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Rapid Multi-Slice T_1 Mapping of Mouse Myocardium: Application to Quantification of Manganese Uptake in α -Dystrobrevin Knockout Mice

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Abstract

Purpose—The aim of this study was to develop a rapid, multi-slice cardiac T_1 mapping method in mice, and to apply the method to quantify manganese (Mn^{2+}) uptake in a mouse model with altered Ca^{2+} channel activity.

Methods—An ECG-triggered multi-slice saturation-recovery Look-Locker method was developed and validated both *in vitro* and *in vivo*. A two-dose study was performed to investigate the kinetics of T_1 shortening, Mn^{2+} relaxivity in myocardium, and the impact of Mn^{2+} on cardiac function. The sensitivity of Mn^{2+} -enhanced MRI in detecting subtle changes in altered Ca^{2+} channel activity was evaluated in a mouse model with α -dystrobrevin knockout.

Results—Validation studies showed strong agreement between the current method and an established method. High Mn^{2+} dose led to significantly accelerated T_1 shortening. Heart rate decreased during Mn^{2+} infusion, while ejection ratio increased slightly at the end of imaging protocol. No statistical difference in cardiac function was detected between the two dose groups. Mice with α -dystrobrevin-knockout showed enhanced Mn^{2+} uptake *in vivo*. *In vitro* patch-clamp study showed increased Ca^{2+} channel activity.

Conclusion—The saturation-recovery method provides rapid T_1 mapping in mouse hearts, which allowed sensitive detection of subtle changes in Mn^{2+} uptake in α -dystrobrevin-knockout mice.

Keywords

manganese-enhanced MRI; Ca^{2+} channel activity; α -dystrobrevin knockout mouse; nNOS; T_1 mapping; Mn^{2+} relaxivity

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INTRODUCTION

Calcium (Ca^{2+}) is the central regulator in cardiac excitation-contraction coupling (1). Ca^{2+} influx via the voltage-gated L-type Ca^{2+} channels, which triggers Ca^{2+} -induced Ca^{2+} release from sarcoplasmic reticulum, is of key importance in cardiac Ca^{2+} cycling. Despite the importance of Ca^{2+} uptake, limited imaging techniques exist for noninvasive measurement of Ca^{2+} channel activity. The emergence of manganese (Mn^{2+})-enhanced magnetic resonance imaging (MEMRI) provides the possibility for *in vivo* assessment of Ca^{2+} uptake (2). Because of its T_1 shortening effect and its long retention in cells, Mn^{2+} has become a useful molecular contrast agent in cardiac MRI (3–5). Unlike gadolinium-based contrast agents which are trapped in the extracellular space (6–8), Mn^{2+} enters the cell via the Ca^{2+} channels (9–12). Hence, the accumulation of Mn^{2+} in myocardium reflects Ca^{2+} channel activity and thus Ca^{2+} influx. As such, MEMRI offers a novel means for measuring Ca^{2+} channel activity in hearts.

T_1 -weighting and T_1 mapping are the two widely used methods to characterize Mn^{2+} uptake in MEMRI (10,13–16). The T_1 -weighting method offers high temporal resolution. However, it does not provide direct quantification of Mn^{2+} concentration in the cells. The T_1 mapping method, in contrast, offers quantitative measurement of the Mn^{2+} accumulation because of the linear relationship between the relaxation rate ($R_1=1/T_1$) and Mn^{2+} concentration within a certain range (4,13,14,17). To quantify the Ca^{2+} channel activity, T_1 mapping with high temporal resolution is required to measure the kinetics of Mn^{2+} uptake (14,15). Previously, a Saturation-Recovery Look-Locker (SRLL) method was developed and validated in our lab for fast cardiac T_1 mapping in mice (18). This method allowed acquisition of single slice T_1 maps of mouse myocardium within 3 min. Despite a significant improvement in temporal resolution, T_1 mapping of a single slice by SRLL provides limited coverage of the whole heart.

Quantification of the concentration of Mn^{2+} within tissue from measured T_1 relaxation times requires knowledge of the relaxivity (r_1) of Mn^{2+} within myocardium, where r_1 is the rate of R_1 increase when Mn^{2+} concentration increases. To date, only a few studies have attempted to quantify r_1 in myocardium *in vivo* (4,13,19). Further, the optimal dose for MEMRI studies is yet to be determined. While high Mn^{2+} dose provides greater sensitivity to detect changes in Ca^{2+} channel activity, a high tissue Mn^{2+} concentration may saturate the relaxation effect induced by Mn^{2+} , leading to a nonlinear increase in R_1 and inaccurate quantification of Mn^{2+} concentration (10,13,19). In addition, high myocardial Mn^{2+} accumulation may negatively impact ventricular function (2,15,20,21). Thus, the optimal Mn^{2+} dose must balance sensitivity and accuracy in delineating Ca^{2+} channel activity while maintaining normal ventricular function.

In the current study, the previous SRLL method was modified to achieve multi-slice T_1 mapping (MSRLL) in mouse heart with no additional time cost. *In vitro* and *in vivo* studies were performed to validate the MSRLL method with the previously established SRLL method. An *in vivo* study with two doses of MnCl_2 solutions was performed to investigate the relationship between R_1 and Mn^{2+} concentrations in myocardium, as well as the impact of Mn^{2+} on cardiac function. The utility of MSRLL was further evaluated in a mouse model

with α -dystrobrevin knockout. Our results show that the MSRL method can provide rapid multi-slice T_1 mapping in mouse heart that allows sensitive detection of subtle changes in altered Ca^{2+} channel activity.

