporter. Mice with cardiac-specific overexpression of GLUT1 have increased myocardial glucose uptake under baseline conditions (78). Following aortic constriction surgery, wild-type mice develop hypertrophy and a subsequent progressive decline in LV function with a reduced cardiac PCr/ATP and a 40% mortality at 8 wk (78). In contrast, GLUT1 overexpressing mice exposed to the same surgery develop the same degree of LV hypertrophy but the hearts are not dilated, function is preserved, PCr/ATP remains normal, and mortality is only 10%. Peroxisome proliferator-activated receptor-α (PPAR\textalpha), a nuclear receptor that regulates the expression of multiple genes controlling both fatty acid uptake and oxidation, was ablated in transgenic mice, and this resulted in a phenotype with normal cardiac contractile function but reduced PCr/ATP at baseline and decreased contractile function and ATP synthesis rates during hemodynamic stress (high Ca\textsuperscript{2+} perfusion) (80). Remarkably, overexpression of GLUT1 in PPAR\textalpha hearts normalized baseline high-energy phosphate levels as well as function and ATP synthesis at increased workload (80). Taken together, these findings demonstrate that further enhancing myocardial glucose use is an effective strategy for preventing the progressive energetic decline and cardiac dysfunction of hearts with impaired PPAR\textalpha activity such as those with pathological hypertrophy (78, 80).

In vivo murine cardiac MRS. TECHNICAL CONSIDERATIONS FOR IN VIVO MURINE CARDIAC MRS. Although the ex vivo isolated, perfused heart preparation allows exquisite control of coronary flow, substrates, temperature, and workload conditions and has provided many important mechanistic insights, it does not perfectly replicate truly physiological in vivo conditions with blood perfusion, hemodynamic loading, and intact neurohumeral and ventricular-vascular interactions, nor does it permit repeated studies in the same animal as pathology develops. Open-chest murine cardiac 31P MRS studies use 31P surface coils placed directly on the surface of the heart and allow for rapid acquisitions under in vivo conditions (74, 144). The approach described by Lee et al. was able to detect a significant reduction of in vivo cardiac PCr/ATP following MI (74), as well as an improvement in PCr/ATP and energetic status when murine cardiac-derived Sca-1+/CD31− progenitor cells were injected into the peri-infarct zone (140). The latter was associated with improved function (EF) and reduced remodeling (140). Because stem cell engraftment and transdifferentiation are typically low, the ability of MRI and MRS to

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quantify factors directly related to the likely benefits of cell therapy, such as wall stress and energetics, underscore the role that MRI and MRS could play in deciphering mechanisms of cellular therapy.

The open-chest in vivo approaches to MRS, however, do not allow for truly noninvasive acquisitions or repeated studies over time in the same animal, and thus completely noninvasive 31P MRS approaches will be reviewed in more detail here. A number of noninvasive image-guided techniques for localizing magnetic resonance spectra from specific regions deep within the body were used to collect human cardiac MR spectra (146a), and these were later adapted for murine heart studies (15, 96, 98). Such approaches typically rely on custom coil assemblies that allow MRI with a 1H-tuned coil and MRS acquisitions with a 31P coil without repositioning the mouse (15).

With this general probe design, one can implement single-voxel or multiple-voxel spatial-localization techniques to acquire MR spectra from the heart while minimizing signals from surrounding tissues like the chest wall and liver. Multiple-voxel techniques offer the opportunity to sample more than one cardiac region, chest muscle, and possibly an external reference phantom in a single acquisition and can be acquired in about the same time as some single-voxel techniques. In practice, a trade-off is made between the acquisition time and the degree of spatial localization, the latter being important for limiting signal from adjacent, noncardiac structures. Fortunately, the in vivo heart is mostly surrounded by lung tissue that offers little 31P NMR signal, with the main sources of 31P signal contamination in cardiac studies arising from the superficial chest muscle and liver. For many practical murine cardiac exams, the combined use of a one-dimensional chemical shift imaging sequence (to localize 31P NMR signals in the anterior-posterior direction from the chest into the heart) and a small diameter 31P receive surface coil (to severely limit signal contamination from the lateral chest and liver, see below) offers a means to acquire cardiac-localized spectra under physiological conditions in tolerable times in anesthetized mice.

