Published in final edited form as:

J Magn Reson Imaging. 2014 February ; 39(2): 307–316. doi:10.1002/jmri.24171.

Magnetic Resonance Characterization of Hepatic Storage Iron in Transfusional Iron Overload

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Abstract

Purpose—To quantify the two principal forms of hepatic storage iron, diffuse, soluble iron (primarily ferritin), and aggregated, insoluble iron (primarily hemosiderin) using a new MRI method in patients with transfusional iron overload.

Materials and Methods—Six healthy volunteers and twenty patients with transfusion-dependent thalassemia syndromes and iron overload were examined. Ferritin- and hemosiderin-like iron were determined based on the measurement of two distinct relaxation parameters: the “reduced” transverse relaxation rate, RR_2 and the “aggregation index,” A, using three sets of Carr-Purcell-Meiboom-Gill (CPMG) datasets with different interecho spacings. Agarose phantoms, simulating the relaxation and susceptibility properties of tissue with different concentrations of dispersed (ferritin-like) and aggregated (hemosiderin-like) iron, were employed for validation.

Results—Both phantom and *in vivo* human data confirmed that transverse relaxation components associated with the dispersed and aggregated iron could be separated using the two-parameter (RR_2 , A) method. The MRI-determined total hepatic storage iron was highly correlated ($r = 0.95$) with measurements derived from biopsy or biosusceptometry. As total hepatic storage iron increased, the proportion stored as aggregated iron became greater.

Conclusion—This method provides a new means for non-invasive MRI determination of the partition of hepatic storage iron between ferritin and hemosiderin in iron overload disorders.

Keywords

iron overload; hepatic storage iron; ferritin; hemosiderin; iron quantification; MRI

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Introduction

In patients with iron overload, the amount of iron in functional and transport pools changes only slightly (1). Almost all of the excess is sequestered in storage forms, as diffuse, soluble and rapidly mobilizable *ferritin iron*, and as aggregated, insoluble and slowly exchangeable *hemosiderin iron*. Ferritin iron is found in virtually all cells, providing both an accessible reserve of iron for synthesis of functional iron-containing compounds and a means of sequestering iron in a soluble, relatively nontoxic form within the cytosol (2). The ferritin iron core is composed of the hydrous ferric oxide mineral ferrihydrite with the approximate formula $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ (3). Recent evidence indicates that iron incorporation and release from ferritin are intrinsic, autonomous properties of the molecule, based on an equilibrium with the concentration of cytosolic low-molecular-weight iron (4). Hemosiderin, formed within secondary lysosomes (siderosomes) from agglomeration of iron cores derived from denatured ferritin (5, 6), seems to help protect against iron toxicity by storing excess iron away from the cytosol (7). The scarce data available suggest that the amounts and distribution of these two forms of storage iron are influenced by the underlying disorder (such as hereditary hemochromatosis, thalassemia, sickle-cell disease and other refractory anemias), by the duration and extent of iron loading, and by the type of therapy (phlebotomy, iron chelation) (8–10). Information is limited, in part, because the sole established means of separately measuring ferritin and hemosiderin iron in tissue requires biochemical analysis of specimens obtained by biopsy. Clinically, only the total storage (ferritin plus hemosiderin) iron concentration of tissues is typically determined. Separate measurements of the two iron storage forms are rarely reported, in part because of the limited tissue samples obtained at biopsy. Progress in understanding the molecular mechanisms underlying iron overload (10) suggests that separate measurement of ferritin and hemosiderin iron would improve our understanding of the pathophysiology of iron-induced tissue damage. Clinically, separate measurements potentially could provide an early indication of iron toxicity and be useful in monitoring treatment by phlebotomy or with different iron-chelating agents (11).

Storage iron within tissues influences the magnetic resonance signal by altering the local magnetic field and strongly alters the signal intensity in both transverse relaxation time (T_2) and effective transverse relaxation time (T_2^*) weighted images (12–28). Ferritin and hemosiderin iron influence MRI signal decay through different mechanisms. At present, MRI methods estimate total storage iron by determination of either transverse relaxation rate (R_2) or effective transverse relaxation rate (R_2^*); no information about the partition of storage iron between ferritin and hemosiderin is provided. The relationship between R_2^* and the total storage iron concentration has been considered to be linear (27). By contrast, the relationship between R_2 and the total storage iron concentration is curvilinear over a clinically relevant range of iron concentrations (24, 27), a phenomenon that is incompletely understood. An alternative approach to the MR characterization of tissue iron has been developed, based on a theoretical model (29), that uses quantitative determinations of the concentrations of dispersed and aggregated iron to measure the total storage iron. In brief, the differences in aggregation and solubility that permit physical separation of ferritin and hemosiderin iron for biochemical measurements also result in distinct MRI signal decay behaviors (29). The signal decay caused by dispersed, soluble ferritin iron has a monoexponential form, and R_2 can be derived from monoexponential fitting to a multiple spin echo (MSE) signal. In contrast, the signal decay caused by aggregated, insoluble hemosiderin iron is non-monoexponential and strongly dependent upon the interecho time. Fitting the model to the multiple sets of MSE data with different interecho spacings permits calculation of a “reduced relaxation rate” (RR_2) and an “aggregation index” (A). The ferritin and hemosiderin iron concentrations can then be estimated from RR_2 and A, respectively, and the total storage iron calculated as their sum.

