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Separate MRI Quantification of Dispersed (Ferritin-like) and Aggregated (Hemosiderin-like) Storage Iron

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Abstract

A new magnetic resonance imaging (MRI) method is proposed for separately quantifying the two principal forms of tissue storage (non-heme) iron: *ferritin iron*, a dispersed, soluble fraction that can be rapidly mobilized, and *hemosiderin iron*, an aggregated, insoluble fraction that serves as a long-term reserve. The method utilizes multiple spin echo (MSE) sequences, exploiting the fact that aggregated iron can induce non-monoexponential signal decay for MSE sequences. The method is validated in vitro for agarose phantoms, simulating dispersed iron with manganese chloride and aggregated iron with iron oxide microspheres. To demonstrate feasibility for human studies, preliminary in vivo data from two healthy controls and six patients with transfusional iron overload are presented. For both phantoms and human subjects, conventional R_2 and R_2^* relaxation rates are also measured in order to contrast the proposed method with established MRI iron quantification techniques. Quantification of dispersed (ferritin-like) iron may provide a new means of monitoring the risk of iron-induced toxicity in patients with iron overload and, together with quantification of aggregated (hemosiderin-like) iron, improve the accuracy of estimates for total storage iron.

Keywords

iron overload; ferritin; hemosiderin; quantification; liver; MRI

INTRODUCTION

Iron overload may result from diseases such as hereditary hemochromatosis (1), or as a result of multiple transfusions of red cells used to treat pathologies such as the thalassemias, sickle

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cell anemia, aplastic anemia and myelosplastic syndromes (2). Non-heme tissue iron concentrations can be elevated many fold above normal levels in liver (3), and clinically significant iron deposition may also occur in other organs including the heart (4). Storage iron occurs in two principal forms: *ferritin iron*, a dispersed, soluble fraction that can be rapidly mobilized, and *hemosiderin iron*, an aggregated, insoluble fraction that serves as a long-term reserve (5). With rising body iron burden, the capacity for safe storage eventually becomes exhausted, and progressively widespread tissue damage begins to occur. The exact mechanisms responsible are incompletely understood, but a number of studies implicate increases in the small, micromolar concentrations of metabolically active iron in the cytosol, leading to oxidative damage that ultimately results in cell death, organ injury and failure (6). Although non-invasive means for direct measurement of cytosolic iron concentrations in vivo are lacking, recent investigations provide compelling evidence that iron entry and exit from ferritin are the result of an equilibrium based on the concentration of cytosolic iron (7). Thus as cytosolic iron concentrations rise, iron accumulates in ferritin as well. These observations suggest that elevated intracellular ferritin iron concentrations may provide a useful indicator of increases in the metabolically active cytosolic iron levels and of a heightened risk of iron-induced toxicity.

Established MRI methods for iron quantification have been based on the measurements of signal intensity ratios or on relaxation rates, such as R_2 and R_2^* , and are intended to estimate the total non-heme iron concentration (4,8–13). Because aggregated iron generates microscopic magnetic field inhomogeneities, which are very efficient in causing spin dephasing, such ratios and rates are, in practice, strongly influenced by hemosiderin iron and thus may not accurately reflect ferritin iron concentrations.

Here we propose a new MRI method for separately quantifying the dispersed and aggregated iron storage pools, thus yielding, in particular, an estimate for the ferritin iron concentration. The method exploits the fact that aggregated iron can induce non-monoexponential signal decay for multiple spin echo (MSE) sequences. By fitting a previously described model (14) to MSE decay data, two parameters, a “reduced relaxation rate” (RR_2) and an “aggregation index” (A), can be determined. The ferritin iron concentration is then calculated from RR_2 , and the hemosiderin concentration is calculated from A . By quantifying tissue ferritin iron, this method may provide a novel, non-invasive measure of short-term changes in cytosolic iron in patients with iron overload. Since this approach results in a more complete characterization of storage iron, it could also potentially lead to more accurate total iron estimates, as compared with methods based on a single metric which inevitably have an associated uncertainty reflecting variations in the relative amounts in the two storage iron pools. Preliminary versions of this method have been briefly described in Refs. ^{15–17}.

This article focuses on the in vitro validation of the method with phantom experiments. Initial in vivo data from human studies are presented to demonstrate the feasibility of applying the technique for hepatic iron quantification in patients with iron overload. In order to give a comparison with established MRI iron quantification methods, standard R_2 and R_2^* relaxation rates are also measured for both phantoms and human subjects.

