Cardio-Chemical Exchange Saturation Transfer Magnetic Resonance Imaging Reveals Molecular Signatures of Endogenous Fibrosis and Exogenous Contrast Media

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Background—Application of emerging molecular MRI techniques, including chemical exchange saturation transfer (CEST)-MRI, to cardiac imaging is desirable; however, conventional methods are poorly suited for cardiac imaging, particularly in small animals with rapid heart rates. We developed a CEST-encoded steady state and retrospectively gated cardiac cine imaging sequence in which the presence of fibrosis or paraCEST contrast agents was directly encoded into the steady-state myocardial signal intensity (cardioCEST).

Methods and Results—Development of cardioCEST: A CEST-encoded cardiac cine MRI sequence was implemented on a 9.4T small animal scanner. CardioCEST of fibrosis was serially performed by acquisition of a series of CEST-encoded cine images at multiple offset frequencies in mice (n=7) after surgically induced myocardial infarction. Scar formation was quantified using a spectral modeling approach and confirmed with histological staining. Separately, circulatory redistribution kinetics of the paramagnetic CEST agent Eu-HPDO3A were probed in mice using cardioCEST imaging, revealing rapid myocardial redistribution, and washout within 30 minutes (n=6). Manipulation of vascular tone resulted in heightened peak CEST contrast in the heart, but did not alter redistribution kinetics (n=6). At 28 days after myocardial infarction (n=3), CEST contrast kinetics in infarct zone tissue were altered, demonstrating gradual accumulation of Eu-HPDO3A in the increased extracellular space.

Conclusions—cardioCEST MRI enables in vivo imaging of myocardial fibrosis using endogenous contrast mechanisms, and of exogenously delivered paraCEST agents, and can enable multiplexed imaging of multiple molecular targets at high-resolution coupled with conventional cardiac MRI scans. (Circ Cardiovasc Imaging. 2015;8:e002180. DOI: 10.1161/CIRCIMAGING.114.002180.)

Key Words: magnetic resonance imaging ◼ magnetization transfer contrast imaging ◼ myocardial infarction

Molecular imaging has emerged as a powerful tool in preclinical research of cancer, cell tracking, and developmental biology,1,2 and emerging molecular MRI techniques including chemical exchange saturation transfer (CEST) can combine molecular and high-quality anatomic imaging.1,3 CEST–MRI uses the exchange of saturated magnetization from endogenous macromolecules,4 chemical contrast agents,1 or engineered reporter genes2 with nearby water molecules to generate contrast on MRI.4 Because each CEST agent generates contrast after radiofrequency irradiation at distinct offset frequencies, multiplexed molecular imaging can be performed in a single session. Application of such techniques to cardiac imaging could prove powerful for combined imaging of molecular targets coupled with structural and functional assessments. However, conventional CEST–MRI methods are poorly suited for cardiac imaging, particularly in small animals with rapid heart rates.

See Clinical Perspective

We introduce a novel, retrospectively cardiorespiratory gated and CEST-encoded steady-state cardiac cine MRI method (cardioCEST) for imaging of fibrosis via endogenous contrast mechanisms and of selectively activated molecular contrast agents. Using endogenous transfer of magnetization between macromolecules in fibrotic scar tissue and surrounding water, we first tested cardioCEST imaging for noninvasive and rapid visualization and quantification of fibrotic scar tissue after surgically induced myocardial infarction (MI) in mice. In the second part of our study, we tested cardioCEST imaging of cardiac circulation with the paraCEST agent Eu-HPDO3A1
in the healthy and infarcted mouse heart. Our results demonstrate both the feasibility of cardioCEST MRI, and its use for multiplex molecular imaging in the heart.

Methods

Animal Models

Adult C57B6 male mice (8–12 weeks of age) were purchased from Harlan Laboratories (Indianapolis, IN). A total of 27 mice were used under protocols approved by the Weizmann Institute Animal Care and Use Committee. MI was surgically induced in 10 mice by permanent left coronary ligation as described by Tarnavski. Mice were anesthetized (ketamine [75 mg/kg IP; Fort Dodge, IA] and xylazine [3 mg/kg IP; VMD, Arendonk, Belgium]), analgized (buprenorphine [0.05 mg/kg IP] and lidocain [1 mg/kg] at the incision site), intubated, and ventilated (12 strokes/min, 200 mL stroke volume, Harvard Mouse Ventilator Model 687, Harvard apparatus, Holliston, MA). A left thoracotomy was performed in the fourth intercostal space, and the left coronary artery was permanently ligated (8-0 prolene suture; Johnson & Johnson Medical Ltd, Wokingham, United Kingdom) at its emergence from under the left atrium.