CARDIAC PCR/ATP IN THE NORMAL MURINE HEART IN VIVO. The first noninvasive in vivo murine cardiac spectra were acquired with image-guided one-dimensional chemical shift imaging (see Fig. 4) that provides a stack of spatially localized 31P spectra from the surface coils, through the chest and to the heart, in ~34 min at 4.7 T (15). This approach enabled combined functional and energetic assessment in the same exam. The in vivo mouse cardiac PCR-to-ATP ratio was found to be 2.0 ± 0.2 (mean ± SD), very close to that previously reported in the normal human heart despite the much higher heart rate in the mouse (15). At about the same time, a single-voxel technique for localizing 31P MRS, called image-selected in vivo spectroscopy (ISIS) method, was adapted to murine studies and demonstrated a similar PCR/ATP in normal mice (2.1 ± 0.04) but a reduction in mice overexpressing bovine growth hormone (1.5 ± 0.13, P < 0.01) (98). During inotropic stimulation that increased heart rate by 40%, the cardiac PCR/ATP did not change, which is similar to prior observations in normal human hearts (96). Taken together, these studies suggest that despite dramatic differences in heart size and heart rates across species from mice to humans, the in vivo PCR/ATP is ~2 in normal hearts and unchanged during at least physiological submaximal levels of increased workload (15, 96, 98).

ALTERED CARDIAC PCR/ATP WITH GENETIC MANIPULATIONS AND IN HEART FAILURE MODELS. A decreased in vivo murine cardiac PCR/ATP, akin to that observed in human heart failure, has been observed in most murine models with contractile dysfunction, including the thoracic aortic constriction, coronary ligation/MI, and doxorubicin cardiotoxicity models. Four weeks after coronary ligation, infarct remodeled mouse hearts were markedly dilated with severely reduced contractile function and a decreased in vivo cardiac PCR/ATP ratio (1.4 ± 0.6)
Thoracic aortic constriction (TAC) results in progressive LV hypertrophy with eventual contractile dysfunction and dilatation. The evolution of this process is ideally suited for a noninvasive imaging/spectroscopy approach that allows serial studies in the same animal. Serial, image-guided in vivo 31P MRS/MRI studies were performed at 3 and 6 wk after TAC surgery (87). Cardiac PCr/ATP declined early at 3 wk along with the development of LV hypertrophy and a decline in EF. The ventricles dilated over the ensuing 3 wk, and, importantly, a reduced cardiac PCr/ATP at 3 wk correlated with EDVs at 6 wk suggesting that abnormal energetics due to pressure overload predict subsequent LV remodeling (87). Serial 31P MRS/1H MRI studies in a mouse model of doxorubicin cardiotoxicity also demonstrated early metabolic changes. Specifically, cardiac PCr/ATP was significantly decreased 6 wk after doxorubicin administration at a time when no systolic or diastolic dysfunction could be detected by MRI (86). With continued administration, metabolic abnormalities progressed and contractile dysfunction developed, suggesting a relationship between cardiac energetics and both LV systolic and diastolic dysfunction. These observations do not prove, but are consistent with, the hypothesis that impaired high-energy phosphate metabolism contributes to doxorubicin-induced myocardial dysfunction. In these studies of progressive myocardial injury, hypertrophy, or remodeling in TAC and doxorubicin hearts, the noninvasive, serial nature of combined 31P MRS/1H MRI uniquely allowed repeated, serial studies of cardiac energetics, structure, and function as these pathophysiological processes evolved.

There are a few in vivo studies of cardiac PCr/ATP in transgenic models. The in vivo myocardial PCr-to-ATP ratio was reported to be higher in GLUT4 null mice. This was attributed to an increase in the total creatine pool but not associated with changes in myocardial [ATP], [ADP] or $\Delta G_{\text{ATP}}$, or skeletal muscle PCr/ATP (144). In contrast, a significant reduction in PCr/ATP was observed in a transgenic cardiomyopathy model with a cardiосpecific inducible NO synthase (iNOS) overexpression and lack of myoglobin (tg-iNOS/wt). These observations suggest that in the absence of efficient cytosolic NO scavenging, iNOS-derived NO critically interferes with the respiratory chain (36).

HIGH-ENERGY PHOSPHATE CONCENTRATIONS AND RATES OF ATP SYNTHESIS. Although the cardiac PCr-to-ATP ratio has been used for decades as an energetic index of myocardial high-energy phosphate metabolism, it alone cannot detect reductions in ATP content or in the rate of ATP synthesis. Techniques to quantify ATP content, using external 31P standards, and to quantify rates of ATP synthesis, using magnetization transfer, were previously developed for noninvasive studies of the human heart (8, 9). Those techniques were recently modified, adapted, and validated in the mouse (47, 48). The first image-guided spatially localized 31P MRS method for quantifying cardiac [PCr] and [ATP] under truly physiological conditions was recently reported and used an external reference phantom (48). This approach rendered a [PCr] and [ATP] of 10.4 ± 1.4 and 5.0 ± 0.9 μmol/g wet wt, respectively, in normal mouse hearts and significantly lower values of 6.7 ± 2.0 and 4.0 ± 0.8 μmol/g wet wt for [PCr] and [ATP], respectively, in TAC hearts. A novel finding of this method is that the reduction of [ATP] in the TAC heart means that the normal well-integrated metabolic machinery of the heart has failed and that the mechanisms that slowly replete ATP pools are inadequate (48).