METHODS

Imaging Method

A schematic diagram of the MSRL pulse sequence is shown in Fig. 1. An ECG-triggered saturation module was applied at the beginning of each phase-encoding step. It consisted of three nonselective 90° radio-frequency pulses to ensure complete saturation of the magnetization. The saturation module was followed by the acquisition of k-space lines of $n \times m$ images using fast low-angle shot (FLASH) method, where n is the number of images acquired along the magnetization recovery curve, and m is the total number of slices. The acquisition of the FLASH images was triggered by the detection of the QRS complex, with 80–100 ms delay after the trigger to ensure that all the images were acquired during mid to late diastole. Multi-slice images were acquired during the diastolic period using the interleaved scheme to minimize the crosstalk between adjacent slices.

Phantom Study

All MRI studies were performed on a horizontal 7T Bruker scanner (Bruker Biospec, Germany) using a 35-mm inner diameter volume coil. The MSRL method was first validated *in vitro* using a multi-compartment phantom with manganese chloride ($MnCl_2$) solutions sealed in 1 mL centrifuge tubes. The $MnCl_2$ concentrations ranged from 30 to 1000 μM . MSRL was applied to acquire T_1 maps of 5 slices with 0.1 mm inter-slice gap. 1-ms Hermite pulses were used for slice excitation. The imaging parameters were: flip angle, 10° ; TE, 1.9 ms; slice thickness, 1 mm; number of averages, 1; field of view, 3.0×3.0 cm²; matrix size 128×64 . For each slice, a total of 10 images that covered 2.4 s of the saturation recovery curve were acquired with a 240 ms interval. For each image, a single k-space line for each of the 5 slices was acquired within 23.6 ms. Single slice SRL images were also acquired to validate the MSRL results. Proton density (M_0) images were acquired with a TR of 3 s and the same flip angle as the MSRL acquisition.

Animals and *In Vivo* Studies

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Case Western Reserve University. 3 month, male FVB mice ($n=19$) were used for *in vivo* validation and the two-dose MEMRI studies. Specifically, mice in prone position were anesthetized with 2% isoflurane and maintained with 0.8–1.8% isoflurane. A 26G $\times$ 19 mm plastic IV catheter (Hospira, Inc., Lake Forest, IL) was inserted into the tail vein and connected to an infusion pump (Braintree Scientific, Inc., Braintree, MA) for Mn^{2+} infusion. ECG, respiration, and body temperature were monitored and recorded by a physiological monitoring system (SA Instruments, Stony Brook, NY). Hot air was blown to the mice to maintain the body temperature at around $35^\circ C$.

To investigate the effect of different sampling intervals on T_1 fitting, two ECG triggering schemes were applied during the acquisition of FLASH images: triggering at every two

heartbeats and triggering at every heartbeat, equivalent to a sampling interval of 2 R-R intervals and 1 R-R interval along the recovery curve, respectively. T_1 maps of three adjacent short-axis slices at mid-ventricular levels were acquired using MSRL. To capture the magnetization recovery at an early phase, the first set of multi-slice images were acquired 40~80 ms after the implementation of the saturation module. All images were acquired during mid- to late-diastole to minimize motion artifact. Imaging parameters were: flip angle, 10° ; TE, 1.9 ms; slice thickness, 1 mm; number of averages, 1; field of view, $3.0 \times 3.0 \text{ cm}^2$; matrix size, 128×64 . During each phase-encoding step, a single k-space line for each of the three slices was acquired within 18.7 ms. For each slice, a total of 10 (triggering at every two heartbeats) or 20 images (triggering at every heartbeat) were acquired, covering approximately ~2.4 s of the saturation recovery curve. SRL images were also acquired using the corresponding triggering scheme to validate the MSRL results. Proton density images (M_0) were acquired with a TR of 10 R-R intervals (1100~1300 ms).

In vivo MEMRI experiments were conducted to demonstrate the utility of MSRL in delineating dynamic changes in T_1 relaxation times. After the acquisition of baseline T_1 maps, MnCl_2 solution was infused through the tail vein catheter at a rate of 0.2 mL/hr for 30 minutes, followed by a 15 minutes washout period. Two different Mn^{2+} concentrations were used: 126 mM (n=9, referred to as the high dose group) and 63 mM (n=10, referred to as the low dose group). T_1 maps for the high dose group were acquired by triggering at every two heartbeats, while those for the low dose group were acquired by triggering at every heartbeat. T_1 maps were acquired continuously during Mn^{2+} infusion and washout. Heart rate was continuously recorded during the whole imaging protocol. Cine FLASH images of the mid-ventricular slices were acquired at baseline and post-contrast to evaluate the impact of Mn^{2+} on ventricular function. Validation study by SRL was performed either before Mn^{2+} infusion (n=5 for each dose group) or after the 15-min washout period (n=5 for the high dose group and n=4 for the low dose group).

To further evaluate the sensitivity of MSRL in detecting subtle changes in Ca^{2+} channel activity, the kinetics of Mn^{2+} -induced R_1 increase was measured in 4 to 5 month old, male α -dystrobrevin knockout mice ($\text{adbn}^{-/-}$, n=5) and their age-matched controls (C57BL/6, n=5). The $\text{adbn}^{-/-}$ mice have been described previously (22). Mn^{2+} infusion used the high dose and image acquisition was triggered at every heartbeat.

Image Analysis

Images were analyzed off-line using MATLAB-based software developed in-house (18). Images were zero-filled to 128×128 matrix during reconstruction. For each slice, T_1 maps of the whole left-ventricle were generated by performing pixel-wise curve fitting. The details on T_1 fitting have been described previously (18). Briefly, the modified relaxation time (T_1^*) and steady-state magnetization (M^*) maps were obtained from pixel-wise, least-square fitting of a three-parameter mono-exponential function to the acquired Look-Locker images. Subsequently, T_1 map was calculated from the T_1^* , M^* , and M_0 maps using the following equation:

$$T_1 = \frac{M_0}{M^*} T_1^*$$

For the analysis of CINE images, epicardial and endocardial contours were traced manually. The ejection ratio was calculated as the percentage change in the area that encompassed the left-ventricle from end-diastole to peak systole.