MRI

MRI was performed on a 9.4T horizontal bore scanner (Bruker Biospec, Ettlingen, Germany), using a cylindrical volume coil for signal excitation and a single channel surface coil for signal reception. Core body temperature and respirations were monitored during imaging (Small Animal Instruments, Stony Brook, New York, NY). Body temperature was maintained at 36.7±0.5°C using thermostated circulating hot water. Anesthesia was induced using 2.0% isoflurane in O₂, and maintained during imaging using 1.25% isoflurane in O₂.

CardioCEST Pulse Sequence Design

CardioCEST, based on a steady-state gradient echo pulse sequence (Figure 1), was designed to generate CEST contrast through the use of frequency-selective radiofrequency irradiation (saturation module) that, after exchange with surrounding water, reduces the initial longitudinal magnetization of the gradient echo readout (readout module) used to acquire image data. Image data were acquired at a constant repetition time and retrospectively gated for cardiac phase and respiratory motion, thus encoding the molecular signature of the target of interest into the steady-state longitudinal magnetization and myocardial signal intensity (for a detailed graphical representation see Figure 1 in the Data Supplement). Saturation module parameters included a train of 28 frequency-selective Gaussian radiofrequency pulses (bandwidth=200 Hz; duration=13.7 ms) with dephasing gradient duration=1 ms, and interpulse delay=5 ms. Readout module parameters included repetition time/echo time=10.2/3.5 ms, with a 1-kHz bandwidth Gaussian excitation pulse (flip angle=10°) and the acquisition of 100 gradient echo readouts. After each CEST preparation, 128 sequence repetitions (saturation and readout modules without a delay between repetitions) were required for acquisition of CEST-encoded cine images. The phase encoding order was linear over the entire acquisition. Additional imaging parameters were FOV=2.56x2.56 cm, Matrix=256x128, slices=1, 6x=100 μm and 6y=200 μm, slice thickness=1 mm, and acquisition time of 2.5 minutes. Images were retrospectively gated using a self-gated navigator algorithm that calculates cardiac and respiratory waveforms, discards lines of k-space acquired during respiratory motion, and bins remaining k-space data based on cardiac phase (IntraGate; Bruker).

The specific parameters of both the saturation and readout modules (flip angle, number of pulses, and the ratio of saturation pulses to repetitions of the readout module) were optimized to produce minimal direct saturation of healthy myocardium when radiofrequency irradiation was applied at offset frequencies far from water resonance, while producing maximal direct saturation when applied on-resonance (Figure 1; Figure II in the Data Supplement). The resulting on-resonance saturation efficiency was 68.3±2.0%, and indirect saturation (measured at ~12 ppm offset from water) was 3.2±2.3%. An additional concern in the design of the cardioCEST protocol was limiting the loss of CEST encoding because of T1-relaxation of surrounding macromolecules. The acquisition of image data at steady state for 1 s after CEST preparation was determined to represent an adequate compromise between preserving CEST contrast and maintaining reasonable scan times. Finally, correction for inhomogeneity of the static magnetic field across the entire field of view of the image is typically performed using water saturation shift referencing to eliminate asymmetrical magnetization transfer ratio (MTR asym) bias. Water saturation shift referencing analysis performed by acquiring CEST-encoded images with low saturation levels (10° saturation flip angle) at offsets of ~1 to 1 ppm with intervals of 0.1 ppm (Figure I in the Data Supplement) revealed a spread of resonant frequencies across the small murine heart that was below the bandwidth of the saturation pulses. Thus water saturation shift referencing correction was not required.