THEORY

We consider both conventional MSE sequences, with N radio frequency (RF) 180° refocusing pulses occurring at the times $(2n - 1)\Delta t$ for $n = 1, 2, \dots, N$, as well as “shifted” MSE sequences with refocusing pulses occurring at the times $\tau, 2\tau + \Delta t, 2\tau + 3\Delta t, 2\tau + 5\Delta t$, etc. Spin echoes thus form at times $2n\Delta t$, for a conventional MSE sequence, and at times $2\tau + 2(n - 1)\Delta t$, for a shifted MSE sequence. In either case, the interecho time is $2\Delta t$, but for the shifted MSE sequence the echoes are displaced by an amount $2(\tau - \Delta t)$. Note that the shifted sequence

reduces to the conventional sequence for $\tau = \Delta t$. An important advantage of the shifted MSE sequence is that by choosing $\tau < \Delta t$ the signal decay curve can be initially sampled at shorter times, which is useful when the signal decay is rapid as occurs in tissues with high iron concentrations.

As has been previously proposed (14), we model the signal decay in iron overloaded liver by

$$S(t) = S_0 e^{-RR_2 t} \exp \left[-A^{3/4} (\Delta t)^{3/4} (t - t_s)^{3/8} \right], \quad [1]$$

where $S(t)$ is the signal amplitude at a time t , S_0 is the initial signal amplitude, RR_2 is the reduced relaxation rate, A is the aggregation index, and t_s is a time shift given by

$$t_s = 2\tau \left[1 - \left(\frac{\tau}{\Delta t} \right)^2 \right]. \quad [2]$$

This form may be regarded as the product of a monoexponential factor and a non-monoexponential factor that describes the more complex effects of diffusion in the spatially inhomogeneous field generated by aggregated iron. We emphasize that Eq. [1] only describes the signal decay at the spin echo times. Non-monoexponential MSE signal decay in liver with iron overload has been demonstrated by several prior studies (15,18–20). Note that Eq. [1] implies that the aggregation index A has dimensions of $(\text{time})^{-3/2}$, and that when $A = 0$, the signal decay predicted by Eq. [1] is monoexponential and RR_2 is then simply equal to the conventional R_2 relaxation rate.

According to our model, the effect of dispersed (ferritin-like) iron is entirely reflected in the reduced relaxation rate, while the effect of aggregated (hemosiderin-like) iron is reflected in the aggregation index. More specifically, we expect the reduced relaxation rate to depend linearly on the dispersed iron concentration according to

$$RR_2 = r_{2,D} C_D + RR_{2,0}, \quad [3]$$

where C_D is the mass of dispersed iron per tissue mass, $r_{2,D}$ is the dispersed iron relaxivity, and $RR_{2,0}$ is the reduced relaxation rate in the absence of dispersed iron. For the aggregation index, our model predicts

$$A = k C_A, \quad [4]$$

where C_A is the mass of aggregated iron per tissue mass and k is a parameter that depends on tissue properties, such as the water self-diffusion coefficient and mean aggregate spacing, as well as on the applied field strength (14). If we assume that the parameter k is approximately constant, then Eqs. [3] and [4] imply that the total iron concentration is given by

$$C_T = \alpha_1 + \alpha_2 RR_2 + \alpha_3 A, \quad [5]$$

with

$$\alpha_1 = -\frac{RR_{2,0}}{r_{2,D}}; \quad \alpha_2 = \frac{1}{r_{2,D}}; \quad \alpha_3 = \frac{1}{k}. \quad [6]$$

In addition, we have

$$C_D = \alpha_1 + \alpha_2 RR_2, \quad [7]$$

and

$$C_A = \alpha_3 A, \quad [8]$$

demonstrating explicitly the separate quantification of dispersed and aggregated iron. When comparing these MRI estimates for C_T , C_D , and C_A with independently determined values (e.g., via biosusceptometry), the MRI estimates will be distinguished by adding “MRI” to the subscript (e.g., $C_{T, MRI}$).

METHODS

Phantoms

To validate the above equations for a case with known concentrations, agarose gel phantoms were prepared with different mixtures of MnCl_2 and iron oxide microspheres designed to mimic varying amounts of ferritin and hemosiderin, as found in iron overloaded liver.

MnCl_2 was chosen to simulate dispersed iron because it has $T_1/T_2 \approx 10$ at 1.5 T (21), comparable to the T_1/T_2 ratio of aqueous ferritin solutions (22) as well as many biological tissues. Another convenient property of MnCl_2 is that the atomic weight of Mn (54.9) is very close to that of Fe (55.8) so that it is reasonable to define, in analogy to liver tissue, a phantom’s total concentration (C_T) simply as the sum of the Mn concentration (C_D) and the microsphere Fe concentration (C_A). Although the metal ion is different, for the purposes of this study MnCl_2 has the essential properties (uniform spatial distribution and appropriate T_1/T_2 ratio) to provide a reasonable model for dispersed hepatic iron. Although it is possible to prepare agarose gels with ferritin, the necessary heating procedure can cause them to be unstable (23); MnCl_2 was therefore utilized to avoid this potential confound.

