Intrinsic Contrast for Characterization of Acute Radiofrequency Ablation Lesions

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Background—Both intrinsic contrast (T1 and T2 relaxation and the equilibrium magnetization) and contrast agent (gadolinium)–enhanced MRI are used to visualize and evaluate acute radiofrequency ablation lesions. However, current methods are imprecise in delineating lesion extent shortly after the ablation.

Methods and Results—Fifteen lesions were created in the endocardium of 13 pigs. A multicontrast inversion recovery steady state free precession imaging method was used to delineate the acute ablation lesions, exploiting T1-weighted contrast. T2 and M0 maps were also created from fast spin echo data in a subset of pigs (n=5) to help characterize the change in intrinsic contrast in the lesions. Gross pathology was used as reference for the lesion size comparison, and the lesion structures were confirmed with histological data. In addition, a colorimetric iron assay was used to measure ferric and ferrous iron content in the lesions and the healthy myocardium in a subset of pigs (n=2). The lesion sizes measured in inversion recovery steady state free precession images were highly correlated with the extent of lesion core identified in gross pathology. Magnetic resonance relaxometry showed that the radiofrequency ablation procedure changes the intrinsic T1 value in the lesion core and the intrinsic T2 in the edematous region. Furthermore, the T1 shortening appeared to be correlated with the presence of ferric iron, which may have been associated with metmyoglobin and methemoglobin in the lesions.

Conclusions—The study suggests that T1 contrast may be able to separate necrotic cores from the surrounding edematous rims in acute radiofrequency ablation lesions. (Circ Arrhythm Electrophysiol. 2014;7:718-727.)

Key Words: arrhythmias, cardiac ▪ magnetic resonance imaging ▪ relaxation

MRI is a powerful tool to detect ablation lesions because of its high soft tissue contrast. Double inversion fast spin echo (DIR) sequence demonstrates intrinsic T1-weighted contrast and quickly detect lesions, but DIR images lack specificity and provide poor border visibility.1 Late gadolinium enhancement (LGE) methods provide high contrast between healthy myocardium and the ablation lesions.3-5 However, acute lesion appearance on the LGE image varies with the time between the Gd-DTPA injection and image acquisition because of the wash-in/wash-out kinetics, particularly in areas associated with microvascular obstruction. Shortly after Gd-DTPA injection, the lesion contains hypoenhanced regions in typical LGE acquisitions. Then Gd-DTPA starts to enter the lesion, and a bright rim becomes visible. As the bright rim expands toward the center of the lesion over the ensuing minutes, wash-out mechanisms also begin to diminish the contrast at the outer border of the lesion. The lesion severity can also affect the wash-in/wash-out kinetics. As a second consideration, a recent study reported that the scar size measured 3 months after ablation with LGE images is <50% of that measured acutely.6 One of the possible reasons for this

Clinical Perspective on p 727

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718
is that the acute lesions depicted in LGE images may consist of regions reflecting both irreversible and reversible damage.6

In this study, intrinsic tissue contrast mechanisms for characterization of the acute RFA lesions were revisited to explain the RF-induced chemical and structural alterations, which change both the T1 and T2 of the tissue. Two different intrinsic contrast MRI methods were used; first, we applied a multicontrast inversion recovery steady state free precession (IR-SSFP) sequence used previously for T1 estimation in LGE.7–13 This sequence is based on a segmented and cardiac-gated SSFP continuous data acquisition after an inversion pulse, which generates multiple images with varying inversion times and degrees to SSFP recovery, weighted by intrinsic tissue T1 and T2. The second method was used to acquire multiple T2-weighted images with fast spin echo (FSE), from which T2 and M* maps were calculated to explain underlying magnetic resonance contrast mechanisms in the RFA lesions.

For comparison to current standards of lesion imaging, T1-weighted DIR and LGE images were also acquired. Corresponding short axis tissue samples were obtained after euthanization. Of particular interest were comparisons among images of lesion contrast-to-noise ratio (CNR) and comparisons of lesion extent with gross pathology. Finally, samples of the lesion and healthy myocardium were analyzed using a colorimetric iron assay to explore the underlying mechanisms of the observed magnetic resonance contrast behavior.

Methods
Histology and colorimetric analysis of the samples were explained in the material in the Data Supplement.

Interventional Procedure
All the procedures were conducted following Sunnybrook Research Institute guidelines and with the approval of the Sunnybrook Research Institute animal care committee (Data Supplement/animal preparation).