CardioCEST Imaging of Cardiac Fibrosis and Scar Formation

CardioCEST was performed at 1, 7, 10, 14, and 21 days after surgically induced MI (n=7). Macromolecular components of fibrotic scar tissue including collagen are excited across a wide bandwidth of radiofrequency irradiation, and subsequently transfer saturated magnetization to surrounding bulk water through a process termed magnetization transfer (MT). We exploited this endogenous contrast mechanism by acquiring a series of images with saturation offsets of ~12, ~9, ~6, ~4, ~2, ~1, 0, 1, 2, 4, 6, 9, and 12 ppm. To normalize for receiver coil sensitivity and to remove the influence of changes in native-T1 relaxation times, a reference scan was performed with saturation flip angle=1° and offset=−15 ppm. Afterward, T1-weighted images were acquired using a cine imaging sequence with identical spatial parameters and flip angle=25° after intraperitoneal injection of gadolinium–diethylene triamine pentaacetic acid (0.1 mmol/kg body weight) via an indwelling intraperitoneal catheter. At 21 days after MI, hearts were excised and scar tissue was visualized in isolated tissue sections with Masson Trichrome staining.
Image analysis was performed on end-diastolic images by defining infarct (hyperintense), border (5 pixels away from infarct zone circumferentially), and remote (remainder) myocardial zones based on signal enhancement in late gadolinium enhancement (LGE) images. Magnetization transfer ratio (MTR) maps were calculated from data acquired 21 days after MI on a pixelwise basis as $\text{MTR}_{\text{ asym}}=\frac{S_{-1\text{ppm}}-S_{15\text{ppm}}}{S_{-1\text{ppm}}} \times 100$ (%), where $S$ represents signal intensity of a given pixel. The acquisition time for a single set of CEST images was 5 minutes after vasodilatation with dobutamine (40 $\text{mg}$ intravenous bolus injection of either Eu-HPDO3A ($n=6$, $0.6$ mg/g), Eu-HPDO3A molecular weight, 553.39 g/mol), which generates CEST contrast at 15 ppm off-resonance from water at $37^\circ$C. Eu-HPDO3A was dissolved in sterile water for intravenous administration. Mice received an intravenous bolus injection of either Eu-HPDO3A ($n=6$, $0.6$ mg/g), Eu-HPDO3A 5 minutes after vasodilatation with dobutamine (40 mg/kg, $n=6$), or saline ($n=5$). Pairs of CEST images (offsets of 15 and $-15$ ppm) were acquired before, and for 30 minutes (6 sets of CEST images) after injection via an indwelling intravenous tail vein catheter. The acquisition time for a single set of CEST images was 5 minutes. Separately, MI was surgically induced ($n=3$) and cardio-CEST was performed 28 days after MI. MTR$_{\text{ asym}}$ maps were calculated from end-diastolic images as $\text{MTR}_{\text{ asym}}=\frac{S_{-1\text{ppm}}-S_{15\text{ppm}}}{S_{-1\text{ppm}}} \times 100$ (%), where $S$ represents the signal intensity of a given pixel.

Changes in MTR$_{\text{ asym}}$ ($\Delta$MTR$_{\text{ asym}}$) over time were calculated as the change over precontrast levels.

**Statistical Analysis**

Statistical analysis was performed using SigmaStat Analytical software (Aspire Software, Ashburn, VA). Comparison of FS, A, $\gamma$, and $\Delta$MTR$_{\text{ asym}}$ between myocardial regions and over time was performed using repeated measures ANOVA with 1 between effect and 1 within effect using Holms-Sidak comparisons. Measurements of MTR$_{\text{ asym}}$ between infarcted and remote zone tissue at 21 days after MI, peak $\Delta$MTR$_{\text{ asym}}$ with Eu-HPDO3A, and end-diastolic diameter of the left ventricle were assessed using 1-way ANOVA comparison. All data are presented as the mean±SD, all graphs are mean±SE. Significance was defined as $P<0.05$ with 2-sided $P$ values.

**Results**

**Molecular Imaging of Fibrotic Scar Formation**

CardioCEST imaging successfully identified fibrotic scar tissue through the use of endogenous MT contrast. Compared to standard cine imaging where scar and remote tissue demonstrate similar signal intensity, CEST encoding reduces the signal intensity of fibrotic scar tissue when compared with nonfibrotic remote tissue (Figure 2). Areas of fibrotic scar tissue can be rapidly and semiquantitatively visualized as areas of reduced MTR$_{\text{ asym}}$ which was significantly reduced in fibrotic scar tissue as compared with healthy remote tissue at 21 days after MI (Figure 2). Furthermore, acquisition of regional spectra serially during infarct healing enabled monitoring of the dynamics of scar formation. Spectra acquired in healthy, border, and infarct myocardial regions at 1 day after MI demonstrated only subtle differences between myocardial regions (Figure 3), consistent with the presence of edema but not scar tissue during the inflammatory stage of infarct healing. In contrast, at 10 days after MI, maturation of scar tissue caused significantly increased MT in infarct zone myocardium, and an intermediate effect in border zone myocardium (Figure 3). The presence of dense scar tissue in infarct zone myocardium was confirmed by Masson Trichrome staining (Figure 3), demonstrating similar spatial localization to signal enhancement after infusion of gadolinium–diethylene triamine pentaacetic acid in all samples. Using identical mice and surgical techniques at our institution, ligation of the