Validation *in vitro* of this model has been reported in agarose phantoms that simulate dispersed (ferritin-like) iron with manganese chloride and aggregated (hemosiderin-like) iron with iron oxide microspheres, together with examination of a small number of subjects to demonstrate the feasibility of human studies (30). The present study focuses on separate measurements of dispersed (ferritin-like) and aggregated (hemosiderin-like) iron in patients with transfusion-dependent thalassemia syndromes and in healthy subjects. We demonstrate that the contributions of dispersed and aggregated iron to the transverse relaxation rate (R_2) can be separated using the parameters RR_2 and A . Finally, the influence of the stimulated echo (31) caused by the present MSE sequence on RR_2 and A (32, 33), and on iron measurement are analyzed based on phantom simulations. We hypothesize that tissue iron quantification based on the two parameters, RR_2 and A , rather than the single parameter, R_2 or R_2^* , will improve estimates of total storage iron. We suggest that separate measurements of dispersed and aggregated iron may contribute to an improved understanding of the pathophysiology of iron overload, potentially provide early warning of an increased risk of iron-induced toxicity and permit rapid monitoring of treatment by phlebotomy or administration of iron-chelating agents.

Materials and Methods

Theory

The MSE signal decay caused by ferritin iron has a monoexponential form, where R_2 may be derived from monoexponential fitting of a MSE signal. The R_2 depends linearly both on the iron concentration and the applied field (34). In contrast, because hemosiderin iron is aggregated in insoluble clusters, the MSE signal decay caused by aggregated iron is *not* monoexponential and has a strong dependence on the interecho time (29). More precisely, the non-monoexponential decay is described approximately by the following analytic form:

$$S = S_0 \times \exp(-RR_2 \times TE) \times \exp(-A^{3/4}(\Delta t)^{3/4}(TE - t_s)^{3/8}). \quad [1]$$

The time shift, t_s , is defined to be:

$$t_s = 2\tau[1 - (\tau/\Delta t)^2], \quad [2]$$

where S_0 is the initial signal intensity, spin echoes form at $TE = 2\tau, 2\tau + 2\Delta t, 2\tau + 4\Delta t, 2\tau + 6\Delta t$, etc., A is the aggregation index, and RR_2 is the “reduced” transverse relaxation rate. The parameter A , accounting for the non-monoexponential term, is primarily sensitive to hemosiderin iron, while the exponential factor RR_2 , incorporating the reduction in the measured R_2 , is primarily sensitive to the ferritin iron (29). It is advantageous to set τ to be less than Δt in order to better sample the initial part of the decay curve, especially when the iron concentration is high. Assuming that the aggregated (hemosiderin-like) iron concentration, C_A , is proportional to the aggregation index A , and the dispersed (ferritin-like) iron concentration, C_D , is linearly related to the ferritin iron, the total iron concentration may be estimated as:

$$C_T = C_D + C_A, \quad [3]$$

where

$$C_D = \alpha_1 + \alpha_2 \times RR_2, \quad [4]$$

$$C_A = \alpha_3 \times A, \quad [5]$$

and α_1 , α_2 , α_3 , are empirical calibration parameters, which can be determined from a best fit of the MRI measurements to independent estimates of the total storage iron concentration derived from biochemical analysis of biopsy specimens (35) or from biosusceptometry (1) using superconducting quantum interference device (SQUID). A unique feature of this approach is that it yields separate estimates for the ferritin-like (C_D) and hemosiderin-like (C_A) iron concentrations.

Human Subjects

Six healthy volunteers and 20 patients with transfusion-dependent thalassemia syndromes and iron overload (19 to 51 years of age, 10 males, and 10 females) were examined in a manner consistent with the Institutional Review Board policies of our institution. Written informed consent was obtained from each subject. The hepatic storage iron concentrations of the patients were monitored by either biosusceptometry ($n = 12$), on the same day as the MRI measurements, or by biopsy ($n = 8$, median interval between MRI and biopsy, 6 months, range 1 – 11 months). Because measurements by biosusceptometry *in vivo* and biochemical analysis *in vitro* of hepatic tissue in biopsy samples are closely correlated ($r = 0.98$; $p < 10^{-5}$) and do not differ significantly (36), the results of the two methods are used interchangeably.

Pulse sequence for *in vivo* MRI

All MR measurements were performed using a Philips 1.5T whole body MR scanner (Philips Medical Systems, Best, The Netherlands) equipped with a five-element phased array cardiac coil. The scanner had a maximum gradient strength of 33 mT/m and a slew rate of 100 T/m/s. For each subject, three slices of 10 mm thickness were acquired with a matrix of 128×128 within a field of view (FOV) of $37 \times 37 \text{ cm}^2$ and with an interslice gap of 1 mm. Each slice was acquired in a double oblique orientation to include both the heart and liver.

To estimate RR_2 and A , three CPMG-based MSE acquisitions were performed using different interecho times, with the first echo at 4 ms (2τ) for all sequences. The imaging protocol includes: a 25-echo sequence with an interecho time ($2\Delta t$) of 4 ms, a 15-echo sequence with an interecho time of 8 ms (i.e., subsequent to the first 4ms echo), and a 10-echo sequence with an interecho time of 15 ms, each with ECG triggering and respiratory navigator gating to minimize cardiac and respiratory motion artifacts. The repetition time (TR) was one heart beat, the parallel imaging SENSE factor was 1.5, the excitation pulse flip angle was 90° , and the refocusing pulse flip angle is 160° , the vendor's default MSE implementation. The image acquisition time was about 176 s for each sequence.