The iron oxide microspheres consisted of magnetite nanoparticles embedded in a polymer matrix (product code UM3CN/5737, Bangs Laboratories, Fishers, Indiana). The spheres had a mean radius of 2.9 μm and an average of 4.4×10^{-9} mg Fe/particle, and these were intended to approximate the effect of hemosiderin aggregates, which are of similar size. We note that the absolute iron concentrations used in our phantoms are roughly 100 times smaller than hemosiderin iron concentrations in iron overloaded liver, but this is compensated by the fact that the specific susceptibility for the microspheres is about 100 times larger (at $B_0 = 1.5$ T) so that the magnetic effects of the microspheres should be similar to those of hemosiderin.

A total of 36 cylindrical sample bottles (1.5 cm in diameter and 15 cm^3 in volume) were prepared with concentrations

$$\begin{aligned} C_D &= m \times 0.0074 \text{ mg Mn/g}, & m &= 0, 1, \dots, 5, \\ C_A &= n \times 0.02 \text{ mg Fe/g}, & n &= 0, 1, \dots, 5. \end{aligned} \quad [9]$$

suspended in 2% agarose gel. The inter-particle distance for the microspheres was 35 μm for the highest iron concentrations. This length scale is comparable to the size of hepatocytes and roughly similar to spacings that separate hemosiderin aggregates in iron overloaded liver (20).

The bottles were immersed in a ~50 mM MnCl_2 aqueous solution bath (to reduce magnetic field inhomogeneities) within a 19 cm diameter cylindrical container prior to imaging. The MnCl_2 was added to the bath to suppress the bath signal so as to avoid contaminating the sample signals. For each scan, the six bottles, arranged in a hexagonal pattern, were imaged simultaneously.

Pulse Sequence—All phantoms were imaged at room temperature on a 1.5 T whole-body MR scanner (Intera, Philips Medical Systems, Best, The Netherlands) equipped with a five-channel phase array receive coil and a gradient system capable of achieving a maximum gradient strength of 33 mT/m and a slew rate of 100 T/m/s. For each pulse sequence, a 10 mm thick single transverse slice (i.e., perpendicular to bottle axes) was acquired near magnet isocenter with a field of view (FOV) of $250 \times 250 \text{ mm}^2$ and an acquisition matrix of 128×128 , resulting in an in-plane resolution of $1.95 \times 1.95 \text{ mm}^2$.

Three different MSE sequences were used, all with a first echo time at $2\tau = 4 \text{ ms}$ and a last echo at 100 ms. One MSE sequence had an interecho time of $2\Delta t = 4 \text{ ms}$ and a total of 25 echoes, one had $2\Delta t = 8 \text{ ms}$ and a total of 13 echoes, and one had $2\Delta t = 16 \text{ ms}$ and a total of 7 echoes. Slice selective refocusing pulses were used with a 180° flip angle and a duration of 0.9 ms. In order to minimize the confounding influence of stimulated echoes, the refocusing slice thickness was set to be three times the excitation slice thickness (24,25). The repetition time (TR) was 2000 ms, the parallel imaging (SENSE) acceleration factor was 2, the total image acquisition time was 182 s for each interecho time, and the excitation flip angle was 90° .

For R_2^* measurement, a 17 echo multiple gradient echo (MGE) sequence was used with a first gradient echo time occurring at 3.15 ms following the initial RF excitation and with an interecho time of 0.86 ms. Other imaging parameters were TR = 1500 ms, image acquisition time = 183 s (reduced slightly by partial phase encoding), and excitation flip angle = 50° .

These sequences were tested by measuring relaxation rates for a 0.675 mM aqueous MnCl_2 solution of MnCl_2 , prepared with distilled water to avoid impurities. The rates were determined from a monoexponential fit to the signal decay data, with only even echoes being utilized for the MSE sequences, as these are relatively insensitive to flip angle errors. The MSE sequences gave R_2 values of 49.5 ± 0.3 , 50.0 ± 0.3 , and $47.9 \pm 1.5 \text{ s}^{-1}$ for interecho times of 4, 8, and 16 ms, respectively, while the MGE sequence gave $R_2^* = 52.8 \pm 0.2 \text{ s}^{-1}$. These correspond to relaxivities of 73.3 ± 0.4 , 74.1 ± 0.4 , 71.0 ± 2.2 , and $78.2 \pm 0.3 \text{ s}^{-1}/\text{mM}$, which are close to the values of $71.4 \text{ s}^{-1}/\text{mM}$ measured with an MSE sequence by Ulmer and coworkers (21) and $74.2 \pm 0.3 \text{ s}^{-1}/\text{mM}$ measured with a single spin echo sequence by St Pierre and coworkers (26). The somewhat higher relaxivity obtained for the MGE sequence may reflect a contribution from macroscopic gradients.

