# Cardiac CEST imaging of diffuse fibrosis

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**Target Audience:** Individuals interested in gadolinium free imaging of diffuse fibrosis, pre-clinical models.

**Purpose:** To demonstrate imaging of diffuse cardiac fibrosis using cardioCEST MRI.

**Introduction:** The emergence of diffuse fibrosis significantly increases a patient's risk of heart failure, arrhythmia, and sudden cardiac death[1]. In recent years, measurement of the extracellular volume fraction using T1-mapping in conjunction with gadolinium has enabled quantification of diffuse fibrosis, however, concerns of nephrotoxicity spur the development of non-contrast techniques. Extracellular matrix proteins exchange saturated magnetization with surrounding bulk water through magnetization transfer (MT). In the presence of diffuse fibrosis, the extracellular water volume is increased relative to healthy tissue, leading to reduced MT. Recently we developed a novel cardiac chemical exchange saturation transfer (CEST) pulse sequence (cardioCEST) that encodes CEST contrast into the steady state longitudinal magnetization of the mouse heart[2]. We sought to test cardioCEST imaging of diffuse fibrosis in a mouse model of chronic Angiotensin II (AngII) stimulation.

**Methods:** ***Pulse Sequence:*** CEST encoding used a saturation pulse train of 88 non-selective Gaussian pulses (flip angle = 270°, bandwidth = 200Hz, duration = 8.8ms). One phase encoding step was performed following each saturation train. A constant repetition time (TR) cine gradient echo sequence (TR/TE = 10.2/3.5 ms, flip angle = 10°) was used to encode CEST contrast into the steady state longitudinal magnetization. Data acquisition was prospectively triggered using combined ECG and respiratory waveforms in order to acquire four averages for each phase encoding step and cardiac phase. Dummy pulses were used to maintain steady state magnetization between heart beats in cases of heart rate variability and during respiratory motion. Images were acquired in 1 mid-ventricular short-axis slice with parameters: FOV=2.56x2.56cm, Matrix = 128x128, slices = 1, and slice thickness = 1mm for all acquired images. ***Imaging:*** All imaging was performed on a 7T Bruker ClinScan (Bruker Biospin, Ettlingen, Germany) using a cylindrical volume coil for excitation and a 4-channel phased array surface coil for reception. Saturation frequency offsets of $\Delta\omega$ = ±20, 15, 10, 6, 3, 1, and 0 ppm were used to obtain z-spectra, and a separate reference scan (offset = 333 ppm, saturation flip angle = 1°) was acquired for signal normalization. ***Image Analysis:*** All images were normalized to the reference (ref) scan (Figure A) in order to account for the receiver coil profile and to normalize for steady state signal. Regional spectra were then measured in the ventricular septum and lateral free wall. For cumulative analysis of CEST contrast, measurements from positive and negative offset frequencies were averaged within each region of interest. The magnetization transfer ratio was measured as $MTR(\omega)=[(S_{ref}-S(\omega))/S_{ref}]*100$. For animals in treatment groups ($T_x$), the change in MT was calculated as $\Delta MT(\omega)=[1-MTR_{Tx}(\omega)/MTR_{Ctl}(\omega)]*100$, where control spectra (Ctl) were generated from animals not undergoing treatment. ***Animal model***: C57Bl/6 male mice (n=11) received either constant infusion of Angiotensin II (1000ng/kg/min, BACHEM, n = 3) or saline (n = 4) via mini osmotic pump (Alzet). A third group (n=4) served as controls. MRI was performed 10 days after pump implantation, with anesthesia maintained using 1.25% isoflurane in oxygen and body temperature maintained using circulating thermostated water. Animals were euthanized immediately after MRI for picrosirius red staining.

**Results:** Representative images acquired with saturation at multiple offset frequencies (Figure A) reveal minimal indirect saturation and significant direct saturation at water resonance. In mice receiving AngII a trend towards hypertrophy was observed (Figure B). Examination of spectra revealed reduced saturation across frequency offsets in the hearts of AngII mice (Figure C,D). When radiofrequency saturation was applied at 15ppm offset from water resonance, MTR was reduced in AngII mice (Septum = 4.51 ± 9.69%, Freewall = 9.64 ± 2.81%) compared to control (Septum = 17.5 ± 3.15%, Freewall = 19.4 ± 1.99%) and saline infused mice (Septum = 11.9 ± 2.89%, Freewall = 16.4 ± 1.93%). AngII mice demonstrated significantly increased ΔMT compared to saline treated mice when saturation was applied at 3ppm offset from water (22.4 ± 7.9% AngII vs. -10.1±5.9%, p = 0.013) and 6ppm offset from water (23.3 ± 18.3% AngII vs. -1.91 ± 8.9%, p = 0.016). Picrosirius red staining of isolated tissue sections confirmed diffuse interstitial fibrosis in AngII treated mice (Figure E).

**Discussion/Conclusion:** Diffuse myocardial fibrosis is characterized by increased extracellular water volume that results in reduced magnetization transfer between matrix macromolecules and bulk water[3,4]. In this study, we used cardioCEST imaging to observe reduced transfer of saturated magnetization from extracellular matrix macromolecules with bulk water in the presence of diffuse interstitial fibrosis. Further adaptation of this technique could enable clinical imaging of diffuse fibrosis without the need for gadolinium.

**References:** **(1)** Wu et al. JACC. 2008; 51(25):2414-21. **(2)** Vandsburger et al. Proc ISMRM. 2014. **(3)** Weber et al MRM. 2009; 62(3): 699-705. **(4)** Leung et al. Proc ISMRM 2014.

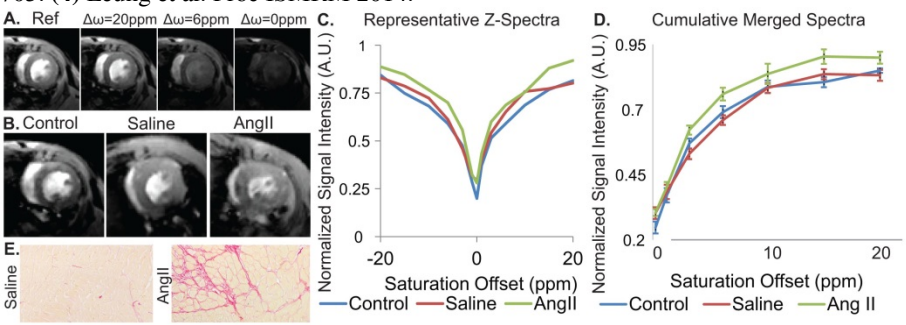


**Figure. A.** Representative cardioCEST images acquired without saturation (ref), and with RF saturation applied at multiple offset frequencies. **B.** End-diastolic images at the mid-ventricle from control mice, and mice receiving infusion of saline or Angiotensin II (AngII). **C.** Representative z-spectra from 3 mice reveal elevated steady state magnetization in AngII mice across the spectrum, reflecting reduced MT in diffusely fibrotic tissue. **D.** Averaged spectra over all mice in the free-wall confirm altered MT patterns in AngII mice. **(E)** Picrosirius red staining confirms diffuse interstitial fibrosis in mice receiving AngII infusions.