In addition, a 17-echo multiple gradient echo (MGE) sequence was implemented for R_2^* measurement, with the first echo time occurring at 2.9 ms following the initial RF excitation, an interecho time of 0.83 ms, TR = one heart beat, and excitation pulse flip angle = 50° . The image acquisition time was 53 s. The MGE acquisitions were performed with ECG triggering and respiratory navigator gating.

Image analysis

Parametric maps for A and RR_2 were calculated. For hepatic iron quantification, a region of interest (ROI) was delineated to cover the majority of liver tissue within an image slice, and was propagated along the echo dimension. The liver tissue was further separated from the blood vessels and signal void regions using a threshold-based segmentation, to avoid signal

contributions from these regions. The three MSE decay data within the liver tissue were rescaled, so that the mean intensities at $t = 4$ ms were identical, to correct for inter-scan differences in intensity scaling. The corrections were typically a few percent of the maximum signal.

The two parameters, RR_2 and A , were estimated by global fitting of the three MSE decay data to the non-monoexponential decay model based on Eq. [1]. The conventional R_2 was obtained using the MSE data with the interecho time 4 ms, and R_2^* was obtained from the MGE data, by fitting these signal decay data to the mono-exponential model. To reduce the effect of background signal on the parameter estimates, the actual functional expression consistently fit to all MSE and MGE data had the form:

$$S_{\text{fit}} = \sqrt{S_{\text{ideal}}^2 + \sigma^2} \quad [6]$$

where S_{ideal} is the ideal (or unbiased) signal and σ is the mean background noise (30, 37). The fits were obtained by using the standard Levenberg-Marquardt method of least squares minimization, with four free parameters (S_0 , RR_2A and σ) derived from the two-parameter model fits, and with three free parameters (S_0 , R_2 or R_2^* , and σ) derived from the mono-exponential (single-parameter) model fits.

The calibration parameters α_1 , α_2 , and α_3 were determined from a linear least squares fit of Eq. [3] to the independent total iron estimates (C_T) derived from the biopsy or biosusceptometry measurements. With this calibration, MRI estimates for the total ($C_{T,MRI}$), dispersed ($C_{D,MRI}$), and aggregated ($C_{A,MRI}$) iron concentrations were determined from Eqs. [3–5]. Linear calibrations between R_2 , R_2^* and iron were estimated using univariate regression.

The background signal must be considered in the fitting procedure, and several techniques have been used by other investigators (14, 15, 24, 27). In addition to assuming a noise bias as described above, we compared R_2 fitting methods proposed by other researchers with our fitting strategy to evaluate the robustness of our approach in the presence of background signals. In general, the other techniques assume either a slow decaying component (14, 24) or a constant (15, 27) added to the signal decay, representing a weak signal contributed by other sources such as bile and blood. Based on the MSE data with the interecho time of 4 ms, we estimated R_2 values with (i) the biexponential method, which uses summation of R_2 from the fast and slow decay components weighted by their population densities, and (ii) the monoexponential method, which contains the second component as a constant (27).

Phantoms

To validate our methods, we conducted a phantom study similar to that of Ref. (30), but with different pulse sequences. Phantoms were prepared, as in Ref. (30), with mixtures of various concentrations of MnCl_2 (0 to 0.675 mM) and iron oxide microspheres (0 to 0.1 mg Fe/ml), to mimic, respectively, the relaxation effects of ferritin and hemosiderin iron found in iron-loaded liver. The iron oxide microspheres were composed of magnetite nanoparticles (UM3CN/5737, Bangs Laboratories, Fishers, Indiana), with a mean radius of 2.9 μm and an average of 4.4×10^{-9} mg Fe/particle. A total of 36 cylindrical sample bottles (1.5 cm in diameter and 15 cm^3 in volume) were prepared with concentrations

$$C_D = m \times 0.0074 \text{ mg Mn/g}, m=0, 1, \dots, 5, \quad C_A = n \times 0.02 \text{ mg Fe/g}, n=0, 1, \dots, 5. \quad [7]$$

suspended in 2% agarose gel and immersed in a ~50 mM MnCl_2 aqueous solution bath. The MnCl_2 was added to the bath both to suppress the bath signal (which could contaminate the sample signal due to artifacts) and to reduce magnetic field inhomogeneities.

The phantom images were acquired using the same MSE sequence designed for the liver study. Six bottles were imaged simultaneously during each scan. Since the molecular weight of Mn (54.9) is close to Fe (55.8), the “total iron” concentration (C_T) was simply the sum of the Mn (C_D) and the microsphere iron (C_A) concentrations.

Finally, stimulated echoes may occur for MSE sequences with inaccurate RF pulse flip angles and imperfect slice selection profile (31). To minimize these, an optimized MSE sequence was implemented on the same MR system with an increased slice selection thickness for the refocusing pulses (19, 30, 32, 33). While the *in vivo* iron measurement was based on the vendor's default (non-optimized) MSE sequence, we evaluated our method on phantoms with both non-optimized and optimized MSE sequence to assess potential systematic effects.

Results

MRI iron measurement for aqueous phantoms

Figure 1 demonstrates the three sets of MSE signal decay in phantoms that contain Mn only (0.015 mg Mn/ml) and that contain both Mn and Fe (0.015 mg Mn/ml, 0.06 mg/ml). As can be seen, the MSE signal decay in phantoms with no microspheres ($C_A = 0$) was independent of the interecho time. With microspheres present, the signal decay, in contrast, depended strongly on the interecho time. Solid lines are the global fits for the multiple decay curves.