Fifteen ablation lesions were created in the left ventricles of 13 healthy swine models, with masses ranging from 20 to 30 kg. Navistar irrigated catheters (2.7 mm [8F], Biosense-Webster, Diamond Bar, CA) were used to deliver 35 Watts for 45 seconds in power controlled mode (Stockert 70 RF, Biosense-Webster, Diamond Bar, CA) to create each lesion in the endocardium of the left ventricle in the healthy pig models under x-ray fluoroscopy guidance (OEC 9800, GE Healthcare, Salt Lake City, UT). Electrophysiology signals were monitored at the target site using CARTO XP (BiosenseWebster, Diamond Bar, CA) to confirm voltage decrease, lesion creation, and catheter contact. RFA delivery was concluded in <20 minutes, and the swine were transferred to the MRI suite.

MRI Protocol
MRI studies were performed using a 1.5-T scanner (Signa HD, GE Healthcare, USA), using a 4-channel cardiac phased array coil. ECG gating and forced breath-holds obtained by shutting the ventilator were used to compensate for the cardiac and respiratory motion, respectively.

Less than 60 minutes after ablation, IR-SSFP and DIR sequences were performed along the short and long axis of the left ventricle in an alternating fashion for 57±27 minutes after initiation of scanning. The IR-SSFP sequence consisted of repeated segmented SSFP readouts after an inversion pulse applied every other heartbeat. A total of 40 images with varying T1 and T2 weightings were acquired over the 2 R-R period (field of view [FOV]=24 cm, slice thickness=5 mm, repetition time/time of echo [TR/TE]=4 ms/1.7 ms, flip angle=45°, readout bandwidth=+83.3 kHz, matrix=192×160, 20 views per segment).

For comparison, T1w DIR (FOV=24 cm, slice thickness: 5 mm, TR/TE=1463 ms/66 ms, bandwidth=+31.25 kHz, matrix=192×160, echo train length=24 images) were also acquired.

Then, 0.2 mmol/kg of gadoliniumdiethylenetriaminepentacetate (Gd-DTPA) was injected for 2-dimensional (2D) LGE imaging (inversion recovery gradient echo [IR-GRE]: FOV=24 cm, slice thickness=5 mm, TR/TE=3.8 ms/2.7 ms, readout bandwidth=+15.3 kHz, matrix=192×160), and multiple images were acquired in a time window <56 minutes after the injection. Variable inversion times (TI) were tested (between 200 and 600 ms) for the 2D LGE imaging, as discussed in detail below. The time delay between ablations and Gd-DTPA injection was <160 minutes. A second dose (0.2 mmol/kg) of Gd-DTPA was injected after finishing the 2D LGE acquisitions, typically 60 minutes after the first injection. Ten minutes later, pigs were euthanized using 50 mg/kg pentobarbital, and in situ high-resolution 3-dimensional (3D) IR-GRE images were acquired for 30 minutes (FOV=24 cm, slice thickness: 2 mm, TR/TE=6.2 ms/3 ms, readout bandwidth=+30.5 kHz, matrix=256×256, TI=500 ms). Thirty minutes after the in situ scans, the hearts were harvested and put into formalin solution, and the in situ protocol was repeated to acquire ex vivo data.

Lesion Borders and Size Measurement
Three different biologically damaged regions in RFA lesions were observed on gross pathology4,7,16: (1) the contact point of the ablation electrode, where the catheter touches the endocardium; (2) the lesion core is the coagulum region and the area of primary damage; and (3) the rim, which is the outermost region of visible damage associated

Figure 1. Representative images illustrating size and contrast-to-noise ratio (CNR) measurements. A, A graphical depiction of radiofrequency ablation lesion and associated anatomy. B, A representative image of gross pathology that was used for size measurements of lesion core and outer border. C and D, Representative images acquired after Gd-DTPA injection showing lesion size and CNR measurements, respectively (inversion time [TI]=500 ms, 35 minutes after injection). E and F, Representative images using inversion recovery steady state free precession (TI=710 ms) with associated measurements of size and CNR, respectively.
with the lesion (Figure 1A). The rim may consist of hemorrhage, hyperemia, and hemostasis. A similar structure can be identified in gross pathology images (Figure 1B) on which the rim looks dark and the lesion core has a white-gray color (Figure 1B). The gross pathology was used for the size comparison because it reflects dimensions before the 10% to 15% shrinkage associated with histopathological processing (Figure 1B). The lesion core appeared pale, and the rim appeared dark in gross pathology, so measuring the borders of the lesion core and the rim was straightforward.