Quantification of Mn²⁺ Concentration and Relaxivity in Myocardium

Hearts from both dose groups were excised and freeze-clamped after imaging studies. The frozen tissues were burned in a furnace (Thermodyne, Salt Lake City, UT) at 600°C for 3 hours. The ashes were dissolved in 20% nitric acid and the Mn²⁺ content was analyzed by a flame atomic absorption spectrophotometer (Buck Scientific, East Norwalk, CT) as previously described (15). Regression analysis was performed to quantify the Mn²⁺ relaxivity in myocardium. Mn²⁺ content at baseline was considered negligible.

Myocyte Isolation

Ventricular myocytes were isolated using the method described by Ren et al with minor modifications (23). Briefly, mice were injected with heparin (1000 units/kg, i.p.) and sacrificed by cervical dislocation. Hearts were quickly excised, cannulated, and perfused with Ca²⁺-free Tyrode solution. The Tyrode solution contained (in mM) 136 NaCl, 5.4 KCl, 1.0 MgSO₄, 1.2 NaH₂PO₄, 10 HEPES, 5.6 glucose, 2 L-glutamine, 5 taurine, and 0.03 L-ascorbic acid, 1X vitamins and amino acids (Gibco, Invitrogen Corporation). The pH of the solution was adjusted to 7.4 with NaOH. Afterwards, hearts were perfused with Tyrode solution containing 0.8 mg/mL collagenase type II (Worthington Biochemical Co., Lakewood, NJ, USA) for 8~10 min. The ventricles were removed from the perfusion column, minced, gently agitated and rinsed. Isolated myocytes were collected and stored in Media 199 (GIBCO, Grand Island, NY, USA) containing 1.8 mM Ca²⁺. All cardiomyocytes were used within 3 hours after isolation. Only rod-shaped myocytes with clear striations were selected.

L-type Calcium Current (I_{Ca}) Measurement

Calcium currents were recorded in isolated ventricular myocytes using conventional whole cell patch clamp techniques. Data acquisition was performed using pClamp 9 software and an Axopatch 200A patch clamp amplifier (Molecular Devices). The pipette solution contained (in mM) 125 CsCl, 20 tetraethylammonium chloride, 5 MgATP, 3.6 creatine phosphate, 10 EGTA, 10 HEPES, pH 7.2. The bath solution contained (in mM) 157 tetraethylammonium chloride, 1 CaCl₂, 0.5 MgCl₂, 10 HEPES, pH 7.4. Patch pipettes had a resistance of 2–4 MΩ. Series resistance compensation (50–80%) was used to minimize voltage control errors. Ca²⁺ current recordings began 3 min after patch rupture. Ca²⁺ currents were normalized to cell capacitance (pA/pF) to account for variations in cell size. Electrophysiologic experiments were performed under continuous flow conditions at 36.5±0.5°C.

Statistical Analysis

All results were expressed as mean $\pm$ standard deviation (SD). Comparison of T_1 values by MSRL and SLL was performed by paired student's t-test and Bland-Altman analysis. Paired student's t-test was also used to compare the heart rate and ejection ratio during Mn^{2+} infusion and washout with that at the baseline. Unpaired Student's t-test was used for comparison between two groups. Two-way repeated measures ANOVA was performed to compare the time courses of R_1 increase and the time courses of heart rate changes during the imaging protocol. P values less than 0.05 were considered statistically significant.

RESULTS

Phantom Study

Total acquisition time was 2 min and 40 s for phantom studies. Shown in Fig. 2a are the T_1 maps of all five slices. There were no statistical differences in measured T_1 values among all five slices. Regression analysis showed a linear relationship between Mn^{2+} concentration and the mean R_1 for each of the five slices (Fig. 2b). There was a strong agreement between T_1 measurements by MSRL and SLL methods (Fig. 2b). The *in vitro* relaxivity of Mn^{2+} measured by MSRL and SLL was also similar (5.01 versus 5.04 $mM^{-1}s^{-1}$).

Pixel-by-pixel Bland-Altman analysis of the difference between MSRL and SLL measurements is shown in Fig. 2c. With short T_1 (<1 s), there was a strong agreement between MSRL and SLL measurements. The trend of increased difference with longer T_1 was due to insufficient coverage of the saturation recovery curves as was demonstrated by previous simulation studies (18).

In Vivo Validation Study

Average acquisition time for *in vivo* studies was 140~166 s (R-R interval: 110~130 ms). Representative T_1 maps from the low dose groups by MSRL and SLL are shown in Fig. 3a. Quantitative comparison showed no difference in T_1 measurements between SLL and MSRL methods both at baseline and after Mn^{2+} washout (Fig. 3b). Pixel-by-pixel Bland-Altman analysis also showed strong agreement in T_1 values measured by MSRL and SLL for both triggering schemes (Fig. 4), especially in post-contrast T_1 mapping. Compared with triggering at every two heartbeats (Fig. 4a), triggering at every heartbeat (Fig. 4b) showed more consistent agreement between MSRL and SLL measurements. In addition, triggering at every heartbeat yielded better separation between the pre- and post-contrast T_1 values. There were also fewer data points with the difference between the two measurements larger than 2 standard deviations.

Representative T_1 maps acquired at baseline and after Mn^{2+} infusion are shown in Fig. 5a&b. The time courses of the R_1 changes for all three slices are presented in Fig. 5c. For each dose group, there were no statistical differences in measured T_1 among all three slices. ANOVA analysis showed significantly larger increase in R_1 for all three slices in the high dose group ($P<0.05$). In the two slices that are distal to the apex, the high dose group also showed significantly larger increase of R_1 from 18 to 27 min during Mn^{2+} infusion ($P<0.05$). The apical slice showed a larger standard deviation in R_1 . As a result, statistical

significance was only detected at 18 min of Mn^{2+} infusion. R_1 values during the washout period were similar between the two dose groups.