Magnetization transfer MRS techniques offer the ability to detect the rates of exchange through specific chemical reactions, and the reactions most commonly studied with 31P MRS magnetization transfer are the CK reaction (PCr$\rightarrow$ATP) and ATP synthesis from inorganic phosphate (P$_i$$\rightarrow$ATP). The general approach, as alluded to above, involves the application of a continuous chemically selective irradiation pulse on a specific resonance (such as the terminal or $\gamma$-phosphate of ATP) to perturb the spins of that resonance and then to observe changes in the resonances of other metabolites in chemical exchange with that resonance (such as in PCr when measuring ATP flux through CK) (see Fig. 5). The conventional magnetization transfer or saturation transfer MRS techniques were very time consuming as they typically required 7–10 MRS acquisitions for a single measurement. However, more rapid methods have been developed that reduce this to 3 to 4 rapid acquisitions, enabling in vivo studies in tolerable times (9, 117, 145). The first in vivo measures of ATP flux through CK in the mouse heart were recently reported (47), and they were based in part on an approach used in human hearts (117). In this approach a triple repetition time saturation transfer (TRiST) method was adapted to mice in which two spatially localized 31P MR spectra were obtained with different repetition times (1.5 and 6 s) in the presence of a saturating irradiation pulse applied to the exchanging CK moiety, viz., $\gamma$-phosphate of ATP at $-2.5$ parts/million (ppm), relative to PCr, and third acquisition with control irradiation applied at +2.5 ppm. During saturation of $\gamma$-phosphate of ATP, the reduction of the PCr resonance is directly related to the unidirectional rate of ATP synthesis through CK (i.e., PCr$\rightarrow$ATP) (145). Despite the massive differences in size and resting rate between murine and human hearts, the rate of ATP synthesis through CK ($\sim 0.3$ μmol·g$^{-1}$·s$^{-1}$) is remarkably similar between these species. TAC murine hearts exhibited a ~30% reduction in $kf$ and a ~50% decline in the rate of ATP synthesis through CK. The reduction in ATP flux was much greater than the reduction in PCr-to-ATP ratio, suggesting that the previously used index of myocardial energetics based on relative metabolite pool sizes underestimates the reduction in myofibrillar ATP delivery in failing hearts. Moreover, the 50% reduction in ATP flux observed in the in vivo failing TAC mouse heart is similar to the 50% reduction in patients with dilated cardiomyopathy (145) and the 65% reduction in patients with pressure-overload hypertrophy and heart failure (124), suggesting that this murine model mimics important bioenergetic aspects of human heart failure (47).

All of these new techniques to measure in vivo myocardial high-energy phosphate pool sizes and ATP synthesis through CK were applied to recently created CK-M conditionally overexpressing mice to test the hypothesis that augmenting CK ATP energy delivery to failing mouse hearts augments contractile function (i.e., that the failing heart is energy starved as...
Fig. 5. In vivo murine cardiac MRI (A), saturation (Sat) transfer $^{31}$P MR spectra (B–D) used to measure PCr/ATP (E), high-energy phosphate concentrations (F and G), and ATP kinetics (H) including the rate of ATP synthesis through CK (I). Note that overexpression of muscle-specific isoenzyme of CK (CK-M) improves metabolism in failing thoracic aortic constriction (TAC) hearts (E, F, and I) and improves contractile function at 4 and 12 wk post-TAC (J and K), although the latter is reversible and dependent on persistent CK-M overexpression (K). MRS, magnetic resonance spectroscopy; EF, ejection fraction; SV, stroke volume; CO, cardiac output. *P < 0.05; **P < 0.01; ***P < 0.001; ΔP < 0.01; #P = 0.07. Reproduced with permission from Gupta et al. (46).
Fig. 6. Top: representative stacked $^{13}$C NMR spectra acquired in the first 60 s following $[2^{-13}$C$]$pyruvate infusion into a perfused rat heart. $[2^{-13}$C$]$Pyruvate was observed at 207.8 ppm. Peaks 1, 2, and 3 represent the metabolic products $[5^{-13}$C$]$glutamate (183.7 ppm), $[1^{-13}$C$]$citrate (181.0 ppm), and $[1^{-13}$C$]$acetyl carnitine (175.2 ppm); peak 4 represents natural abundance $[1^{-13}$C$]$pyruvate (172.8 ppm, left inset); peak 5 represents $[2^{-13}$C$]$pyruvate hydrate; and peaks 7 and 8 represent $[2^{-13}$C$]$lactate and $[2^{-13}$C$]$alanine. Bottom: summary of the metabolic fate of infused $[2^{-13}$C$]$pyruvate along with the measured parameters of the observed metabolites in normal and ischemic isolated hearts. See Schroeder et al. (121) for definitions of symbols. Reproduced with permission from Schroeder et al. (121).
related to CK metabolism (46). These studies demonstrated that CK-M overexpression significantly increased ATP flux through CK ex vivo and in vivo but did not alter contractile function in normal mice. In failing hearts induced by TAC, CK-M overexpression increased the rate of ATP synthesis through CK and improved cardiac PCr/ATP and [PCr]. Importantly, this improvement in CK energy metabolism in failing hearts improved multiple indexes of contractile function, both at rest and in response to dobutamine stimulation. CK-M overexpression also increased survival in failing hearts. In addition, when the CK-M transgene overexpression was turned off after the development of heart failure, the contractile improvement resolved. All of these observations provide arguably the first direct evidence that the failing heart is energy starved as it relates to CK metabolism (46). The ability of noninvasive MRI/MRS to provide serial measures of LV function and energy metabolism in the same animal as heart failure developed, and the CK expression was manipulated enabled new and potentially important insights into the role of impaired energy metabolism in heart failure.