Two different lesion sizes were reported: the maximum linear distance across (1) the lesion core and (2) the outer border, which contained the whole lesion including the rim (Figure 1A). The RFA lesions had a different appearance on the intrinsic contrast and 2D LGE images. The 2D LGE images showed both hyper- and hypoenhanced regions, whose sizes depended on the time after Gd-DTPA injection (Figure 1C). The lesion seems as a hypoenhanced region at an early time point; as the contrast agent diffuses into the lesion, a bright rim becomes visible. The outer border of the bright rim was considered as the lesion border in 2D LGE images at all time points (Figure 1C and 1D). Because both hyper- and hypoenhanced regions exist in these images, the CNR was measured as the contrast between the inner hypoenhanced area and surrounding hypoenhanced region.

However, the IR-SSFP sequence depicted lesions as hypoenhanced regions (Figure 1C), so the maximum linear distance between 2 points on the periphery of a hypoenhanced region was assigned as the size of the lesions on both IR-SSFP images.

The measured sizes were compared with the gross pathology outer border size, which includes the rim, and lesion core. The size measurements were performed manually by 2 different observers, and intraobserver correlation was also reported.

The CNR of the IR-SSFP images was defined as the mean MRI signal in the lesion core minus the mean signal of the surrounding tissue, divided by the SD of the background noise, which was obtained from an air-filled region outside the heart (Figure 1F). Because the 2D LGE images changed over time after injection, the CNR of the 2D LGE images was defined as the mean of the hypoenhanced rim minus the mean of the hypoenhanced lesion core, divided by the background noise (Figure 1D). Because the MRI intensity tended to vary as a function of the distance from the MRI coil, the surrounding tissue was chosen to be unalbled tissue in the immediate vicinity of the ablation lesion.

### Results

#### Histology

The RFA lesions usually had a teardrop appearance. A narrower lesion width was observed subendocardially with a greater width 2 to 3 mm below the endocardial surface, which might be explained by the flow cooling effect at the endocardial surface.

Histology (Figure 2R–2U) showed different deformation results and borders (Figures 1A and 2P) similar to gross pathology results (Figure 2O). The catheter contact zone consisted of severe muscular damage with loss of vascular and cellular structure (Figure 2R). The lesion core looked pale in gross examination and was composed of relatively preserved intact muscular tissue with intralosomal hematoma. The rim was dark in gross pathology, with intact cardiac muscle cellular structure, a few inflammatory cells, and mild edema. The dark color was because of hemorrhage in this region (Figure 2S). The left side of Figure 2S is the healthy myocardium, and the outer border of the rim of the ablation lesion was also visible. Figure 2T showed the myocytes close to the rim, and Figure 2U depicts the region closer to the catheter contact point. Thermal damage was more severe close to the contact point as expected. Overall, thermal damage gradually decreased from the catheter contact point to rim.

#### Imaging

The DIR image shows a bright region larger than the lesion because of the $T_1$ signal that made it likely as a result of edema, and the lesion borders were mostly inconclusive (Figure 2A).

The IR-SSFP images provided multiple contrasts of the lesion, healthy myocardium, edema, and blood. The first image was noisy because of rapid signal variation after inversion (Figure 2A). During the signal recovery, images with different inversion times show different contrasts. Immediately after the inversion pulse ($TI=95$ ms), the myocardium was nullled except for the broad region in and around the lesion (Figure 2C), which corresponds with the hypoenhanced edematous region in the DIR images (Figure 2A). Moreover, in images associated with longer TIs in the same sequence (Figure 2E–2I), the lesion was clearly demarcated with sharp borders, further isolated by the reduced blood signal in these images.

The appearance of the lesions in 2D LGE images varied with time after the Gd-DTPA injection (Figure 2K [t=20 minutes] and Figure 2L [t=45 minutes]). As shown in Figure 2K, between 5 and 20 minutes after Gd-DTPA injection, the lesion consisted largely of a hypoenhanced region; however, at times >25 minutes, the lesion appearance changed, and a hypoenhanced rim around the hypoenhanced area was observed.

To confirm, 3D LGE postmortem before organ harvesting (in situ, Figure 2M) and 3D LGE postmortem after fixation (ex vivo, Figure 2N) images were acquired.