The impact of Mn^{2+} accumulation on heart rate and ejection ratio is shown in Fig. 6. Comparing to the baseline, a 6.1% and 4.2% decrease in heart rate was observed in the high and low dose groups, respectively (Fig. 6a). ANOVA analysis detected no difference in the time courses of heart rate between the two dose groups. Fig. 6b shows the ejection ratio of the mid-ventricular slice at baseline and post-contrast. Comparing to the baseline, there was a slight increase in ejection ratio in both dose groups ($P < 0.05$).

Mn^{2+} Concentration and Relaxivity in Myocardium

Mn^{2+} accumulation in myocardium was significantly higher in the high dose group ($P < 0.05$, Fig. 7a). A progressive increase in R_1 was observed when the Mn^{2+} concentration was low. However, this increase reached a plateau at higher Mn^{2+} concentration (Fig. 7b). Regression analysis using data only from the low dose group yielded an r_1 of $6.02 \text{ mM}^{-1}\text{s}^{-1}$ with an R^2 of 0.87. When using data from both dose groups, the fitted r_1 was only $2.97 \text{ mM}^{-1}\text{s}^{-1}$ with a reduced R^2 of 0.53.

***In Vivo* MEMRI on $\text{adb}n^{-/-}$ Mice**

Shown in Fig. 8a are the time courses of the R_1 changes for all three slices in $\text{adb}n^{-/-}$ mice and their controls. There was a significantly larger increase in R_1 in $\text{adb}n^{-/-}$ mice for all three slices by ANOVA analysis ($P < 0.05$). Unpaired Student's t-test also showed significantly larger increase in R_1 from 3 to 18 min during Mn^{2+} infusion in $\text{adb}n^{-/-}$ mice. Calculated Mn^{2+} concentration maps at 18 min during Mn^{2+} infusion for control and $\text{adb}n^{-/-}$ mice are shown in Fig. 8b. Mean Mn^{2+} uptake calculated from R_1 changes is in agreement with that reported in literature (13). Unpaired Student's t-test revealed a significantly higher Mn^{2+} uptake in $\text{adb}n^{-/-}$ mice (Fig. 8c). No difference in R_1 was observed during late infusion (18~30 min) and washout (30~45 min).

Alteration in Ca^{2+} Currents in $\text{adb}n^{-/-}$ Mice

No difference was observed in cell capacitance between $\text{adb}n^{-/-}$ and control myocytes (144 ± 9 vs. 163 ± 17 pF). Fig. 8d shows the capacitance normalized current-voltage relationship for I_{Ca} . While peak I_{Ca} density (at 0 mV) was not significantly different between the control and $\text{adb}n^{-/-}$ mice, I_{Ca} density was greater in $\text{adb}n^{-/-}$ myocytes at -10 and -20 mV ($P < 0.05$). Boltzman analysis revealed a 4.5 mV hyperpolarizing shift in the potential for half-maximal activation ($V_{1/2}$) of I_{Ca} , from -19.8 mV in control myocytes to -24.3 mV in the $\text{adb}n^{-/-}$ mice (Fig. 8e).

DISCUSSION

In the current study, we present a rapid, multi-slice T_1 mapping method that allows the measurement of T_1 in mouse myocardium at ~3 min temporal resolution. *In vitro* and *in vivo* validation studies demonstrated that the accuracy of our current method is comparable to that of the single-slice method developed and validated previously. The utility of this method in monitoring the dynamics of Mn^{2+} -induced T_1 changes during Mn^{2+} infusion and

subsequent washout was demonstrated in a two-dose *in vivo* MEMRI study. The sensitivity of rapid T_1 mapping in detecting subtle changes in Ca^{2+} channel activity was also evaluated in an MEMRI study comparing the $\text{adbn}^{-/-}$ mice and their controls. In addition, Mn^{2+} relaxivity in myocardium, which allows the absolute quantification of tissue Mn^{2+} content from measured T_1 , was estimated. Finally, Mn^{2+} -induced changes in cardiac function were also evaluated.

The accuracy of T_1 estimation is dependent on the sampling of the T_1 recovery curve. In cardiac T_1 mapping, the sampling interval is determined by the ECG triggering scheme. To avoid mis-triggering caused by the switching gradients, a triggering scheme at every two heartbeats can be used, leading to a sampling interval of 2 R-R intervals, which may give rise to insufficient coverage of the ascending portion of the recovery curve, especially at shorter T_1 values. Alternatively, triggering at every heartbeat doubles the number of data points and improves the accuracy of T_1 fitting. To minimize mis-triggering, the acquisition of the FLASH images needs to be implemented at an earlier phase during diastole (mid- to late-diastole) such that the QRS complex of the ECG signal is less affected by the switching gradients. However, mis-triggering may still occur occasionally, especially when heart rate fluctuates. Further, since a mouse heart has no real period of diastasis, the slices acquired at mid to late diastole were at slightly different phases of diastole. The intra-slice T_1 variations observed in the current study may be partially attributed to measurement errors caused by mis-triggering or motion artifact, which may diminish the sensitivity of detecting true T_1 heterogeneity in a single T_1 map. However, as the current method is aimed at following the dynamics of Mn^{2+} uptake with multiple acquisitions of T_1 maps during Mn^{2+} infusion, such pixel-wise variations are likely to appear random over the time course of Mn^{2+} infusion.

T_1 estimation in the current study used a fixed data sampling interval that was calculated from the average heart rate during acquisition. Hence, variations in heart rate will lead to mis-assignment of the data points that can impact the accuracy of T_1 estimation. For example, a 10% change in heart rate will lead to a 10% error in T_1 estimation. In our current study, changes in heart rate (typically <10% and mostly around 5%) developed progressively during the ~3 min data acquisition. Since the acquisition of the center k-space lines, which reflects the majority of changes in signal intensity, occurred in even less time, the errors introduced by the mis-assignment of the data points should be much smaller than 10%.