Image Analysis—Signal decay data for the sample bottles were obtained by averaging the signal intensities for each echo over square regions of interest (ROI) consisting of 25 voxels. In order to correct for any variation in the recovery of the longitudinal magnetization, the MSE

decay data corresponding to the three different interecho times were rescaled so that the mean signal intensities at $t = 4$ ms were identical; these corrections were typically just a few percent of the signal. The decay data were then fit to our proposed model based on Eq. [1] in order to estimate the parameters RR_2 and A . From the MGE data, R_2^* values were obtained by fitting to a conventional monoexponential form. In addition, a conventional R_2 estimate was obtained by fitting the MSE signal decay data for the $2\Delta t = 4$ ms data to a monoexponential.

To reduce the effect of background noise on our parameter estimates, the actual functional expression fit to the data had the form

$$S_{\text{fit}} = \sqrt{S_{\text{ideal}}^2 + \sigma^2}, \quad [10]$$

where S_{ideal} is the ideal signal (for a given model) in the absence of noise and σ is the background signal. This noise correction procedure is an approximate method motivated by theoretical models of noise in magnitude images (27–30). The background signal σ was treated as a free parameter and was thus determined from the decay data. In all cases, the “signal-to-noise ratio” estimate for the first echo was greater than 150 for the MSE sequences and 20 for the MGE sequence.

For the MGE sequences, all 17 echoes were employed in the fit, but for the MSE sequences only the even echoes were used in order to minimize the effect of flip angle errors. The fits were obtained by least squares minimization based on a Numerical Recipes implementation of the Levenberg-Marquardt method with errors for the parameter estimates being derived from the covariance matrix (31). The fits for the MGE sequences had three free parameters: R_2^* , the initial signal intensity S_0 , and the background signal σ . The data for the different MSE sequences ($2\Delta t = 4, 8, 16$ ms) were fit globally to Eqs. [1] and [10] with RR_2 and A assumed to be the same for all three interecho times. Thus there were a total of four free parameters (S_0 , RR_2 , A and σ) for the fits used to determine our model parameters. In analogy with the MGE data analysis, the fits used to estimate the conventional R_2 had three free parameters (with only the even echoes being used).

Influence of Solvent on Relaxation Rate Calculations—The microspheres are supplied as aqueous suspensions containing both a surfactant (sodium dodecyl sulfate) and free polymer chains. This solvent was incorporated into the phantoms in proportion to the iron concentration and also influences MRI signal decay. To control for this, a sample of the microsphere suspension was centrifuged to separate out the solvent. A bottle with 2% agarose gel containing the solvent was then prepared, with the concentration of solvent being the same as for the phantoms with 0.1 mg Fe/g. A second bottle was also prepared with a pure 2% agarose gel. By using the MSE sequence with an interecho time of 4 ms, the R_2 values for the two bottles were found to be $23.7 \pm 0.4 \text{ s}^{-1}$ and $7.7 \pm 0.2 \text{ s}^{-1}$, respectively, leading to a net R_2 effect of $16.0 \pm 0.4 \text{ s}^{-1}$ for the solvent. The relaxation rates (i.e., RR_2 , R_2 , R_2^*) measured for the phantoms were all corrected by subtracting out this solvent contribution (in proportion to the iron concentration) so that the solvent’s effect on the phantom results would be negligible.

Calibration—The calibration parameters α_1 , α_2 , and α_3 were determined from a least squares fit of Eq. [5] to the data, using only the known (i.e., prepared) values for C_T with no input from the values of C_D or C_A for the individual samples. With this calibration, MRI estimates for the total ($C_{T, MRI}$), dispersed ($C_{D, MRI}$), and aggregated ($C_{A, MRI}$) Fe/Mn concentrations for each sample bottle were then found from Eqs. [5], [7], and [8].

Human subjects

Two normal volunteers without prior history of iron overload diseases (ages 23 and 25 yr) and six patients with thalassemia major (ages 18, 19, 23, 32, 36, and 43 yr) were recruited for this study under a protocol approved by the Columbia University Institutional Review Board. Informed consent was obtained from all subjects.

For the thalassemia subjects, the hepatic iron concentrations, as measured with superconducting quantum interference device (SQUID) biosusceptometry, were 2.97, 2.01, 2.46, 1.17, 3.09, and 2.79 mg Fe/g wet tissue. Since normal hepatic liver iron is typically in the range of 0.05 to 0.5 mg Fe/g (32), normal subjects were assumed to have iron concentrations of 0.3 mg Fe/g for data plotting purposes.

Human imaging

Human imaging utilized the same scanner and pulse sequences as for the phantom experiments, with identical slice thickness and MSE echo times. However, the FOV was $302 \times 403 \text{ mm}^2$ and the acquisition matrix was 96×128 . In addition, the interecho times for the MGE sequences were slightly different (1 ms in most cases). Both the MSE and MGE image acquisitions were performed under free breathing due to their long acquisition times. Respiratory gating based on navigator echoes and cardiac triggering was employed in order to reduce motion and flow artifacts. Image acquisition times for the MSE and MGE sequences were typically 5 to 10 min each.