The 2D LGE images with various TI values were acquired to determine the optimal TI for lesion visualization. Figure 3A to 3F show images with TI values of 250, 300, 350, 400, 500, and 600 ms, which were acquired in the time window 22±4 minutes after the Gd-DTPA injection. Although the 2D LGE method could visualize the lesion borders when TI value was adjusted (red and yellow arrows), these borders were difficult to see in DIR-FSE images (Figure 3G). Using the DIR-FSE sequence, only 4 of the 15 lesion borders could be clearly resolved. However, the multicontrast IR-SSFP sequence provided good depiction of both the lesions and the edema in various images with different TIs. The IR-SSFP image at an early inversion time ($TI=89$ ms) provided similar lesion pattern and signal contrast to that in the DIR-FSE image, likely reflecting edema (Figure 3H). However, the lesion extent was clearly visualized with IR-SSFP at $TI=717$ ms (Figure 3I); the lesion configuration in these images was validated against the 3D LGE in situ (Figure 3J) and ex vivo (Figure 3K) images, as well as against gross pathology (Figure 3L and 3N) and histology (Figure 3M and 3O) images.

Figure 4 depicts the results from one animal where 2 RFA lesions were generated in close proximity to each other in the posterior wall. Figure 4 shows similar results to those in the previous figures except for one important difference: although all the other sequences depicted the 2 close lesions as one big lesion, the IR-SSFP image (Figure 4B) was able to visualize properly the 2 distinct lesions on the posterior wall.

#### Magnetic Resonance Relaxometry

$T_1$ maps show a large area of diffuse elevated $T_1$ in and around the ablation region (Figure 5B). The $M_0^*$ map (Figure 5E) depicts well the RFA lesion observed on the gross pathology
and IR-SSFP images. $M_+^*$ depends on true equilibrium ($M_0$), $T_1$, and the RR interval ($M_+^* = M_0 (1 - e^{-RR/T_1})$). Because the RR interval was relatively short in our porcine model (600–700 ms), the $M_+^*$ term is $T_1$ weighted. Both the $T_2$ and $M_+^*$ maps show that the lesion core and the surrounding areas could have increased $T_2$, whereas the lesion core alone could have a shortened $T_1$.

Figure 5D and 5F shows 2 different heart phases of the IR-SSFP series: the first phase was acquired in the first R-R with a short inversion time ($TI = 123$ ms), and therefore, this image has bright blood while the second phase was acquired later in the second R-R ($TI = 661$ ms) when the blood signal was null. Signal enhancement around the ablation region in the first phase corresponds to the area of elevated $T_2^*$, whereas the lesion core alone could have a shortened $T_1$.

The edematous region emerged as the area of high $T_2$ values near the ablation and which surrounds the lesion. The healthy myocardium was defined on the same slice far from the lesion. Compared with healthy myocardium, $M_+^*$ increased by 85% and 30% in the lesion and edematous region, whereas $T_2$ increased in the lesion and edematous region by about the same amount (30–35 ms).

Lesion Size Measurement
Statistical tests were conducted to validate the association between the lesion size of inner core on gross pathology and the various 2D LGE images. The $T_2^*$ and relative $M_+^*$ values are shown in the Table. The lesions appeared as the area of high signal intensity on $M_+^*$ map near the ablation, as confirmed by 3D LGE images and gross pathology.

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A total of 16 lesions were analyzed. The inner core size measurements provided a strong correlation with IR-SSFP sizes ($n=16$; Pearson correlation coefficient $r=0.942$; $P=6e^{-8}$; confidence interval, 0.836–0.980), and a 2-tailed $t$ test showed that while these measurements were significantly different ($P=0.0015$; $t(15)=-3.88$), the mean difference was small (–0.09 cm). The inner core size was also significantly smaller than LGE ($P=26e^{-8}$; $r=-8.8$; mean difference=–0.27 cm). LGE size measurements provided a strong correlation with outer border size ($n=16$; Pearson correlation coefficient $r=0.914$; $P=77e^{-8}$; confidence interval, 0.764–0.970), and a 2-tailed $t$ test showed that these measurements were not significantly different ($r=0.1016$; mean difference=0.04 cm). However, the outer border was found to be significantly larger than IR-SSFP ($P=26e^{-4}$; $r=-8.8$; mean difference=–0.27 cm). LGE size measurements provided a strong correlation with outer border size ($n=16$; Pearson correlation coefficient $r=0.914$; $P=77e^{-4}$; confidence interval, 0.764–0.970), and a 2-tailed $t$ test showed that these measurements were not significantly different ($r=0.1016$; mean difference=0.04 cm). However, the outer border was found to be significantly larger than IR-SSFP ($P=1.83e^{-4}$; $t(15)=9.131$; mean difference=0.22 cm). Comparing the imaging methods, the IR-SSFP method results were found to be significantly smaller than the LGE results ($n=16$; $P=4.9e^{-5}$; $t(15)=-5.61$, mean difference=–0.17 cm). Last, a Bland–Altman analysis comparing the inner core sizes and IR-SSFP sizes (with inversion times between 650 and 900 ms) showed that the differences were within 2 SDs, and the variance seemed to remain constant as the lesion size increased (Figure 6A). The Bland–Altman analysis also showed that the lesion sizes in the LGE images matched well the outer border of the lesions in gross pathology (Figure 6D). DIR images were useful for edema delineation, but the borders of the lesions were barely visible with this sequence; therefore, only 4 lesion sizes were measured. Intraobserver correlation coefficients were determined as follows: IR-SSFP=0.988, DIR=0.947, and LGE=0.936. CNR values in 2D LGE (7.03±3.04) were higher than in IR-SSFP (5.85±2.61) and DIR (6.58±4.10).