The steady-state myocardial PCr/ATP, [ATP], and [PCr] reflect, in part, mitochondrial oxidative phosphorylation regulation. ATP synthesis by oxidative phosphorylation and glycolytic exchange (Pi → ATP) can also be measured by 31P NMR magnetization transfer techniques similar to those used to measure ATP flux through CK, except that changes are noted in the Pi resonance while the γ-phosphate of ATP is selectively irradiated (39). A recently reported 31P magnetization saturation transfer method, as first applied in a porcine model, can provide in vivo measures of unidirectional ATP turnover through both CK and Pi → ATP, promising important energetic insights in the future (152).

Although the focus of this summary of in vivo studies has been on 31P MRS, it should be mentioned that in vivo studies with 1H MRS are now being reported. Localized in vivo 1H MRS, using the point-resolved spectroscopy (PRESS) approach, a single-voxel technique, was applied to the mouse heart and water-suppressed spectra from the interventricular septum detected creatine, taurine, carnitine, and lipids. Reduced creatine signals were detected by PRESS in creatine-deficient GAMT KO hearts (120) and elevated creatine in creatine transport protein overexpressor hearts (106).

Stable isotopes and cardiac MRS. Stable isotope administration is not required for 31P MRS studies of high-energy phosphates or of 1H MRS studies of lipids and creatine. However, 18O administration has been used in concert with 31P MRS of heart extracts to calculate ATP synthesis rates through multiple energy transfer reactions including CK and adenylate kinase (108, 109). 2H NMR has been used with 2H2O to quantify perfusion and intracellular water volumes (2, 101). However, 2H-labeled carbon substrates and 2H NMR have not been used to study myocardial metabolism, mostly because of the limited chemical shift dispersion (~7 ppm) and broad peaks of 2H NMR spectra that result in metabolite peak overlap. In contrast, the chemical shift range of 13C MRS is very wide (~200 ppm), and this minimizes signal overlap and allows easier discrimination of specific metabolites and of 13C enrichment within specific carbon positions of a given metabolite. 13C-enriched substrates are routinely used with 13C MRS because together they allow the noninvasive quantification of myocardial substrate competition and use in the beating heart, with identification of the appearance of the 13C nucleus from a labeled substrate into specific carbon positions within metabolites in utilized metabolic pathways as well as the measurement of metabolic rates (4, 5, 80, 129, 130). A detailed description of 13C MRS techniques, 13C substrate labeling strategies, analysis approaches, and cardiac applications are beyond the scope of this discussion, and thus readers are referred to prior reviews on this topic (16, 25, 65, 84, 85). It is probably worth mentioning that critical comparisons of the advantages/disadvantages of 13C MRS and gas chromatography-mass spectrometry for assessing myocardial metabolism were previously published (27, 28). A particularly exciting recent advance is the development of means to hyperpolarize noble gases and some 13C-containing substances to increase the NMR signal by up to a factor of ~100,000 (84, 85). This dramatic increase in 13C NMR signal can be used to generate metabolic maps/images and/or to track metabolism in real time (45, 121) (Fig. 6).

Concluding Remarks

In summary, a combined cardiovascular MRI/MRS exam offers a noninvasive means to quantitatively and serially assess myocardial structure, mass, function, perfusion, and tissue characteristics along with myocardial metabolism, including that of the high-energy phosphates, in a single study in the mouse. The approach requires sophisticated instrumentation and high-field superconducting magnets but can be accomplished in times tolerated by chronically ill animals even during acute interventions, such as adrenergic stimulation. Although the techniques and technology continue to be refined, murine cardiovascular MRI/MRS is now a powerful, evolving means to assess the anatomic, functional, and metabolic consequences of specific genetic manipulations under physiological conditions and repetitively over time as induced pathophysiology develops.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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Review