Mean signal decay data were obtained for the human images by averaging the signal intensities over ROI that included the entire liver regions contained within the acquired slices. As for the phantom data, the $t = 4 \text{ ms}$ intensities were used to rescale the MSE data to correct for differences in longitudinal magnetization recovery. The in vivo signal decay data were fit in an identical manner as for the phantoms, yielding estimates for RR_2 , A , R_2 , and R_2^* , with only the even echoes being used for the MSE data.

As for the phantoms, the iron concentration calibration parameters were obtained from a least squares fit of Eq. [5], with the SQUID biosusceptometry iron concentration values used for C_T . This then allowed $C_{T, MRI}$, $C_{D, MRI}$, and $C_{A, MRI}$ to be determined for each subject.

RESULTS

Phantoms

Representative signal decay data and fits for the phantom sample bottles are shown in Fig. 1, demonstrating that the decay can be accurately modeled by Eqs. [1] and [10]. The MSE decay when no microspheres are present ($C_A = 0$) is nearly independent of the interecho time and essentially monoexponential. With microspheres present, the signal decay, in contrast, depends strongly on the interecho time and a departure from monoexponentiality is apparent.

The fitted values for RR_2 varied from 8.5 to 61.0 s^{-1} , while the fitted values for A varied from 0 to $0.103 \text{ ms}^{-3/2}$. A least squares fit of Eq. [5] to the full set of phantom results yields the calibration parameters:

$$\begin{aligned}\alpha_1 &= -(8.5 \pm 1.7) \times 10^{-3} \text{ mg Mn/g}, \\ \alpha_2 &= (8.7 \pm 0.4) \times 10^{-4} \text{ s} \cdot (\text{mg Mn/g}), \\ \alpha_3 &= 0.93 \pm 0.02 \text{ ms}^{3/2} \cdot (\text{mg Fe/g}).\end{aligned}\tag{11}$$

The corresponding values for $C_{T, MRI}$, $C_{A, MRI}$, and $C_{D, MRI}$ as functions of the actual concentrations are plotted in the left column of Fig. 2. Linear regression yields coefficients of determination of $r^2 = 0.99, 0.99$, and 0.96 , respectively, demonstrating a high correlation between predicted and true concentrations when our model is used.

Alternatively, the conventional relaxation rate parameters R_2 (middle column of Fig. 2) and R_2^* (right column of Fig. 2) are not all simple functions of C_T , C_A , and C_D . The linear regression coefficients of determination for R_2 are $r^2 = 0.60, 0.26$, and 0.72 , indicating that this parameter is only moderately correlated with the concentrations, while for R_2^* , the corresponding coefficients of determination are $r^2 = 0.92, 0.99$, and 0.005 , indicating a high correlation with the total and aggregated concentration but a poor correlation with the dispersed concentration. Clearly, neither conventional relaxation rate can be used to accurately estimate separate concentrations for both the aggregated and dispersed components.

Human studies

Mean signal decay and the corresponding fits for a normal subject and a patient are shown in Fig. 3. For the patient, a departure from monoexponential decay can be detected, even well above the noise level. As for the phantom results, the data can be accurately modeled with Eqs. [1] and [10].

Figure 4 demonstrates the image quality obtainable in liver with our MSE sequences. For most subjects, breathing artifacts were largely eliminated by the use of navigator echoes and respiratory gating. Also shown in Fig. 4 are the corresponding parametric maps for RR_2 and A . For the group of subjects studied, these maps were relatively uniform within the liver, with the exception of features associated with vasculature and, in some cases, imaging artifacts. This demonstrates that the imaging method has a sufficiently high signal-to-noise ratio to allow for the voxel-by-voxel fitting to our model (at least for the iron levels considered). However, the quantitative results discussed below were obtained by first averaging the signal over the entire liver slice prior to fitting to Eqs. [1] and [10] rather than simply averaging the parametric maps. The former approach has the important advantage of allowing the quality of the nonlinear fits to be carefully assessed, which is more difficult to do on a voxel-by-voxel basis.

The fitted values for RR_2 varied from 17.0 to 44.5 s^{-1} , and the fitted values for A varied from 8.4×10^{-4} to $0.142 \text{ ms}^{-3/2}$. These are very similar to the ranges for RR_2 and A found for the phantoms, confirming that the phantoms provided reasonable models for MSE signal decay in iron overloaded liver.