The solvent of the microspheres, which is in proportion to the iron concentration, was found to influence MRI signal decay, with a net R_2 effect of $133.0 \pm 4 \text{ s}^{-1}/(\text{mg Fe/g})$ estimated as discussed in a previous study (30). In order to minimize the solvent's influence on the phantom results, the phantom R_2 and RR_2 values were corrected by subtracting out this net R_2 effect.

We noticed that in the present study, the estimated relaxivity of the MnCl_2 phantom was approximately $59.1 \text{ s}^{-1}/\text{mM}$ at 1.5T, which is lower than the published values of $74.2 \text{ s}^{-1}/\text{mM}$ measured with a single spin echo sequence (22) and $71.4 \text{ s}^{-1}/\text{mM}$ measured with the MSE sequence (38). We believe this underestimation to be mainly due to stimulated echoes introduced by the imperfect slice selection profile and reduced refocusing RF pulse flip angle (19, 30–33). When the refocusing slice thickness is increased by an empirically determined factor of three, the relaxivity then becomes consistent with the literature values.

The *in vivo* image data in the present study were acquired using the vendor-supplied, non-optimized MSE sequence, i.e., without increasing the slice selection thickness for the refocusing RF pulses. This difference prompted investigation of the relationship between RR_2 and A values derived from the present and optimized MSE sequences (32, 33). A linear regression analysis ($r = 0.99$) verified that the stimulated echo effects contributed to an underestimation of RR_2 (Figure 2a) and an overestimation of A (Figure 2b). The relationship between the measurements made with the vendor-supplied and optimized MSE sequences appears to be linear, supporting the feasibility of calibrating RR_2 and A (as measured with the vendor-supplied sequence) for total and compositional iron quantifications. A least squares fit of Eq. [3] to the full set of phantom results yields the calibration parameters

$$\alpha_1 = -(11.3 \pm 1.9) \times 10^{-3} (\text{mg Mn/g}), \quad \alpha_2 = (11.8 \pm 0.56) \times 10^{-4} \text{ s} \cdot (\text{mg Mn/g}), \quad \alpha_3 = (0.75 \pm 0.015) \text{ ms}^{3/2} \cdot (\text{mg Fe/g}), \quad [8]$$

Figure 3 demonstrates the estimation of Mn and Fe concentration maps for phantoms containing various Fe concentrations (0 to 0.1 mg/ml) and a fixed Mn concentration (0.015 mg/ml) using the two-parameter method. The phantom in the center (red arrow) contains no

Mn and Fe. The phantom on the top (yellow arrow) contains Mn only. Figure 3b and 3c are Mn and Fe maps estimated by Eqs. [4] and [5], respectively. Figure 3d is the summation of Mn and Fe, simulating the mapping of total iron concentration.

The MRI determined total iron concentrations estimated by Eqs. [3–5] and the calibration parameters (Eq. [8]) are strongly correlated (Pearson correlation coefficient $r = 0.99$), and in excellent agreement with the true iron concentrations (mean difference was 0.0 mg Fe/g, and 95% limits of agreement were 0.006 and -0.005 mg Fe/g).

***In vivo* MRI liver iron measurement**

Figure 4 demonstrates the compositional iron concentration maps in a healthy (Figure 4a) and an iron overloaded liver (Figure 4d). The liver ROI template is indicated by red arrow. The mean RR_2 and A from the six healthy subjects were $20.8 \pm 4.0 \text{ s}^{-1}$ and $0.0029 \pm 0.0020 \text{ ms}^{-3/2}$, respectively, representing the normal range of relaxation values, with an assumed total storage iron concentration of 0.15 mg Fe/g wet weight.

A least squares fit of Eq. [3] to the total storage iron concentration determined by biopsy or biosusceptometry measurements ($n = 20$) yielded the calibration parameters:

$$\alpha_1 = -(0.28 \pm 0.30)(\text{mg Fe/g}), \alpha_2 = (5.9 \pm 2.0) \times 10^{-2} \text{ s} \cdot (\text{mg Fe/g}), \alpha_3 = (33 \pm 3) \text{ ms}^{3/2} \cdot (\text{mg Fe/g}), \quad [9]$$

The ferritin (Figures 4b and 4e) and hemosiderin (Figures 4c and 4f) maps were calculated based on Eq. [4–5] using the two-parameter (RR_2 , A) method. The total storage iron concentration map (sum of the ferritin and hemosiderin) is shown in Figure 4g.

The total iron concentration calculated based on the R_2 and R_2^* are demonstrated in Figure 4h and 4i, respectively, for the iron overload liver. The linear calibrations are:

$$C_{T_R_2} = (0.13 \text{ s} \times R_2 - 2.5)(\text{mg Fe/g}), \quad C_{T_R_2^*} = (0.0135 \text{ s} \times R_2^* - 0.5)(\text{mg Fe/g}). \quad [10]$$

There were only traces of hemosiderin iron in normal liver as can be seen from the fact that varying the interecho time had little to no effect on the signal decay (Figure 5). In the iron-loaded liver tissue, elevated ferritin and hemosiderin iron are evident. The signal decay depends strongly on the interecho time (Figure 5). The total iron in this specific iron overload liver estimated by R_2^* (5.1 mg Fe/g) is greater than that from the two-parameter (RR_2 , A) method, from R_2 , and from biopsy, or 2.6, 1.9, and 2.4 mg Fe/g wet weight, respectively.