**Figure 2.** CardioCEST of fibrotic scar tissue. 
A, Anatomic short-axis reference image (saturation flip angle=1°) acquired 21 days after myocardial infarction (MI) demonstrates a thinned wall with preserved signal (white arrows) in scar tissue. B, The image acquired at the same location and cardiac phase with CEST encoding (saturation flip angle=270°; offset=-1 ppm) demonstrates reduced signal within the fibrotic scar (white arrows). C, Measurement of the magnetization transfer transfer ratio (MTR) from images (A) and (B) reveals reduced MTR in areas of fibrotic scar (white arrows) compared with noninfarcted remote tissue. D, Images acquired at the end-systolic cardiac phase confirm the absence of contraction in the area of scar tissue. E, Reduced signal intensity in fibrotic scar is still observed at end-systole in CEST-encoded image. F, MTR measured with a saturation offset of −1 ppm was significantly lower in fibrotic scar tissue compared with healthy tissue at 21 days after MI ($P<0.05$ vs Remote).
coronary artery consistently results in collagen volume fraction measurements of 71±4% in infarct zone tissue at the end of infarct healing.13

To quantitatively image the deposition of fibrotic scar tissue using cardioCEST imaging, we modeled the spectra of fibrotic scar tissue as the convolution product of healthy remote spectra (normal) with a Lorentzian function (Scar Function) that describes the MT effects of macromolecules in fibrotic tissue (Figure 4). Changes in γ during the time course of infarct healing revealed statistically significant line broadening in infarct zone spectra starting at 10 days after MI (Figure IV in the Data Supplement). However, although statistically significant, the effects were still subtle at a maximum of 0.12 ppm. In contrast, significant MT contrast from fibrotic scar tissue reduced the steady-state signal in infarct zone myocardium starting at 7 days after MI, which resulted in statistically significant reduction of A in infarct zone myocardium (Figure 4).

Because the magnitude of A decreases with increasing MT from fibrotic scar tissue, the FS was designed as a more intuitive measure that increases with greater MT from fibrotic scar tissue. The FS increased rapidly between 7 and 10 days after MI in infarct zone tissue and remained elevated for the duration of infarct healing (Figure 4). In parallel, fractional shortening decreased significantly between 1 and 21 days after MI.

**Figure 3.** Spectral changes resulting from fibrotic scar formation. A, Late gadolinium enhancement image reveals hyperintense areas of fibrotic scar tissue 10 days after myocardial infarction (MI; blue dotted line). Border zone tissue (orange dotted line) was defined as the area 5 pixels away from the infarct zone along the circumferential direction, and remote tissue as the remainder (red dotted line). B, The spatial distribution of fibrotic scar was confirmed at 21 days after MI by Masson Trichrome staining. C, Normalized tissue spectra acquired 1 day after MI reveal minimal divergence between infarct, border, and remote tissues. D, Representative normalized spectra acquired 10 days after MI reveal significantly reduced steady-state signal in infarct tissue when CEST encoding is applied at frequencies offset from water resonance. In border zone tissue, which is heterogeneous compared with the densely fibrotic scar tissue, an intermediate effect is observed in acquired spectra. With normalized tissue spectra, a value of 1 indicates the same value between reference and MT/CEST encoded scans, with any divergence resulting from MT/CEST or indirect saturation.