A least squares fit of Eq. [5] to the full set of human results for RR_2 and A obtained from the mean liver signal decay data yields the calibration parameters:

$$\begin{aligned}\alpha_1 &= -(0.54 \pm 0.61) \text{ mg Fe/g}, \\ \alpha_2 &= (5.7 \pm 2.0) \times 10^{-4} \text{ s} \cdot (\text{mg Fe/g}), \\ \alpha_3 &= 18 \pm 4 \text{ ms}^{3/2} \cdot (\text{mg Fe/g}).\end{aligned}\quad [12]$$

These are substantially larger in magnitude than for the phantoms, reflecting the fact that ferritin and hemosiderin are less efficient than MnCl_2 and iron oxide microspheres in causing MRI signal decay, although this was compensated for in the experimental design by using lower concentrations for the phantoms. We emphasize that the units in Eq. [12] refer to wet tissue weight.

The dependences of R_2 , R_2^* , $C_{T, MRI}$ on the total hepatic iron concentration, as determined by SQUID biosusceptometry, are shown in Fig. 5 along with the linear regression lines. The

coefficients of determination are similar in all three cases and indicate a good correlation with total iron. Also shown in Fig. 5 is a plot of the fractional ferritin concentration (i.e., $C_{D, MRI}/C_{T, MRI}$) versus C_T , which suggests that the proportion of iron in the form of ferritin decreases with increasing total iron. Qualitatively similar behavior has been reported by Zuyderhoudt and coworkers (33) in a study where ferritin and hemosiderin concentrations were separately measured for biopsy samples from iron overload patients.

DISCUSSION

Iron-free ferritin (apoferritin; Mr 440,000) consists of 24 protein subunits arranged in a spherical shell that can sequester up to 4,500 atoms in a hydrous ferric oxide mineral ferrihydrite core. Both iron release and incorporation into ferritin seem to be intrinsic, autonomous properties of the molecule (5). With low levels of cytosolic iron, soluble iron-containing ferritin is present in the cytosol as randomly dispersed ferritin particles. With increasing cytosolic iron, the concentrations of dispersed ferritin increase and small clusters of ferritin particles appear, still soluble and spread throughout the cytosol. With further increases in cytosolic iron, ferritin is collected in lysosomes by fusion of ferritin clusters with lysosomal membranes, by autophagocytosis, or both. Digestion of ferritin within secondary lysosomes (siderosomes) leads to denaturation of ferritin protein subunits and to aggregation of the ferritin iron cores, processes thought to result in the formation of amorphous, insoluble masses of hemosiderin. Production of hemosiderin, enclosed within siderosome membranes, seems to sequester the excess iron away from the cytosol and protect against iron toxicity. Hemosiderin characteristically has a much slower cellular turnover than ferritin. Overall, the available evidence suggests that the metabolically active cytosolic iron is in rapid equilibrium with soluble, dispersed ferritin for short-term storage while exchange with the long-term aggregates of iron within hemosiderin is both sluggish and limited.

While established MRI iron quantification methods (4,8–13) provide estimates for total iron concentrations, they cannot be expected to accurately quantify ferritin iron levels, particularly in patients with high iron levels where the ferritin can be only a small fraction of the total iron concentration. The method proposed here, in contrast, explicitly provides estimates for both ferritin and hemosiderin iron. This is of potential clinical significance, as the above considerations suggest that ferritin iron may be a better indicator of cellular/tissue toxicity.

We have validated our technique for phantoms consisting of agarose suspensions containing $MnCl_2$ to simulate dispersed ferritin iron and iron oxide microspheres to simulate aggregated hemosiderin iron. The MRI estimates were highly correlated with the true concentrations. In R_2 nor R_2^* , which are addition, neither, conventionally used for iron quantification, showed a high correlation with the $MnCl_2$ concentration, indicating a relative insensitivity to the dispersed component. This is most probably a result of an enhanced efficiency, due to the generation of magnetic field inhomogeneities, for aggregated iron in causing MRI signal dephasing. A similar enhanced relaxation efficiency of hemosiderin compared to ferritin also likely occurs in vivo for iron overloaded liver.

For in vivo liver iron quantification, we have given a preliminary test of feasibility. Our in vivo results are qualitatively similar to those for the phantoms, although comprehensive validation was not possible with a limited number of subjects and due to the fact that individual ferritin and hemosiderin concentrations were not known. In particular, the values for the calibration parameters given by Eq. [12] should be regarded as provisional. Nonetheless, the observation shown in Fig. 5d that the fractional ferritin concentration decreases with increasing total iron, in qualitative consistency with prior studies (33), is encouraging.

There are two technical features of the method described here that we view as critical for accurate quantification. The first is the increased slice thickness for the refocusing pulses to reduce stimulated echo effects, as has been discussed in detail elsewhere (24,25). The incorporation of this feature is a key distinction relative to the related methods used in the prior work of Refs. 15–17. Second, by using two or more MSE sequences with different interecho times, the possibility of obtaining spurious results due to partial volume effects, which can also cause non-monoexponential signal decay, is greatly diminished, due to the specific interecho time dependence predicted by Eq. [1]. Such partial volume effects could occur because of signal from blood or bile.