While the imaging sequence was designed to perform multi-slice T_1 mapping that can cover the whole heart, T_1 mapping at the apical and basal slices will be less robust because myocardium at these two levels has more pronounced through-plane motion. In the current study, we imaged three adjacent 1 mm thick slices to demonstrate the utility of this method, which might be sufficient for most cardiac applications using the AHA 17 segment model. Further, previous studies have indicated the need for respiratory motion correction (24,25). However, simultaneous ECG and respiratory triggering will lead to non-uniform sampling intervals and prolonged data acquisition time. Since the focus of the current study is to develop a fast T_1 mapping method that can capture the kinetics of Mn^{2+} uptake in murine myocardium, no respiratory triggering was used to account for respiratory motion. While this inevitably introduces errors, a previous study by Streif et al (26) showed little influence

of respiratory motion on T_1 mapping when the ratio of the breathing rate and the heart rate was low (about 1:8 in their study). This ratio was about 1:10 in our current study. Hence, the relatively low respiration rate (~50 cycles/min) in our study likely limited respiratory artifact beyond that which would be found when respiration occurs at a more physiological rate of 100–120 cycles/min. The extent to which the Mn^{2+} uptake is affected by this low respiratory rate needs further investigation.

The current method provides fast T_1 mapping via the use of saturation pulses, which eliminates the requirement of long waiting time to reestablish equilibrium magnetization. The time interval between the saturation modules (TR) was 20 R-R intervals, which covered the initial ascending portion of the recovery curve to up to ~2.4 s. In a previous study, we have evaluated the impact of the incomplete coverage of the saturation recovery curve on fitting accuracy by computer simulation (18). Our results suggest that a TR of 2.4 s is sufficient to yield accurate estimations for a wide range of T_1 values (0.2 to 1.7 s) that encompass possible myocardium T_1 changes from baseline to the end of contrast infusion in MEMRI experiments. While using longer TR can further improve the accuracy of T_1 estimation, the temporal resolution will be decreased.

To further improve the temporal resolution, only 64 lines at the center of k-space were acquired with one data average, leading to low spatial resolution and partial volume effects that will limit its ability to detect small areas with abnormal Ca^{2+} uptake. On the other hand, improving the spatial resolution to 128 phase-encoding lines will quadruple the acquisition time to achieve comparable SNR, leading to decreased temporal resolution that will not be sufficient to capture the dynamics of Mn^{2+} -induced R_1 increase. Alternatively, the combination of the current method with other fast imaging methods, such as compressed sensing, can be used to provide both high temporal and spatial resolution.

Both dose groups showed progressive increase in R_1 during Mn^{2+} infusion. The increase in R_1 was relatively slow at the early stage of Mn^{2+} infusion, potentially because of the binding of Mn^{2+} to plasma proteins (2,19,27–29). With the increase of free Mn^{2+} concentration in plasma, a fast increase in R_1 occurred at the later stage of Mn^{2+} infusion. During Mn^{2+} washout, only a slight decrease in R_1 was observed, suggesting prolonged Mn^{2+} retention in cardiac myocytes (10,11). The two dose groups showed no difference in R_1 during the washout period. However, myocardial Mn^{2+} content was significantly higher in the high dose group (Fig. 7a). Previous studies have also reported an increase in Mn^{2+} accumulation in myocardium but similar R_1 increase at high Mn^{2+} infusion dose (13,19). These results suggest that T_1 shortening may saturate at high Mn^{2+} concentrations in myocardium.

The saturation of the T_1 shortening effect led to a plateau in R_1 increase at high Mn^{2+} concentration (Fig. 7b). Previously, Waghorn *et al.* reported the same saturation effect in mouse myocardium with a Mn^{2+} concentration above ~100 $\mu\text{g/g}$ dry weight, or 0.47 mM using a wet-to-dry ratio of 4.05 (13,19), which is consistent with our current data. As a result, regression analysis using data from both dose groups resulted in an underestimation of Mn^{2+} relaxivity in myocardium by more than 50%. Using data only from the low dose group, the estimated Mn^{2+} relaxivity in myocardium is $6.02 \text{ mM}^{-1}\text{s}^{-1}$, which is in agreement with that reported in literature (4,13).

The current study also demonstrated the sensitivity of MEMRI to alterations in Ca^{2+} channel activity in mice deficient in α -dystrobrevin, i.e., the $\text{abdn}^{-/-}$ mouse. The gene product, α -dystrobrevin, is a cytoplasmic protein of the dystrophin-glycoprotein complex. Grady et al. previously demonstrated the absence of sarcolemmal neuronal nitric oxide synthase (nNOS) associated with α -dystrobrevin disruption in $\text{abdn}^{-/-}$ mice (22). Hence, the observed changes in the current study may be attributed to alterations in nitric oxide signaling on Ca^{2+} cycling. The effects of nNOS on calcium cycling have been investigated in nNOS-deficient ($\text{nNOS}^{-/-}$) mice previously (30–33). These *in vitro* studies demonstrated either elevated (31–33) or normal (30) basal Ca^{2+} current in $\text{nNOS}^{-/-}$ myocytes. More recently, Vandsburger et al. also observed increased Mn^{2+} uptake in $\text{nNOS}^{-/-}$ mice *in vivo* (16). Similar to the findings by Vandsburger et al., the $\text{abdn}^{-/-}$ mice also showed a greater and faster R_1 increase compared to the wildtype mice (Fig. 8a), suggesting an increase in Ca^{2+} influx via the Ca^{2+} channels in myocardium, which was confirmed by the patch-clamp study *in vitro* (Fig. 8d&e). These data support an inhibitory role of sarcolemmal nNOS on Ca^{2+} channels.