**Figure 4.** Modeling the effect of fibrotic scar on the MR signal enables quantitation of the Fibrotic Score during infarct healing. A, The spectrum of fibrotic tissue (blue) can be modeled because the product of a healthy tissue spectrum (red) convolved with a Lorentzian function ( Scar Function) that accounts for the MT effects of macromolecular components of fibrotic scar. The parameters A (magnitude) and γ (linewidth) were optimized via RMS minimization of the difference between the convolution product (black dotted line) and the infarct zone data. B, The magnitude of the Lorentzian function decreased significantly immediately after myocardial infarction (MI) in infarct zone tissue, remaining lower than at 1 day after MI for the duration of infarct healing. In border zone tissue, A decreased by day 10 after MI, but did not remain decreased. C, Fibrotic Score increased most rapidly between 7 to 10 days after MI in infarct zone myocardium and remained elevated for the duration of infarct healing, reflecting the deposition of fibrotic scar tissue. In border zone myocardium, fibrotic score increased significantly at 10 days after MI, but decreased thereafter, probably reflecting the effects of infarct extension (*P<0.05 vs day 1, †P<0.05 vs day 7, ‡P<0.05 vs Border, and §P<0.05 vs day 10).
during scar formation and maturation (18±6% day 1 versus 10±4% day 21, P<0.05). In border zone myocardium, FS increased significantly by day 7 after MI, but then decreased at subsequent time points. We defined myocardial regions of interest at each time point based on enhancement patterns at LGE. Subsequently, infarct extension into previously border zone tissue at later time points led to spatial reclassification of the border zone at LGE to include a larger fraction of healthy tissue by day 21 (Figure 4). Important hemodynamic and imaging measurements are detailed in the Table I in the Data Supplement.

Molecular Imaging of Eu-HPDO3A Redistribution in Cardiac Circulation
The average MTR_{asym} before intravenous infusion of Eu-HPDO3A was −0.2±1.7%. Maps of MTR_{asym} acquired before and 7.5 minutes after intravenous infusion of Eu-HPDO3A (Figure 5) demonstrate significant increases in cardiac MTR_{asym} after myocardial circulatory redistribution of Eu-HPDO3A. Peak changes in MTR_{asym} over preinfusion levels (ΔMTR_{asym}) were significantly higher in mice receiving infusion of Eu-HPDO3A than those receiving saline (Figure 5). To assess whether cardioCEST was sensitive to increased blood volume during vasodilatation, dobutamine (40 mg/kg, intravenous bolus) was infused in a separate group of mice and, after 5 minutes, MTR_{asym} measurements were repeated with Eu-HPDO3A. Peak ΔMTR_{asym} values were significantly higher after vasodilatation with dobutamine compared with after infusion of either Eu-HPDO3A or saline (Figure 5). Despite differences in peak ΔMTR_{asym}, the time course of ΔMTR_{asym} was similar with and without dobutamine (Figure 5), and remained higher than saline infusions at all time points ≤22.5 minutes after infusion. Changes in physiological parameters are detailed in Table I in the Data Supplement.

At 28 days after surgically induced MI, remote zone tissue demonstrated similar peak ΔMTR_{asym} and MTR_{asym} redistribution kinetics to healthy, noninfarcted tissue after infusion of Eu-HPDO3A (Figure 6). In contrast, ΔMTR_{asym} at 7.5 minutes after injection was significantly reduced in infarct zone tissue as compared with remote tissue (Figure 6). In addition, MTR_{asym} kinetics reflected a gradual and delayed accumulation of Eu-HPDO3A in infarct zone myocardium, with minimal washout during the 30 minutes of observation (Figure 6). Additional physiological and imaging parameters are detailed in Table I in the Data Supplement.