Total storage iron, ferritin- and hemosiderin-like iron concentrations in liver ROI were calculated for all subjects. The mean total storage iron estimated by (RR_2 , A) and biopsy/biosusceptometry are 3.26 ± 2.25 and 3.34 ± 2.32 mg Fe/g, respectively, for the 20 patients. The mean total iron estimated by (RR_2 , A) for normal subjects is 1.04 ± 0.22 mg Fe/g. As shown in Figure 6a, a strong correlation ($r = 0.95$) was found between the total iron measured by (RR_2 , A) and that determined by biopsy or biosusceptometry. Strong correlations were also found between the R_2 based iron measurements and the true iron concentrations ($r = 0.88$). In contrast, the R_2^* based iron measurements were saturated for high iron overload cases (> 5 mg Fe/g), due to significant signal drop out at the earliest echo, and showed weak correlation with the true iron concentrations ($r = 0.36$). However, if these high overload cases are excluded, the correlation increases ($r = 0.80$) (data not shown).

Figure 6b shows the fractional hemosiderin iron concentration as a function of the biopsy and biosusceptometry derived total iron concentration, suggesting that the proportion of the

total storage iron existing as hemosiderin grows as the total iron concentration increases, which is consistent with the results of the previous study (30).

Comparison of R_2 and RR_2 measurements in phantoms

The aggregation index A is independent of the Mn concentrations (30), and as shown in Figure 2b, proportional to the microsphere iron concentration. The dependencies of R_2 and RR_2 on the microsphere iron concentration can be indirectly represented by their relationships with A (Figure 7a). There is no correlation between RR_2 and A ($r = 0.06$).

There are two sources of relaxations contributing to R_2 , that from the $MnCl_2$, a diffuse, soluble component, R_{2_Mn} , and that from the iron oxide microspheres, an aggregate, insoluble component, R_{2_IO} , and one may plausibly write

$$R_2 = R_{2_Mn} + R_{2_IO} \quad [11]$$

The dependency of R_2 on A ($r = 0.63$) was used to analyze the two R_2 components. By subtracting the RR_2 from R_2 , a linear curve fitting

$$Y = 0.23ms^{1/2} \times A \quad [12]$$

was found to best represent the dependency part of R_2 on A , and can be used to estimate the R_{2_IO} component in R_2 . The R_{2_Mn} can be estimated by simply subtracting the R_{2_IO} from R_2 .

As shown in Figure 7b, the R_2 values (circles) are loosely distributed due to the presence of microspheres that affect R_2 but not RR_2 . High correlations ($r = 0.99$) and excellent agreement were found between the R_{2_Mn} and RR_2 (dots), indicating that the RR_2 relaxation rate is mainly associated with the $MnCl_2$, thus equivalent to the soluble component of R_2 , the R_{2_Mn} .

Comparison of R_2 and RR_2 measurements in liver

Similarly, for iron overload liver, there are two sources of relaxations contributing to R_2 , that from the ferritin, R_{2_F} , and that from the hemosiderin, R_{2_H} , and thus

$$R_2 = R_{2_F} + R_{2_H} \quad [13]$$

The dependencies of R_2 and RR_2 on the hemosiderin iron concentrations can be estimated by their relationships with A (Figure 7c). Little correlation was found between RR_2 and A ($r = 0.17$), suggesting that the relaxation rates derived from the two forms of signal decay are independent for the iron overloaded liver. The dependent part of R_2 on A ($r = 0.82$), which is associated with the hemosiderin iron and may be estimated from the linear fit $R_{2_H} = 0.23 ms^{1/2} \times A$, was subtracted from the R_2 to calculate the R_{2_F} component.

The comparisons between R_2 versus RR_2 and R_{2_F} versus RR_2 are demonstrated in Figure 7d. As expected, the R_2 values (circles) are loosely correlated with RR_2 values ($r = 0.41$). There are two outliers of R_2 values indicated by an arrow, representing two high hepatic iron overload cases, in which the proportions of storage iron in the hemosiderin form are approximately 95%. Nevertheless, with the subtraction of R_{2_H} , excellent correlations were found between the R_{2_F} and RR_2 (dots) values ($r = 0.99$), confirming that the RR_2 relaxation rate is mainly associated with the ferritin iron, and is equivalent to the R_{2_F} component in R_2 .

Comparison of R_2 measurements using different fitting procedures

Finally, strong correlations were found between the present R_2 estimates assuming a noise level as a fitting parameter in Eq. [6] and bi-exponential R_2 measurements ($r = 0.99$) (14), and between the present and the mono-exponential R_2 measurements assuming a constant bias component ($r = 0.99$) (27), which confirmed the robustness of our fitting strategy in the presence of background signal and noise bias, and verified the improvement of the total iron measurement using the two parameters (RR_2 , A) derived from the same fitting strategy.

Discussion

The proposed MRI method allows for the separate quantification of diffuse (ferritin-like) and aggregated (hemosiderin-like) storage iron in liver. The present two-parameter (RR_2 , A) model, the modified MSE sequences that contain three different interecho times, and the parallel imaging and motion suppression gating system provided high quality images yielding accurate estimates of total as well as compositional (ferritin- and hemosiderin-like) iron concentrations in the liver. The validity and feasibility of the two-parameter method is supported by both phantom and *in vivo* human data in the present study. Consistent with the results in Ref. (30) and (39), the proportion of hepatic iron stored as ferritin, as determined by the two-parameter method, decreases as the total storage iron concentrations increase. The resulting MRI total hepatic iron measurement was highly correlated with iron concentration estimated from the biopsy or biosusceptometry methods. The ability to separately measure ferritin and hemosiderin iron may be clinically useful to evaluate patients with iron overload and to monitor the results of treatment with phlebotomy or chelation with higher specificity and sensitivity.