Two helpful, but less critical, features are the use of shifted MSE sequences and the restriction of the MSE fits to only even echoes. One advantage of the shifted MSE sequences is that they allow the signal decay to be sampled at shorter times. Additionally, having the first echo for each MSE sequence occur at the same time provides a convenient means of consistently scaling the signal intensities and of assessing quality issues such as those that arise from subject motion. Limiting the fits to only the even echoes reduces the influence of flip angle errors, which tend to be less for even echoes, on the parameter estimations.

Although our fitting procedure involved four free parameters (S_0 , RR_2 , A and σ) compared to three (S_0 , R_2 or R_2^* , and σ) for a standard monoexponential fit of a single decay curve, the fits were robust due to the simultaneous fitting of all three decay curves with the same set of four parameters. In all the fits used for Figs. 2 and 5, convergence using the Levenberg-Marquardt method was rapid and consistently yielded a single solution with no significant sensitivity to the initial parameter estimates.

In order to reduce the effects of motion artifacts, respiratory gating was employed for human imaging, which resulted in relatively long acquisition times. However, it should be possible to substantially reduce the acquisition time in future studies with the application of a recently developed breathhold fast spin echo MSE sequence (34).

It is important to bear in mind that our approach relies on several idealizations. First, the analytic form of Eq. [1] is an approximation (14). In particular, the derivation of Eq. [1] assumes that the RF refocusing pulses have an infinitesimal duration, while the actual refocusing pulses have durations of several milliseconds. This is a potential source of systematic errors, especially when the signal decay during the refocusing pulse duration is significant, as may be the case for high iron concentrations. The underlying theory also assumes perfect RF pulse flip angles, and inaccuracies may arise in practice from flip angle errors, which can generate stimulated echoes, even though our sequences were optimized to reduce this effect. Another assumption is that the typical spacing, L , between iron aggregates should be greater than or comparable to the diffusion length scale $\sqrt{D\Delta t}$, where D is the water diffusion coefficient. For liver with our choice of interecho times, this means that L should be roughly 1 to 2 μm or larger. The effect of microscopic field inhomogeneities that vary on a scale much smaller than this will be diffusionally averaged and contribute to RR_2 rather than A . As discussed by Ghugre and coworkers (20), the spatial pattern of hemosiderin deposition in iron overloaded liver is complex with multiple apparent length scales. However, a principal length scale roughly corresponds to the size of the hepatocytes, which is 20 to 30 μm and hence consistent with our observation of significant contributions to A .

That the interecho time thus sets an effective resolution for our method implies that both RR_2 and A may vary somewhat with the choice of interecho times, as has been noted for A in prior studies (14,20). As a consequence, both the accuracy of the method and the values for the calibration parameters may depend on the details of MSE sequence timings, which should be considered if comparing and interpreting RR_2 and A values obtained using different sets of

interecho times. We emphasize that this interecho dependence for A is not included in our fitting procedure for the MSE data; as a consequence, the fitted values for A represent a type of average for the range of interecho times employed.

As proposed by Vymazal and coworkers for brain iron (35), an alternative to the approach presented here is to use the longitudinal relaxation rate R_1 to quantify diffuse iron. However, in contrast to RR_2 , R_1 depends nonlinearly on the ferritin loading factor (i.e., the number of iron atoms per ferritin molecule), which means that R_1 is a function of the ferritin loading factor, as well as the ferritin iron concentration (36).

The primary motivation for developing a method for separately quantifying the diffuse and aggregated iron storage pools is the potential clinical importance, as discussed above, of separately estimating tissue ferritin iron concentrations in vivo with MRI. Our method may also lead to more accurate quantification for total non-heme tissue iron than established MRI techniques, because the latter typically rely on a single MRI-measurable parameter that is affected differently by the two forms of iron. Hence, two iron overload patients with the same total iron concentrations but different relative proportions of ferritin and hemosiderin iron could yield significantly different MRI estimates for total iron using conventional approaches. Variability in the relative proportions is thus an effective source of measurement noise. Our method, in contrast, should be more robust with respect to differences in ferritin/hemosiderin ratios, since each component is individually characterized. Our method, however, may be difficult to apply to some patients with very high hepatic iron concentrations due to rapid decay of the MSE signal.

CONCLUSION

In this initial study, we have proposed a method for separate quantification of diffuse and aggregated storage iron and have provided an initial validation with phantom experiments. Our in vivo results demonstrate the feasibility of applying this method in liver, although our sample size is too small to reliably assess the method's accuracy for human studies. Further validation will compare MRI estimates for the two iron pool concentrations with tissue estimates obtained directly from liver explants. While this paper has focused on hepatic iron quantification, our method could also be applied to iron quantification for other organs, such as heart and pancreas, which are frequently affected in iron overload disorders.