Discussion
Myocardial fibrosis is increasingly associated with arrhythmias and adverse cardiac events, and early identification is emerging as a priority clinical target in high-risk patients including millions with reduced renal function. Although LGE is the standard of care for fibrosis imaging, the potential nephrotoxicity of gadolinium in patients with reduced renal function restricts diagnosis to highly invasive, painful, and often inaccurate endomyocardial biopsies, hindering the development of novel therapies. Detection of fibrotic tissue through CEST encoding is a direct detection process, thus obviating the need for chelated gadolinium salts. The results of our study reveal that cardioCEST can identify cardiac fibrotic tissue both rapidly and qualitatively, and can be used for semiquantitative mapping via spectral modeling. Changes in FS during the process of infarct healing paralleled the time course of scar formation and maturation as established by previous histological studies including those from our institution. In addition, high-density scar in myocardial regions of interest demonstrating heightened FS was confirmed histologically in all samples. Clinically, cardioCEST of myocardial fibrosis could enable diagnosis and risk stratification of large high-risk patient cohorts currently contraindicated to LGE. Furthermore, because sensitivity to fibrosis increases with spatial resolution, cardioCEST could potentially enable fibrosis imaging in tissues difficult to image with LGE including the right ventricle and both atria.
Previously, 2 studies have used MT-weighted cardiac magnetic resonance imaging (CMR) to differentiate infarcted from stunned myocardium,19 and to visualize changes in cardiac tissue composition20 using MT preparation with on-resonance saturation. In the first study, MT-weighted CMR of the dog heart early after surgically induced MI resulted in moderate signal enhancement in infarct zone myocardium.19 In the second study, changes in MTR in infarct zone myocardium were observed in patients immediately after acute MI.20 We did not observe consistent changes in infarct zone MTR during the acute phase of MI (day 1) when radiofrequency irradiation was applied on resonance. This probably results from different MT/CEST-weighting strategies between previous studies, which used high radiofrequency irradiation to maximize direct saturation of water to create positive contrast from MT, and our study which used lower radiofrequency-irradiation to limit indirect saturation of water so as to heighten detection of fibrosis at greater CEST-offset frequencies.

CEST–MRI has been used with a single saturation module and rapid spin-echo readout in stationary organs to detect degenerative disease processes21,22 and to track CEST-reporter gene expressing cancer cells.2 In 1 previous study, CEST-weighted imaging was performed using an ECG-gated rapid gradient echo technique in the presence of slow heart rates.23 However, rapid heart rates in small animals on the order of 400 to 700 bpm obstruct the application of either method for cardiac CEST imaging (Figure I in the Data Supplement). Steady-state cardioCEST imaging as reported here overcomes these limitations and is optimized to enable imaging of multiple targets, including the circulation, matrix targets, or potentially paraCEST labeled or CEST-reporter gene expressing cells.

In previous studies using Eu-HPDO3A, MTR asym of ≈60% was generated when Eu-HPDO3A was dissolved in solution, and ≈6% when isolated inside the cytosol of cells.1 The myocardial biodistribution of gadolinium-HPDO3A, which is a chemically similar Lanthanide (III) complex to Eu-HPDO3A, is well established from numerous clinical studies. In healthy tissue, Eu-HPDO3A probably rapidly equilibrates between the intravascular and extracellular fluid compartments similar to gadolinium chelates. Because Ln-HPDO3A complexes are unable to cross the intact membranes of healthy cardiomyocytes, our measures of peak ΔMTR asym probably reflect the combined CEST contrast generated by Eu-HPDO3A within the myocardial circulation and the extracellular space. In response to vasodilatation with dobutamine, increased peak MTR asym values probably reflect CEST contrast generated by the combined effects of both increased myocardial blood volume and more rapid redistribution of Eu-HPDO3A to the extracellular compartment because of faster heart rates. The dobutamine-induced increase in peak ΔMTR asym parallels increases in perfusion in response to stimulation with dobutamine in previous studies,24 as well as changes in myocardial circulatory blood volume in healthy tissue similar to widely established measures using gadolinium-HPDO3A complexes. Finally, accumulation of Eu-HPDO3A in infarcted myocardium resulted in steadily elevated ΔMTR asym at 20 minutes after infusion, reflecting a combination of reduced perfusion and increased extracellular distribution volume similar to LGE. However, in contrast to conventional LGE–CMR, which is performed after the completion of all anatomic imaging and further requires a 10-minute delay after injection of gadolinium contrast agents, paraCEST agents can be injected before MR examination without altering the underlying contrast on cine images, and then selectively excited to visualize infarcted myocardium without a delay time. As a result, the complexity of preclinical CMR can be simplified by both reducing scan times and eliminating the need for an indwelling catheter for delivery of gadolinium. One important consideration is that the CEST contrast generated by Eu-HPDO3A is pH-dependent and may, therefore, be altered in severely ischemic tissues.