**Colorimetric Iron Assay for Ferric Iron**

The results from the iron assay showed that the tissue samples from the lesion core were found to have much higher ferric iron content than samples from the healthy myocardium (Figure 7C). For 5 different samples, the optical densities of ferric iron in the lesions were >10× those in the healthy myocardium.

**Discussion**

**Imaging**

MRI is a powerful tool for visualizing the structural differences of tissues. Intrinsic contrast shows $T_1$ and $T_2$ changes, which reflect complex structural and physiological responses such as interstitial edema, hyperemia, conformational changes, and tissue coagulation. 3D LGE primarily reflects contrast agent distribution determined by changes in membrane permeability and microvascular obstruction in the acute setting. We think that the intrinsic contrast methods are more useful for characterization of the acute RFA lesions.
LGE: Acquisition Time and Inversion Time Dependence

Lesion appearance in the 2D LGE images depends on the time delay after injection. As evident on Figure 2K (t=20 minutes) and Figure 2L (t=45 minutes), the lesion consisted mainly of a hypoenhanced region; but after 25 minutes, the lesion appearance changed and the hyperenhanced rim around the hypoenhanced area became visible. Wash-out kinetics also affect lesion appearance; wash-out of the Gd-DTPA starts from the outermost rim and extends to the center over time. These mechanisms may in turn depend on other parameters such as perfusion, as well as size and location of the lesion.

Lesion appearance in 2D LGE also depends on selected TI. Researchers have been using TI values between 200 and 350 ms to null the myocardium and highlight lesions. In this study, nulling the healthy myocardium resulted in unsatisfactory image quality. As shown in Figure 3, the images with TI equal to 500 to 600 ms provided acceptable visualization of the RFA lesion.

IR-SSFP: Multiple Intrinsic Contrast Images

Although 2D LGE has extensively been used for RFA lesion characterization in clinical practice, the intrinsic contrast methods have been used rarely because of poorer depiction of lesion borders. That said, the IR-SSFP sequence has advantages over the standard LGE methods. First, the IR-SSFP approach can yield MRI lesion assessment earlier after lesion creation than the current LGE methods because the intrinsic contrast methods do not rely on the wash-in and wash-out of the Gd-DTPA. In the LGE method, the complete enhancement of the lesion core takes 60 to 90 minutes after Gd-DTPA injection for acute
lesions. However, the physiological changes because of the RFA can be monitored immediately using the intrinsic contrast IR-SSFP, which may make magnetic resonance–guided electrophysiology operations more favorable in the future.3,5,21–23

Second, because it is based on intrinsic contrast only, IR-SSFP can be repeated after the creation of multiple RFA lesions without degradation of image contrast, as opposed LGE methods which may require waiting for the wash-out of Gd-DTPA, which may take >600 minutes.6 Therefore, an LGE method is impractical for magnetic resonance–guided electrophysiology operations where multiple Gd-DTPA injections may be necessary.