The study on the $\text{abdn}^{-/-}$ mice also suggests that the current method is sensitive to 16% increase in Ca^{2+} channel activity observed by the patch-clamp method. Numerous studies have observed a wide range of variations in Ca^{2+} channel activity in diseased hearts. Taking hypertensive hearts as an example, existing literature on Ca^{2+} channel activity has reported no change (34–36), up to more than 2-fold increase (37), or ~46% decrease (38). Whether the current method has the sensitivity to detect altered Ca^{2+} channel activity at different stages of the disease requires careful evaluation.

Using the current method, we did not observe a difference in Mn^{2+} uptake at apex, mid-ventricle and base in $\text{abdn}^{-/-}$ mice, suggesting that changes in Ca^{2+} current are similar at different short-axis levels. The utility of the multi-slice T_1 mapping method would be better demonstrated in a mouse model with differences in Ca^{2+} channel activity at basal and apical levels. This pattern of Ca^{2+} channel alteration has been observed in a few disease models. In a rabbit model of drug-induced long QT type 2, the males showed a 22% increase in basal peak Ca^{2+} channel current as compared to the apex (39). In Takotsubo cardiomyopathy in humans, a transient wall motion abnormality of left ventricular apex that may lead to heterogeneous changes in Ca^{2+} channel activity (40). However, mouse models for these diseases have not been established.

Decreased heart rate was observed during Mn^{2+} infusion and subsequent washout (Fig. 6a). In contrast, a slight increase in ejection ratio was observed at the end of the imaging protocol (Fig. 6b). Previous studies on isolated perfused hearts (41) and papillary muscles (42) reported a negative inotropic effect of Mn^{2+} during the wash-in phase, followed by a positive inotropic effect during washout. While the decrease in heart rate during Mn^{2+} infusion and the slight recovery during the washout phase, as well as the increased ejection ratio at the end of Mn^{2+} washout observed in the current study, are consistent with these previous findings, one cannot rule out the possibility that prolonged anesthesia and increased blood volume due to Mn^{2+} infusion may also impact the hemodynamics of the heart. As such, further investigation is necessary to elucidate the impact of Mn^{2+} on cardiac inotropy and chronotropy.

In summary, an ECG-triggered, multi-slice saturation-recovery Look-Locker method was developed for fast cardiac T_1 mapping in mice. Validation studies showed strong agreement between the current method and the previously validated single-slice saturation-recovery Look-Locker method. The utility of MEMRI by MSRL in detecting subtle changes in cellular Ca^{2+} current was evaluated in a study involving $\text{adbn}^{-/-}$ mice. Our results suggest that fast T_1 mapping by MSRL enabled the delineation of the kinetics of Mn^{2+} uptake in mouse myocardium, which allowed sensitive detection of a small increase in Ca^{2+} channel activity in $\text{adbn}^{-/-}$ mice.

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References

1. Bers DM. Cardiac excitation-contraction coupling. *Nature*. 2002; 415:198–205. [PubMed: 11805843]
2. Wendland MF. Applications of manganese-enhanced magnetic resonance imaging (MEMRI) to imaging of the heart. *NMR Biomed*. 2004; 17:581–94. [PubMed: 15761947]
3. Cory DA, Schwartzenuber DJ, Mock BH. Ingested manganese chloride as a contrast agent for magnetic resonance imaging. *Magn Reson Imaging*. 1987; 5:65–70. [PubMed: 3586874]
4. Nordhøy W, Anthonen HW, Bruvold M, Jynge P, Krane J, Brurak H. Manganese ions as intracellular contrast agents: proton relaxation and calcium interactions in rat myocardium. *NMR Biomed*. 2003; 16:82–95. [PubMed: 12730949]
5. Nordhøy W, Anthonen HW, Bruvold M, Brurak H, Skarra S, Krane J, Jynge P. Intracellular manganese ions provide strong T_1 relaxation in rat myocardium. *Magn Reson Med*. 2004; 52:506–14. [PubMed: 15334568]
6. Kim RJ, Chen EL, Lima JA, Judd RM. Myocardial Gd-DTPA kinetics determine MRI contrast enhancement and reflect the extent and severity of myocardial injury after acute reperfused infarction. *Circulation*. 1996; 94:3318–3326. [PubMed: 8989146]
7. Saeed M, Wendland MF, Watzinger N, Akbari H, Higgins CB. MR contrast media for myocardial viability, microvascular integrity and perfusion. *Eur J Radiol*. 2000; 34:179–195. [PubMed: 10927160]
8. Klein C, Schmal TR, Nekolla SG, Schnackenburg B, Fleck E, Nagel E. Mechanism of late gadolinium enhancement in patients with acute myocardial infarction. *J Cardiovasc Magn Reson*. 2007; 9:653–658. [PubMed: 17578720]
9. Anderson M. Mn ions pass through calcium channels. A possible explanation. *J Gen Physiol*. 1983; 81:805–827. [PubMed: 6308126]
10. Hu TC, Pautler RG, MacGowan GA, Koretsky AP. Manganese-enhanced MRI of mouse heart during changes in inotropy. *Magn Reson Med*. 2001; 46:884–90. [PubMed: 11675639]
11. Skjold A, Kristoffersen A, Vangberg TR, Haraldseth O, Jynge P, Larsson HBW. An apparent unidirectional influx constant for manganese as a measure of myocardial calcium channel activity. *J Magn Reson Imaging*. 2006; 24:1047–55. [PubMed: 17024667]
12. Hunter DR, Haworth RA, Berkoff HA. Cellular manganese uptake by the isolated perfused rat heart: a probe for the sarcolemma calcium channel. *J Mol Cell Cardiol*. 1981; 13:823–32. [PubMed: 6271977]
13. Waghorn B, Edwards T, Yang Y, Chuang K-H, Yanasak N, Hu TC-C. Monitoring dynamic alterations in calcium homeostasis by T_1 -weighted and T_1 -mapping cardiac manganese-enhanced MRI in a murine myocardial infarction model. *NMR Biomed*. 2008; 21:1102–11. [PubMed: 18780285]