In conventional CEST acquisitions, a spin-echo readout is used after saturation, leading to a T2-dependent decay in CEST contrast. In cardioCEST, the CEST contrast is lost through the contribution of inflowing blood in which the CEST effect is not encoded into the steady-state longitudinal magnetization, and from exchange with relaxing stationary protons. Reducing the number of gradient, echo acquisitions in each sequence repetition could enhance CEST contrast by reducing the aforementioned factors. However, in our early protocol development, we found that reducing the number of acquisitions after saturation from 100 to 70 had minimal

**Figure 6.** CardioCEST MRI After myocardial infarction (MI). A, Midventricular short-axis anatomic reference image reveals significant wall thinning (white arrows) in an area of scar tissue at 28 days after MI. B, Asymmetrical magnetization transfer ratio (MTR asym) map acquired before an injection of Eu-HPDO3A demonstrates uniformly low MTR asym throughout the heart. C–F, Representative maps of MTR asym acquired serially after injection of Eu-HPDO3A demonstrate rapid redistribution and gradual washout of Eu-HPDO3A within healthy noninfarcted myocardium, and slow accumulation within infarct zone myocardium. G, ΔMTR asym increased rapidly and decreased slowly in remote, noninfarcted myocardium, differing significantly from infarct zone myocardium at all time points except 12.5 minutes after injection. In infarct zone myocardium, ΔMTR asym increased slowly ≤17.5 minutes after injection, remaining consistently elevated thereafter (*P<0.05 vs Infarct, †P<0.05 vs 12.5 minutes).
effect on spectral shape, and resulted in a 30% increase in scan time (Figure V in the Data Supplement). Although further reduction in the number of acquisitions would probably yield greater gains in CEST sensitivity, the increased scan times would become prohibitive for the kind of dynamic imaging performed in this study. Importantly, sensitivity to CEST encoding is greatest immediately after conclusion of the saturation module, and acquisition of the center of $k$-space at this time point would act to heighten CEST sensitivity. Owing to limitations in the pulse sequence programming environment, our acquisition of $k$-space was constrained to a semirandom order, thereby somewhat reducing the CEST contrast in our images. In future studies, combination of a targeted centric $k$-space acquisition scheme combined with novel compressed sensing methods could improve both sensitivity and speed of cardioCEST.

An important limitation of this method is that measurements must be performed on image sets with a consistent number of averages which, because images are retrospectively reconstructed, can be affected by variations in heart rate or respiration. We took great care to maintain consistent body temperature and anesthesia during imaging, which resulted in regular pulse and respiratory rates. Furthermore, the number of averages in reconstructed images was not significantly different between acquisitions (Table I in the Data Supplement), suggesting that measurements of MTR and FS accurately reflected underlying changes.

The steady-state cine cardioCEST method developed in this study represents several exciting potential advances for translational molecular CMR. First, imaging of fibrosis with cardioCEST eliminates the need for gadolinium, potentially enabling examination in high-risk patient populations now excluded from critical CMR protocols. Second, cardioCEST in combination with cell labeling and CEST-reporter genes can enable preclinical imaging of multiple aspects of cell fate decisions in regenerative medicine. And finally, given the broad spectral range available for CEST-encoded imaging, and the specific resonance frequencies of CEST agents, multiplexed molecular and functional imaging can be performed in the same imaging session to examine the heart from the level of cell fate decisions up to global structural and functional changes.

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**Disclosures**

Drs Aime and Delli Castelli are inventors of the CEST agents used in this work. The other authors report no conflicts.

**References**


Molecular imaging with MRI offers a unique opportunity to combine multiscale examination of the diseased heart with traditional high-quality anatomic and functional MRI. Application of CEST-encoded cardiac magnetic resonance imaging in combination with CEST-reporter genes to preclinical models of cardiac regeneration could help to elucidate mechanisms promoting stem cell survival and differentiation. Ultimately, as barriers to gene therapy and engineered stem cells for cardiac regeneration are overcome, the ability to track the efficacy of gene therapy, as well as cell fate decisions in vivo with molecular MRI could prove invaluable. Moreover, clinical translation of cardioCEST fibrosis imaging could enable diagnosis and risk stratification in high-risk patients contraindicated to gadolinium contrast agents who are currently excluded from standard of care diagnostics. At present, patients with compromised renal function suffer high rates of cardiovascular mortality. Without adequate tools to quantify changes in tissue composition, a major barrier exists to the development of novel and potentially life-saving therapies that could mitigate adverse remodeling and prevent cardiovascular mortality. The translation of cardioCEST fibrosis imaging could enable both the earlier diagnosis and risk stratification in such patients, and could for the first time allow novel therapies to be tested and the outcome to be quantified.
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Supplemental Material

Supplemental Table. Physiological and measurement parameters for all studies.