Last, the multicontrast IR-SSFP method provides a series of images from one breath-hold. IR-SSFP is a T2/T1 weighted sequence after reaching the steady state but the weightings of T1 and T2 and hence the contrast between the tissues changes between the inversion and the recovery (Figure 7). This allowed improved discrimination of the lesion core from the adjacent tissue as shown using the simulation (Figure 7A), the region of interest-based signal analysis (Figure 7B), and in the lesion images (Figures 2–5). Therefore, the IR-SSFP images at early inversion times (TI\approx90 \text{ms}, Figures 2C and 3H) demonstrated a T2 weighting and sensitivity to edema (see DIR images, Figures 2A, 3G, and 4A), which may be useful for prediction of early recurrence of arrhythmias; more T1-weighted images were acquired at TI\approx800 \text{ms}, which clearly depicted the lesion core. The changing contrast effect was also confirmed by the IR-SSFP simulation (Figure 7A, bottom). This result suggests that IR-SSFP may be adequate in depicting both lesion core and edematous regions.

The region of interest-based signal measurements depicted the IR-SSFP signal evolution over time for the lesion and healthy myocardium (Figure 7B), and these measurements seem to correlate with the signal variation of lesion in the computer simulation (Figure 7A).

In this study, the lesion appearances in the both IR-SSFP and 2D LGE images were consistent, with one exception. In one of the experiments, an accidental acute infarct was created by the operator. The IR-SSFP sequence could delineate the RFA lesions from the infarct because the infarct was not visible with this sequence. However, in the 2D LGE image, both the lesion and the acute infarct resulted in the same contrast.

Considering the effects of beat-to-beat variation or arrhythmia, there are 2 potential concerns on a segmented acquisition sequence, such as IR-SSFP: (1) different segments might be acquired at different phases of heart cycle that may lead to artifacts in the image. Because the IR-SSFP is retrospectively gated similar to a CINE acquisition, the motion artifact is greatly reduced. (2) The gated inversion pulse in the sequence may introduce artifacts because of different contrast weighting on segments. Although the second concern is more challenging to correct fully, arrhythmia rejection functionality in the IR-SSFP sequence makes sure that the inversion pulse is not played out in case of arrhythmia. This ensures that a steady state is almost reached before each inversion pulse. With an altered acquisition protocol, allowing acquisition of different TI-weighting at similar cardiac phases, one could get parametric maps from this sequence, reflecting signal recovery in the presence of an SSFP train. This should provide improved delineation of lesions determined by T1 effects. Adjustment

Table. Relaxometry Analysis

<table>
<thead>
<tr>
<th>Region of Interest (ROI)</th>
<th>Mean $T_2$, ms</th>
<th>$M_o^*$ Map</th>
</tr>
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<tbody>
<tr>
<td>Healthy myocardium (HM)</td>
<td>47 (42–53)</td>
<td>0%</td>
</tr>
<tr>
<td>Edematous region</td>
<td>79 (65–103)</td>
<td>31%</td>
</tr>
<tr>
<td>Lesion</td>
<td>83 (69–98)</td>
<td>85%</td>
</tr>
</tbody>
</table>

The table shows the values of $T_2$ and $M_o^*$ ($100\%\cdot(ROI−ROI_{HM})/ROI_{HM}$) in the lesion, edematous region, and healthy myocardium.
of the method to allow such gated acquisition across multiple heartbeats is a work in progress.

In this study, our main focus was the characterization of the lesions using T1-based images and its underlying mechanisms. We analyzed the T2 and Mo maps for the completeness of the study and added 5 animals for this purpose.

**Lesion Size Measurement**

In our experiments, it was observed that IR-SSFP images with TIs in the range of 700 to 900 ms depicted well the lesion core, excluding the rim (Figure 6A). However, the 2D LGE lesion images were well matched with the area including the rim (Figure 6D). After the ablation procedure, the lesion becomes electrically isolated. To the electrophysiologist, reversibility of the RF ablation’s damage to the myocardium damage will seem as the restoration of electric conduction. The histological observations made in this study suggest that such reversibility is more likely to occur in the lesion’s rim than in the lesion core because of reduced cell destruction in the rim.24,25

The more specific depiction of the lesion core by the IR-SSFP method can be observed on. Only the IR-SSFP images showed 2 separate lesions as opposed to one big lesion in the images of the other methods (Figure 4B); the histology data similarly demarcated less cell destruction in the rim, which consists of hemorrhage, between the 2 lesions. Although a chronic study is necessary for a more accurate analysis of the lesion reversibility, the IR-SSFP images (Figure 4B) may better indicate gaps and incomplete RFA lines that can cause recurrence of arrhythmias.