Note that the calibration parameters for phantoms are substantially smaller in magnitude than that for the livers, due to the fact that ferritin and hemosiderin are less efficient than $MnCl_2$ and iron oxide microspheres in causing MRI signal decay. Therefore the range of the absolute concentrations used in the phantoms is smaller than that of the iron overloaded livers, although the magnetic and relaxation effects are similar.

The established MRI iron quantification methods (12–15, 17–19, 22–24, 27, 28) provide total iron estimates, but do not separately determine ferritin iron levels in patients with iron overload, for which ferritin may constitute only a fraction of the total iron concentration. In our phantom study, varying the proportion of Mn and microspheres in phantom mixtures of different C_T may yield the same estimates for total iron using the single-parameter (R_2 and R_2^*) approach. The variability in the relative fraction of iron in dispersed ferritin and clustered hemosiderin may explain, at least in part, the curvilinear relationship between R_2 and liver iron concentration in Ref. (24). Compared to the present two-parameter method, the single-parameter methods were less strongly correlated with the total iron concentration.

It should be noted that the two R_2 components, R_{2_F} and R_{2_H} , can be determined empirically by removing the dependencies of R_2 on the parameter A using the two-parameter method. The RR_2 correlated excellently with the R_{2_F} , indicating that it is mainly associated with the dispersed iron. This is validated by the phantom study, which further confirmed the capability of the two-parameter method to separately measure the two forms of storage iron, ferritin and hemosiderin, using the RR_2 and A parameters. The conventional R_2 depends strongly on the interecho time when hemosiderin iron is presented, and it alone is not able to quantify the ferritin and hemosiderin iron separately.

Motion artifacts, noise bias, slowly varying components from blood and bile, and partial volume effects can contribute to non-monoexponential signal decay, and are therefore potential confounding factors for our two-parameter method. ECG triggering and respiratory

navigator gating can be used to effectively correct for the cardiac and respiratory motion. Recently, a breath-hold multi-echo fast spin-echo (FSE) strategy (40) was proposed for measurement of R_2 in the liver and heart, which reduced imaging time to a breath-hold duration of about 20 s. When calculating iron concentration in liver, the blood vessels can be excluded based on their image intensity in the MSE images. Overall, an advantage of the present MSE acquisition strategy with multiple interecho times is that it helps mitigate the confounding effects caused by the background noise and slowly decaying components, due to the specific interecho time dependency predicted by Eq. [1]. The background noise defined in the two-parameter model also reduces the estimation error. Moreover, the shifted MSE acquisition allows the first echoes to be acquired at the same time, so that the signal decay from different acquisitions can be sampled at shorter times and scaled consistently to correct for the longitudinal magnetization recovery variations or intra-scan differences, minimizing the estimation error for the relaxation parameters. As a result, the total iron quantified by the present two-parameter model showed the best correlation with the true iron concentration in both phantom and human studies *in vivo*.

In our study, the global fitting to estimate the four parameters (S_0 , RR_2 , A and σ), and the monoexponential fitting to estimate the three parameters (S_0 , R_2/R_2^* , and σ) are robust and rapid. There is no strict requirement for initial parameter estimates. The fitting and estimates can be applied to signal decay in ROI within an iron-loaded tissue, or on a pixel-by-pixel basis to map the relaxation parameters. Image acquisition with higher spatial resolution can be implemented for measuring iron concentration in small tissues or organs.

The stimulated echo effects introduced by the imperfect slice selection profile and inaccurate RF pulse flip angle, based on the vendor's default MSE implementation, resulted in an underestimation of RR_2 , and an overestimation of A . Regardless of the sources of the stimulated echoes, their effects on the estimation of RR_2 and A can be calibrated to permit accurate quantification of total as well as compositional (ferritin- and hemosiderin-like) iron concentrations. This feature demonstrates the feasibility of applying the two-parameter method in clinical settings having either the standard (default) or optimized MSE sequences. However, the variations in calibration parameters should be expected due to the variations of RR_2 and A estimated using different scanners and sequences (30). Nonetheless, although the two-parameter method appears to be robust with respect to the stimulated echo effects, the refocusing slice thickness and RF pulse flip angle should be consistent throughout a single study, and MSE sequences with minimal stimulated echo effects should be implemented across scanners for accurate R_2 -based iron quantification.

In conclusion, we report MRI measurement of dispersed (ferritin-like) and aggregated (hemosiderin-like) hepatic storage iron in patients with transfusional iron overload. This method may provide important new information about the partition of storage iron between ferritin and hemosiderin in other forms of iron overload, and could potentially improve the accuracy of conventional single-parameter (R_2 or R_2^*) techniques for hepatic iron quantification *in vivo*. MRI detection of increases in ferritin iron could potentially provide a non-invasive early indicator of expansion of the cytosolic iron pool leading to an elevated risk of iron-induced tissue toxicity in the liver, heart, and other tissues. Finally, MRI measurement of ferritin iron may offer a means to rapidly monitor the effects of treatment of iron overload by phlebotomy or administration of iron-chelating agents.

Acknowledgments

Grant Support: National Institutes of Health R01-DK069373, R01-DK066251, and R01-DK049108; American Heart Association 0730143N; Hong Kong Research Grant Council GRF HKU 7794/07M; and the St. Giles Comprehensive Sickle-Cell – Thalassemia Program.