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</tr>
<tr>
<td></td>
<td>Eu-HPDO3A</td>
<td>28</td>
<td>443 ± 12</td>
<td>61 ± 4</td>
<td>17.3 ± 0.1</td>
</tr>
</tbody>
</table>

Heart rate was measured in beats per minute, and respiratory rate in breaths per minute. All measures are presented as mean ± standard deviation.
Supplemental Figure 1. Diagram of cardioCEST acquisition and steady state CEST encoding, and comparison to ECG-triggered CEST imaging. (A) For cardioCEST imaging, following CEST preparation modules with a train of off-resonance saturation pulses (blue boxes), 100 excitation pulses were played out at a constant interval of 10ms maintaining the longitudinal magnetization at steady state (green boxes). Acquired data was retrospectively gated, in order to remove k-space data acquired during the respiratory motion and bin remaining data based on cardiac phase. (B) CardioCEST is unique that in any voxel in which the CEST effect takes place, the initial longitudinal magnetization of the voxel at the start the Imaging step (step D) is weighted by the CEST contrast generated from the encoding step (steps A-C). By continuously exciting the longitudinal magnetization at a constant TR, the CEST effect is
encoded into the steady state signal. (C) In an ECG-triggered and respiratory gated cardiac
CEST acquisition, the CEST weighting of the longitudinal magnetization at the start of image
acquisition would be highly variable and would not be encoded into the steady state. As a result,
the CEST effect would be rapidly lost because of (a) periods without RF excitation during
breathing leading to a loss of steady state magnetization as shown in the first interval, (b)
variable periods between the end of saturation and readout leading to variable CEST weighting
as shown in the second interval, and (c) heart rate variability resulting in inconsistent steady state
magnetization.
Supplemental Figure 2. (Left) Example spectra demonstrating the effect of saturation flip angle on indirect saturation of water signal. In conventional CEST experiments, saturation with high flip angles results in significant indirect saturation at frequency offsets far from water resonance. In our study, we sought to limit indirect saturation while still obtaining maximal direct saturation at water resonance. Experimental optimization resulted in the use of a train of 28, 270° saturation pulses followed by 100 gradient echo readouts. (Right) Water saturation shift referencing (WASSR) correction for field inhomogeneity was initially performed on early scans in our study. A representative WASSR spectra from the whole heart demonstrates a myocardial resonant frequency offset by 0.1 ppm from water resonance. The average offset over initial scans was 0.1 ± 0.1 ppm (n = 4), which falls well below the bandwidth of the saturation pulses used in this study (bandwidth = 0.5 ppm). For this reason, WASSR correction was not performed in the remainder of the study.
Supplemental Figure 3. Representative MTR spectra at 21 days after MI. (A)

Representative MTR spectra from 3 mice (M1-M3) are shown for remote tissue (solid lines) and corresponding infarct tissue (dotted lines). Differences between infarct and remote spectra are seen in all examples. (B) The average over these spectra reveals differences in MTR across all frequencies in the spectrum. (C) The difference in MTR between the healthy and fibrotic tissue regions (ΔMTR) is higher at the saturation frequency offset of -1 ppm with greater consistency compared to other offsets. Based on this, MTR was calculated only for offsets of -1 ppm for rapid visualization of scar tissue (Figure 2).
Supplemental Figure 4. γ increased significantly starting at 10 days after MI in infarct and border zone tissue, but remained elevated only in infarct zone tissue. While statistically significant differences were measured, the magnitude of such changes were subtle in comparison to changes in the magnitude variable A. (*P <0.05 vs. Day 1, †P<0.05 vs. Day 7, ‡P<0.05 vs. Border, §P<0.05 vs. Day 10).
Supplemental Figure 5. Effect of number of acquisitions on MT/CEST sensitivity. In the early stages of protocol development we examined whether reducing the number of acquisitions following each saturation period enhanced MT/CEST sensitivity of cardioCEST. These experiments were performed on excised hearts from healthy mice. We found that reducing the number of acquisitions from 100 (blue line) to 70 (red line) resulted in a modest improvement in sensitivity at a cost of increased scan time. While reducing the number of gradient echo acquisitions by a significant number would likely increase MT/CEST contrast significantly, the rather dramatic increase in scan time could prove prohibitive for some cardioCEST applications.