14. Chuang K-H, Koretsky A. Improved neuronal tract tracing using manganese enhanced magnetic resonance imaging with fast T(1) mapping. *Magn Reson Med*. 2006; 55:604–11. [PubMed: 16470592]
15. Chen Y, Payne K, Perara VS, Huang S, Baba A, Matsuda T, Yu X. Inhibition of the sodium-calcium exchanger via SEA0400 altered manganese-induced T1 changes in isolated perfused rat hearts. *NMR Biomed*. 2012; 25:1280–5. [PubMed: 22434695]
16. Vandsburger MH, French BA, Kramer CM, Zhong X, Epstein FH. Displacement-encoded and manganese-enhanced cardiac MRI reveal that nNOS, not eNOS, plays a dominant role in modulating contraction and calcium influx in the mammalian heart. *Am J Physiol Heart Circ Physiol*. 2012; 302:H412–9. [PubMed: 22058155]
17. Strijkers GJ, Mulder WJM, van Tilborg GAF, Nicolay K. MRI contrast agents: current status and future perspectives. *Anticancer Agents Med Chem*. 2007; 7:291–305. [PubMed: 17504156]
18. Li W, Griswold M, Yu X. Rapid T1 mapping of mouse myocardium with saturation recovery Look-Locker method. *Magn Reson Med*. 2010; 64:1296–303. [PubMed: 20632410]
19. Hu TC-C, Chuang K-H, Yanasak N, Koretsky A. Relationship between blood and myocardium manganese levels during manganese-enhanced MRI (MEMRI) with T1 mapping in rats. *NMR Biomed*. 2011; 24:46–53. [PubMed: 20665900]
20. Gerdin B, McCann E, Lundberg C, Arfors KE. Selective tissue accumulation of manganese and its effect on regional blood flow and haemodynamics after intravenous infusion of its chloride salt in the rat. *Int J Tissue React*. 1985; 7:373–380. [PubMed: 4055262]
21. Seo Y, Satoh K, Watanabe K, Morita H, Takamata A, Ogino T, Murakami M. Mn-bicine: a low affinity chelate for manganese ion enhanced MRI. *Mag Reson Med*. 2011; 65:1005–1012.
22. Grady RM, Grange RW, Lau KS, Maimone MM, Nichol MC, Stull JT, Sanes JR. Role for alpha-dystrobrevin in the pathogenesis of dystrophin-dependent muscular dystrophies. *Nat Cell Biol*. 1999; 1:215–20. [PubMed: 10559919]
23. Ren J, Wold LE. Measurement of Cardiac Mechanical Function in Isolated Ventricular Myocytes from Rats and Mice by Computerized Video-Based Imaging. *Biol Proced Online*. 2001; 3:43–53. [PubMed: 12734580]
24. Vandsburger MH, Janiczek RL, Xu Y, French BA, Meyer CH, Kramer CM, Epstein FH. Improved arterial spin labeling after myocardial infarction in mice using cardiac and respiratory gated look-locker imaging with fuzzy C-means clustering. *Magn Reson Med*. 2010; 63:648–57. [PubMed: 20187175]
25. Kober F, Iltis I, Cozzone PJ, Bernard M. Myocardial blood flow mapping in mice using high-resolution spin labeling magnetic resonance imaging: influence of ketamine/xylazine and isoflurane anesthesia. *Magn Reson Med*. 2005; 53:601–6. [PubMed: 15723407]
26. Streif JUG, Nahrendorf M, Hiller K-H, Waller C, Wiesmann F, Rommel E, Haase A, Bauer WR. In vivo assessment of absolute perfusion and intracapillary blood volume in the murine myocardium by spin labeling magnetic resonance imaging. *Magn Reson Med*. 2005; 53:584–92. [PubMed: 15723416]
27. Scheuhammer AM, Cherian MG. Binding of manganese in human and rat plasma. *Biochim Biophys Acta*. 1985; 840:163–169. [PubMed: 3995083]
28. Missy P, Lanthers MC, Grignon Y, Joyeux M, Burnel D. In vitro and in vivo studies on chelation of manganese. *Hum Exp Toxicol*. 2000; 19:448–456. [PubMed: 11125715]
29. Cowan DM, Fan Q, Zou Y, Shi X, Chen J, Aschner M, Rosenthal FS, Zheng W. Manganese exposure among smelting workers: blood manganese-iron ratio as a novel tool for manganese exposure assessment. *Biomarkers*. 2009; 14:3–16. [PubMed: 19283519]
30. Barouch LA, Harrison RW, Skaf MW, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature*. 2002; 416:337–339. [PubMed: 11907582]
31. Khan SA, Skaf MW, Harrison RW, Lee K, Minhas KM, Kumar A, Fradley M, Shoukas AA, Berkowitz DE, Hare JM. Nitric oxide regulation of myocardial contractility and calcium cycling: independent impact of neuronal and endothelial nitric oxide synthases. *Circ Res*. 2003; 92:1322–9. [PubMed: 12764022]