References

1. Brittenham GM, Badman DG. Noninvasive measurement of iron: report of an NIDDK workshop. *Blood*. 2003; 101(1):15–19. [PubMed: 12393526]
2. Arosio P, Ingrassia R, Cavadini P. Ferritins: a family of molecules for iron storage, antioxidation and more. *Biochim Biophys Acta*. 2009; 1790(7):589–599. [PubMed: 18929623]
3. Chasteen ND, Harrison PM. Mineralization in ferritin: an efficient means of iron storage. *J Struct Biol*. 1999; 126(3):182–194. [PubMed: 10441528]
4. De Domenico I, Vaughn MB, Li L, et al. Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. *EMBO J*. 2006; 25(22):5396–5404. [PubMed: 17082767]
5. Iancu TC. Ferritin and hemosiderin in pathological tissues. *Electron Microsc Rev*. 1992; 5(2):209–229. [PubMed: 1581551]
6. Miyazaki E, Kato J, Kobune M, et al. Denatured H-ferritin subunit is a major constituent of haemosiderin in the liver of patients with iron overload. *Gut*. 2002; 50(3):413–419. [PubMed: 11839724]
7. Wixom RL, Prutkin L, Munro HN. Hemosiderin: nature, formation, and significance. *Int Rev Exp Pathol*. 1980; 22:193–225. [PubMed: 7005144]
8. Brittenham GM. Iron-chelating therapy for transfusional iron overload. *New Engl J Med*. 2011; 364:146–156. [PubMed: 21226580]
9. De Domenico I, Vaughn MB, Paradkar PN, Lo E, Ward DM, Kaplan J. Decoupling ferritin synthesis from free cytosolic iron results in ferritin secretion. *Cell Metab*. 2011; 13:57–67. [PubMed: 21195349]
10. Fleming RE, Ponka P. Iron Overload in Human Disease. *N Engl J Med*. 2012; 366:348–359. [PubMed: 22276824]
11. De Domenico I, Ward DM, Kaplan J. Specific iron chelators determine the route of ferritin degradation. *Blood*. 2009; 114:4546–4551. [PubMed: 19671920]
12. Bonkovsky HL, Rubin RB, Cable EE, Davidoff A, Rijcken TH, Stark DD. Hepatic iron concentration: noninvasive estimation by means of MR imaging techniques. *Radiology*. 1999; 212(1):227–234. [PubMed: 10405746]
13. Clark PR, Chua-Anusorn W, St Pierre TG. Proton transverse relaxation rate (R2) images of liver tissue; mapping local tissue iron concentrations with MRI [corrected]. *Magn Reson Med*. 2003; 49(3):572–575. [PubMed: 12594762]
14. Clark PR, Chua-anusorn W, St Pierre TG. Bi-exponential proton transverse relaxation rate (R2) image analysis using RF field intensity-weighted spin density projection: potential for R2 measurement of iron-loaded liver. *Magn Reson Imaging*. 2003; 21(5):519–530. [PubMed: 12878262]
15. Clark PR, St Pierre TG. Quantitative mapping of transverse relaxivity (1/T(2)) in hepatic iron overload: a single spin-echo imaging methodology. *Magn Reson Imaging*. 2000; 18(4):431–438. [PubMed: 10788721]
16. Ernst O, Sergeant G, Bonvarlet P, Canva-Delcambre V, Paris JC, L'Hermine C. Hepatic iron overload: diagnosis and quantification with MR imaging. *AJR Am J Roentgenol*. 1997; 168(5):1205–1208. [PubMed: 9129412]
17. Gandon Y, Olivie D, Guyader D, et al. Non-invasive assessment of hepatic iron stores by MRI. *Lancet*. 2004; 363(9406):357–362. [PubMed: 15070565]
18. Jensen PD. Evaluation of iron overload. *Br J Haematol*. 2004; 124(6):697–711. [PubMed: 15009057]
19. Pell GS, Briellmann RS, Waites AB, Abbott DF, Lewis DP, Jackson GD. Optimized clinical T2 relaxometry with a standard CPMG sequence. *J Magn Reson Imaging*. 2006; 23(2):248–252. [PubMed: 16416434]
20. Pierre TS, Chan P, Bauchspeiss K, et al. Synthesis, Structure and Magnetic Properties of Ferritin Cores with Varying Composition and Degrees of Structural Order: Models for Iron Oxide Deposits in Iron-Overload Diseases. *Coordination Chemistry Reviews*. 1996; 151:125–143.
21. Poon CS, Henkelman RM. Practical T2 quantitation for clinical applications. *J Magn Reson Imaging*. 1992; 2(5):541–553. [PubMed: 1392247]