The CNR measurements of the RFA lesions have been used to quantify the lesion visualization by researchers. However, CNR measurement suffers from poor standardization because of the lack of homogeneous signal in the lesion and spatial variation in coil sensitivity. Here, to minimize coil-shading effects, the region of healthy myocardium was chosen in the vicinity of the ablation lesion (Figure 1D and 1F). Although 2D LGE resulted in higher lesion-to-healthy myocardium CNR compared with IR-SSFP in individual images, the varying contrast of IR-SSFP across images at different TIs provided a richer data set for lesion separation (Movie in the Data Supplement). Measuring the CNR values of the DIR images was difficult because of the lack of sharp lesion borders; therefore, the reliability of the measurements was questionable in these data sets.

The current 2D version of the sequence would work directly in clinical practice for lesion verification and longitudinal characterization because the patient heart rate is lower than pigs. The acquisition scheme of the sequence can also be modified to provide quantitative parametric maps, which would likely improve the delineation of lesions. In addition, other groups have demonstrated capacity to generate 3D T1-weighted maps. In the longer run, 3D high-resolution IR-SSFP sequence and fast acquisition techniques, such as compressed sensing, would add benefit to the field, and our group is working on these methods.

The underlying mechanism seems to be change in the state of iron in blood and myocardium during ablation. Under this assumption, one can directly translate the results to patient studies. The current 2D version of the sequence would work directly in clinical practice for lesion verification and longitudinal characterization because the patient heart rate is lower than that for pigs. The acquisition scheme of the sequence can also be modified to provide quantitative parametric maps, which would likely improve the delineation of lesions. In addition, other groups have demonstrated capacity to generate 3D T1-weighted maps. In the longer run, 3D high-resolution IR-SSFP sequence and fast acquisition techniques, such as compressed sensing, would add benefit to the field, and our group is working on these methods.

![Figure 6. Radiofrequency ablation lesion size: Bland–Altman analysis of gross pathology- vs imaging-based lesion size. The difference between the lesion size measured with inversion recovery steady state free precession images and (A) inner core (B) outer border sizes as measured by gross pathology in cm vs the average size of a given lesion. Similarly, the differences between the lesion size measured with 2-dimensional late gadolinium enhancement images and (A) inner core (B) outer border sizes as measured by gross pathology in cm vs the average size of a given lesion. Red lines indicate 95% confidence limits. Note that, use of DIR images for estimating borders resulted in large errors.](http://circep.ahajournals.org/)}
Ferric Iron and T1 Shortening in RFA Lesions

Hemoglobin gets converted to methemoglobin when the blood is heated. Methemoglobin contains ferric iron instead of ferrous iron (Fe2+), which is found in oxyhemoglobin, deoxyhemoglobin, and myoglobin. Although more detailed analysis is necessary for a full proof, our initial observation shows that the underlying contrast mechanism (T1 shortening), which is depicted by IR-SSFP images, is likely because of the change of the iron state inside the lesion core, specifically the transformation to ferric iron. Meanwhile, measured lesion size in LGE images is time dependent because of the wash-in/wash-out kinetics of the gadolinium in the lesion.

Further investigation might be useful to confirm the effect of the ferric iron using different MRI tools such as T1 and T2 relaxometry maps. Edema causes a slight increase in T1 value; therefore, the effect of edema is expected to be slight signal decrease on T1-weighted images. However, T1-weighted IR-SSFP images (TI=800 ms) depicted hyperintense region, which suggests a decrease of the T1 value; we think this is because of the ferric iron deposition in the lesion core. Changes in T2, which should better reflect the impact of edema are seen in the T2 map and DIR images and generally cover a significantly larger area that affected by the reduction in T1.

Conclusions

The proposed intrinsic contrast methods demonstrated the significance of T1 and T2 contrast in the characterization of acute RFA lesions. The multi echo fast spin echo yielded relaxometry maps, which permitted an improved understanding of the underlying contrast mechanisms. IR-SSFP images showed strong T1-dependent contrasts between the ablation lesions and normal myocardium, which exhibited high correlation with histological and gross pathology findings. It was also shown that the T1 shortening in the lesions is correlated with the higher ferric iron concentration compared with healthy myocardium. These findings suggest that imaging thermal injury in the myocardium using the IR-SSFP approach, during or immediately after ablation, has the potential to provide a reliable estimation of the extent of myocardial damage and potential reversibility of that damage. More generally, the IR-SSFP depiction of intrinsic T1 contrast provided more reliable and consistent RFA lesion characterization than seen with LGE.