32. Sears CE, Bryant SM, Ashley EA, Lygate CA, Rakovic S, Wallis HL, Neubauer S, Terrar DA, Casadei B. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. *Circ Res.* 2003; 92:e52–9. [PubMed: 12623875]
33. Burger DE, Lu X, Lei M, Xiang F-L, Hammoud L, Jiang M, Wang H, Jones DL, Sims SM, Feng Q. Neuronal nitric oxide synthase protects against myocardial infarction-induced ventricular arrhythmia and mortality in mice. *Circulation.* 2009; 120:1345–54. [PubMed: 19770398]
34. Brooksby P, Levi aJ, Jones JV. The electrophysiological characteristics of hypertrophied ventricular myocytes from the spontaneously hypertensive rat. *J Hypertens.* 1993; 11:611–22. [PubMed: 8397240]
35. Brooksby P, Levi AJ, Jones JV. Investigation of the mechanisms underlying the increased contraction of hypertrophied ventricular myocytes isolated from the spontaneously hypertensive rat. *Cardiovasc Res.* 1993; 27:1268–77. [PubMed: 8252588]
36. Cerbai E, Barbieri M, Li Q, Mugelli A. Ionic basis of action potential prolongation of hypertrophied cardiac myocytes isolated from hypertensive rats of different ages. *Cardiovasc Res.* 1994; 28:1180–7. [PubMed: 7954620]
37. Keung EC. Calcium current is increased in isolated adult myocytes from hypertrophied rat myocardium. *Circ Res.* 1989; 64:753–763. [PubMed: 2522834]
38. Alvin Z, Laurence GG, Coleman BR, Zhao A, Hajj-Moussa M, Haddad GE. Regulation of L-type inward calcium channel activity by captopril and angiotensin II via the phosphatidylinositol 3-kinase pathway in cardiomyocytes from volume-overload hypertrophied rat hearts. *Can J Physiol Pharmacol.* 2011; 89:206–15. [PubMed: 21423294]
39. Sims C, Reisenweber S, Viswanathan PC, Choi B-R, Walker WH, Salama G. Sex, age, and regional differences in L-type calcium current are important determinants of arrhythmia phenotype in rabbit hearts with drug-induced long QT type 2. *Circ Res.* 2008; 102:e86–100. [PubMed: 18436794]
40. Komamura K, Fukui M, Iwasaku T, Hirofumi S, Masuyama T. Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment. *World J Cardiol.* 2014; 6:602–9. [PubMed: 25068020]
41. Horner WH, Kligfield P. Antidysrhythmic and dose-related hemodynamic effects of manganese in perfused isolated rabbit hearts. *J Cardiovasc Pharmacol.* 1982; 4:149–56. [PubMed: 6176792]
42. Vierling W, Reiter M. An intracellularly induced positive inotropic effect of manganese in guinea-pig ventricular myocardium. *Naunyn Schmiedeberg's Arch Pharmacol.* 1979; 306:249–53. [PubMed: 471077]

Figure 1. MSRLT pulse sequence

After the saturation module, 10 or 20 sequential ECG-triggered, multi-slice FLASH acquisitions separated by an interval τ were implemented. Each block on the relaxation curve represents a multi-slice acquisition module. To minimize cardiac motion artifact, a trigger delay was applied before the FLASH acquisitions such that all the images were acquired at mid-to-late diastole.

Figure 2. T_1 mapping of the multi-compartment phantom

a. T_1 maps of the five imaging slices. Phantoms 1–8 contain $MnCl_2$ solutions with the following concentrations (μ M): 1000, 900, 700, 500, 300, 200, 100, and 30. **b.** Quantitative comparison of the T_1 measurements by MSRL and SRLL. **c.** Bland-Altman plot of the difference between T_1 measured by MSRL and SRLL. The middle dotted line is the mean of the difference. The upper and bottom dotted lines are the mean plus and minus two standard deviations, respectively.

Figure 3. *In vivo* T₁ mapping

a. Representative T₁ maps at pre- (left) and post-contrast (right) acquired by MSRL (top) and SRLL (bottom), respectively. **b.** Comparison of T₁ mapping by MSRL and SRLL.

Figure 4. Bland-Altman plots for the comparison of T_1 measurements by MSRL and SLL *in vivo*

a. High Mn^{2+} dose group. **b.** Low Mn^{2+} dose group. The middle dotted line is the mean of the difference. The upper and bottom dotted lines are the mean plus and minus two standard deviations, respectively.

Figure 5. R_1 changes in dynamic MEMRI study

a&b. Pre- and post-contrast T_1 maps of the apical (left), mid-ventricular (middle), and basal slices (right), respectively. **c.** Time courses of R_1 changes. * $P < 0.05$ high dose versus low dose, with the color of * indicating the corresponding slice.

Figure 6. The impact of Mn^{2+} accumulation on heart rate and ejection ratio
a. Time courses of the heart rate at low and high Mn^{2+} doses. **b.** Ejection ratio of the mid-ventricular slice at pre- and post-contrast. * $P < 0.05$ compared to heart rate at pre-contrast in the high dose group; † $P < 0.05$ compared to heart rate at baseline in the low dose group; # $P < 0.05$ compared to ejection ratio at pre-contrast.

Figure 7. Mn²⁺ concentration and relaxivity in myocardium

a. Post-contrast Mn²⁺ concentration in myocardium by flame atomic absorption spectrophotometry. *P<0.05 high dose versus low dose. **b.** Mn²⁺ relaxivity in myocardium by linear regression analysis. The solid and dashed lines represent the regression analysis using data from the low dose group and both dose groups, respectively.

Figure 8. Mn^{2+} uptake and Ca^{2+} channel activity in $adb n^{-/-}$ mice

a. Time courses of R_1 changes by MEMRI. **b.** Representative Mn^{2+} concentration maps at 18 min during Mn^{2+} infusion in $adb n^{-/-}$ and control mice. **c.** Average Mn^{2+} concentration at 18 min of Mn^{2+} infusion. **d.** Capacitance normalized current-voltage relationship. **e.** Boltzman analysis of the potential for half-maximal activation ($V_{1/2}$) of the calcium current. * $P < 0.05$ compared to control mice. The colors of * in panel **a** indicate the corresponding slice.