22. St Pierre TG, Clark PR, Chua-Anusorn W. Single spin-echo proton transverse relaxometry of iron-loaded liver. *NMR Biomed.* 2004; 17(7):446–458. [PubMed: 15523601]
23. St Pierre TG, Clark PR, Chua-Anusorn W. Measurement and mapping of liver iron concentrations using magnetic resonance imaging. *Ann N Y Acad Sci.* 2005; 1054:379–385. [PubMed: 16339686]
24. St Pierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood.* 2005; 105(2):855–861. [PubMed: 15256427]
25. Storey P, Thompson AA, Carqueville CL, Wood JC, de Freitas RA, Rigsby CK. R2* imaging of transfusional iron burden at 3T and comparison with 1.5T. *J Magn Reson Imaging.* 2007; 25(3): 540–547. [PubMed: 17326089]
26. Thomsen C, Wiggers P, Ring-Larsen H, et al. Identification of patients with hereditary haemochromatosis by magnetic resonance imaging and spectroscopic relaxation time measurements. *Magn Reson Imaging.* 1992; 10(6):867–879. [PubMed: 1461084]
27. Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood.* 2005; 106(4):1460–1465. [PubMed: 15860670]
28. Wood JC, Ghugre N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. *Hemoglobin.* 2008; 32(1–2):85–96. [PubMed: 18274986]
29. Jensen JH, Chandra R. Theory of nonexponential NMR signal decay in liver with iron overload or superparamagnetic iron oxide particles. *Magn Reson Med.* 2002; 47(6):1131–1138. [PubMed: 12111959]
30. Jensen JH, Tang H, Tosti CL, et al. Separate MRI quantification of dispersed (ferritin-like) and aggregated (hemosiderin-like) storage iron. *Magn Reson Med.* 2010; 63(5):1201–1209. [PubMed: 20432291]
31. Williams CF, Redpath TW, Smith FW. The influence of stimulated echoes on contrast in fast spin-echo imaging. *Magn Reson Imaging.* 1996; 14(4):419–428. [PubMed: 8782180]
32. Sammet CL, Swaminathan SV, Tang H, et al. Measurement and correction of stimulated echo contamination in T(2)-based iron quantification. *Magn Reson Imaging.* 2012
33. Wu EX, Kim D, Tosti CL, et al. Magnetic resonance assessment of iron overload by separate measurement of tissue ferritin and hemosiderin iron. *Ann N Y Acad Sci.* 2010; 1202:115–122. [PubMed: 20712781]
34. Vymazal J, Zak O, Bulte JW, Aisen P, Brooks RA. T1 and T2 of ferritin solutions: effect of loading factor. *Magn Reson Med.* 1996; 36(1):61–65. [PubMed: 8795021]
35. Overmoyer B, McLaren C, Brittenham G. Uniformity of liver density and nonheme (storage) iron distribution. *Arch Pathol Lab Med.* 1987; 111(6):549–554. [PubMed: 3579513]
36. Brittenham GM, Sheth S, Allen CJ, Farrell DE. Noninvasive methods for quantitative assessment of transfusional iron overload in sickle cell disease. *Semin Hematol.* 2001; 38(1 Suppl 1):37–56. [PubMed: 11206960]
37. Henkelman RM. Measurement of signal intensities in the presence of noise in MR images. *Med Phys.* 1985; 12(2):232–233. [PubMed: 4000083]
38. Ulmer JL, Mathews VP, Hamilton CA, Elster AD, Moran PR. Magnetization transfer or spin-lock? An investigation of off-resonance saturation pulse imaging with varying frequency offsets. *AJNR Am J Neuroradiol.* 1996; 17(5):805–819. [PubMed: 8733952]
39. Zuyderhoudt FM, Sindram JW, Marx JJ, Jorning GG, van Gool J. The amount of ferritin and hemosiderin in the livers of patients with iron-loading diseases. *Hepatology.* 1983; 3(2):232–235. [PubMed: 6832714]
40. Kim D, Jensen JH, Wu EX, Sheth SS, Brittenham GM. Breathhold multiecho fast spin-echo pulse sequence for accurate R2 measurement in the heart and liver. *Magn Reson Med.* 2009; 62(2):300–306. [PubMed: 19526516]

Figure 1. Multiple MSE signal decay in phantoms (0.015 mg Mn/ml) without and with Fe (0.06 mg/ml). Signal intensity is in natural log scale.

Figure 2. Comparison of RR_2 and A in phantoms using the non-optimized and optimized MSE sequences. (a) Estimation of RR_2 in phantoms with various Mn concentrations (0 to 0.037 mg/ml); (b) Estimation of A in phantoms with various Fe concentrations (0 to 0.1 mg/ml) and a fixed Mn concentration (0.015 mg/ml).

Figure 3.

Estimation of Mn and Fe concentration maps from phantoms containing various Fe concentrations (0 to 0.1 mg/ml) and a fixed Mn concentration (0.015 mg/ml). (a) Phantom image; (b) Mn map; (c) Fe map; (d) C_T (Fe+Mn) map. The center phantom (red arrow) contains no Mn and Fe. The top phantom (yellow arrow) contains Mn only. The color bar represents a range of 0 to 0.12 mg Fe/ml.

Figure 4. Compositional iron concentration maps in a healthy (a–c) and an iron overload liver (d–i, 2.4mg Fe/g). (a) Normal liver; (b) Ferritin map; (c) Hemosiderin map; (d) Iron overload liver; (e) Ferritin map; (f) Hemosiderin map; (g) Total iron estimated by RR_2 , A; (h) Total iron estimated by R_2 ; (i) Total iron estimated by R_2^* . The liver ROI templates are indicated by red arrows. The color bar represents a range of 0 to 10 mg Fe/g wet weight.

Figure 5. Multiple MSE signal decay in the healthy and iron overload livers. The liver regions for acquiring the signal decays are indicated in Figure 4a and 4d.

Figure 6.

Comparison of total iron concentration between the MRI estimations using the two-parameter (RR_2 , A) method and the biopsy/biosusceptometry measurements. (a) Total iron quantification using the two-parameter method; (b) Fractional hemosiderin iron concentration (C_A/C_T).

Figure 7.

Separation of the R_2 components in phantom and in liver. (a) Phantom: the dependencies of R_2 and RR_2 on A ; (b) Phantom: comparisons between RR_2 and R_2 , and between RR_2 and R_{2_Mn} ; (c) Liver: the dependencies of R_2 and RR_2 on A ; (d) Liver: comparisons between RR_2 and R_2 , and between RR_2 and R_{2_F} . The arrow points to two high hepatic iron overload cases containing high proportions of hemosiderin iron.