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Disclosures

None.
References


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SUPPLEMENTAL MATERIAL

Animal Preparation

Before the procedure, the pigs were fasted overnight. The pigs were sedated by IM injection of ketamine (15mg/Kg) and atropine (0.05mg/Kg). After sedation, the pigs were weighed and intubated; IV access was obtained by way of a 22Ga angiocatheter in the marginal ear vein. Then, burprenorphine (1ml/10Kg, IM) and duplocillian (2ml, IM) were given. The animals received continuous IV lactated ringers solution and remained on a ventilator with 2L/min of oxygen and Isoflurane given to effect. Once in a surgical plane of anesthesia, an incision was made on the neck and the carotid artery was exposed and isolated. Once the carotid artery was isolated, a 2.0 sterile silk ligature was placed at the proximal and distal ends of the artery and an 8F introducer and sheath was inserted into the carotid artery. The pigs were heparinized (approximately 100 IU/Kg, IV) and a lidocaine drip (3 mg/kg/hour) was started. Amiodarone (75mg, IV), an anti-arrhythmic drug, was administered as required.

MR Relaxometry

In addition to the above imaging sequences, data was acquired in a subset of pigs (n = 5) to produce \( T_2 \) and \( M_0^* \) maps to examine in more detail the specific intrinsic relaxation behavior producing lesion/myocardium conspicuity before contrast agent injection. Multi-echo fast-spin-echo (MEFSE) images were acquired (FOV = 26cm, slice thickness = 5mm, TR = 697ms, TE = 5/25/45/65ms, bandwidth = +62.5 kHz, matrix = 160 × 128, echo train length = 16, total scan time of 16 heartbeats per slice). From the resulting images a standard 2-parameter fit (\( S = M_0^* e^{-\frac{TE}{T_2}} \)) was used to map absolute \( T_2 \) values and percentage difference of the \( M_0 \) values from the healthy myocardium, which was defined as \( 100 \times (ROI - ROI_{HM}) / ROI_{HM} \).

Region of Interest (ROI) Data Analysis

Two different ROI based analyses were performed. First, mean values within ROIs located in the lesion core, adjacent edematous region, and remote healthy myocardium regions were compared in the \( T_2 \) and \( M_0^* \) maps (Table 1). Second, signal values within ROIs located in the lesion core, remote healthy myocardium, and blood zones were examined in the IR-SSFP images.
In addition, an IR-SSFP sequence was simulated (MATLAB, version 7.6; Mathworks Inc., Natick, MA) for signal and contrast characteristics of the “lesion” (T_1 = 800ms, T_2 = 80ms), “edematous tissue” (T_1 = 1200ms, T_2 = 80ms), “healthy tissue” (T_1 = 1000ms, T_2 = 40ms) to better understand the varying contrasts present in the IR-SSFP images (TR/TE = 4/1.7ms, flip angle = 45°).

**Histology**

The histological processing was completed in three weeks after each pig experiment. The hearts were put into a 10% formalin solution just after the in-situ imaging and remained there for at least 24 hours. They were then placed inside an aluminum container, which was subsequently filled with Cavex CA37 dental alginate gel (Cavex, Haarlem, Netherland) to facilitate slicing. A commercially available meat slicer was used to obtain 5mm thick short axis slices. The gel was then removed and both sides of the slices were photographed for gross pathology. Slices were kept overnight in a second formalin solution and loaded into a tissue processor. Processed tissue samples were embedded in paraffin for sectioning and 5µm thick whole-mount sections were prepared using a microtome. These sections were stained using hematoxylin–eosin (H&E). Lastly, the slides were digitized with 20X resolution using a TISSUEscope digitizer (Biomedical Photometrics, Waterloo, ON, Canada). Histology images were used to validate thermal damage regions.

**Colorimetric Iron Assay for Ferric Iron (Fe³⁺)**

In samples taken immediately after sacrifice from a separate set of lesions (n = 5) created by the same RFA procedure as previously described, where sacrifice occurred typically 45 minutes post-ablation, the ferric iron content was calculated using an iron assay kit (product# K 390-100, BioVision, Mountain View, CA) and compared with that from adjacent healthy tissue. All tissue samples were kept at -80°C until they were processed. The tissue samples (ablated and healthy myocardium) were homogenized using a glass homogenizer to avoid any metal interaction. Then, the iron assay was processed according to manufacturer's instructions, and the optical densities were measured at 593nm in a microplate reader.
**Supplementary Video:** Varying contrast of IR-SSFP across images at different TI’s provided a richer data set for lesion separation.