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References

1. Batts KP. Iron overload syndromes and the liver. *Mod Pathol* 2007;20:S31–39. [PubMed: 17486050]
2. Porter JB. Concepts and goals in the management of transfusional iron overload. *Am J Hematol* 2007;82:1136–1139. [PubMed: 17968973]
3. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. *N Engl J Med* 2000;343:327–331. [PubMed: 10922422]
4. Jensen PD. Evaluation of iron overload. *Br J Haematol* 2004;124:697–711. [PubMed: 15009057]
5. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1996;1275:161–203. [PubMed: 8695634]

6. Le Lan C, Loréal O, Cohen T, Ropert M, Glickstein H, Pouchard M, Deugnier Y, Le Treut A, Breuer W, Cabantchik ZI, Brissot P. Association between hepatic damage, iron overload and incidence of redox-active iron in the plasma. *Blood* 2005;105:4527–4531. [PubMed: 15671444]
7. Bou-Abdallah F, Zhao G, Mayne HR, Arosio P, Chasteen ND. Origin of the unusual kinetics of iron deposition in human H-chain ferritin. *J Am Chem Soc* 2005;127:3885–3893. [PubMed: 15771525]
8. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;22:2171–2179. [PubMed: 11913479]
9. Gandon Y, Olivie D, Guyader D, Aubé C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. *Lancet* 2004;363:357–362. [PubMed: 15070565]
10. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855–861. [PubMed: 15256427]
11. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005;106:1460–1465. [PubMed: 15860670]
12. Alustiza JM, Castiella A, De Juan MD, Emparanza JJ, Artetxe J, Uranga M. Iron overload in the liver diagnostic and quantification. *Eur J Radiol* 2007;61:499–506. [PubMed: 17166681]
13. Wood JC, Ghugre N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. *Hemoglobin* 2008;32:85–96. [PubMed: 18274986]
14. Jensen JH, Chandra R. Theory of nonexponential NMR signal decay in liver with iron overload or superparamagnetic iron oxide particles. *Magn Reson Med* 2002;47:1131–1138. [PubMed: 12111959]
15. Tang H, Jensen JH, Sheth S, Swaminathan S, Azabagic A, Tosti C, Brittenham GM, Brown TR. MR measurement of ferritin and hemosiderin iron in patients with iron overload. *Proc Intl Soc Mag Reson Med* 2005;13:1880.
16. Sheth S, Tang H, Jensen JH, Altmann K, Prakash A, Printz BF, Hordof AJ, Tosti CL, Azabagic A, Swaminathan S, Brown TR, Olivieri NF, Brittenham GM. Methods for noninvasive measurement of tissue iron in Cooley's anemia. *Ann N Y Acad Sci* 2005;1054:358–372. [PubMed: 16339684]
17. Tosti CL, Tang H, Jensen JH, Sheth S, Swaminathan SV, Hultman K, Azabagic A, Altmann K, Prakash A, Printz BF, Brown AL, Hordof AJ, Olivieri NF, Brittenham GM, Brown TR. MR estimation of myocardial ferritin iron and hemosiderin iron in thalassemia major. *Proc Intl Soc Mag Reson Med* 2006;14:1201.
18. Thomsen C, Wiggers P, Ring-Larsen H, Christiansen E, Dalhoj J, Henriksen O, Christoffersen P. Identification of patients with hereditary haemochromatosis by magnetic resonance imaging and spectroscopic relaxation time measurements. *Magn Reson Imaging* 1992;10:867–879. [PubMed: 1461084]
19. Bulte JW, Miller GF, Vymazal J, Brooks RA, Frank JA. Hepatic hemosiderosis in non-human primates: quantification of liver iron using different field strengths. *Magn Reson Med* 1997;37:530–536. [PubMed: 9094074]
20. Ghugre NR, Coates TD, Nelson MD, Wood JC. Mechanisms of tissue-iron relaxivity: nuclear magnetic resonance studies of human liver biopsy specimens. *Magn Reson Med* 2005;54:1185–1193. [PubMed: 16215963]
21. 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;17:805–819. [PubMed: 8733952]
22. Vymazal J, Zak O, Bulte JW, Aisen P, Brooks RA. T1 and T2 of ferritin solutions: effect of loading factor. *Magn Reson Med* 1996;36:61–65. [PubMed: 8795021]
23. Gossuin Y, Burtea C, Monseux A, Toubeau G, Roch A, Muller RN, Gillis P. Ferritin-induced relaxation in tissues: an in vitro study. *J Magn Reson Imaging* 2004;20:690–696. [PubMed: 15390148]
24. 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;23:248–252. [PubMed: 16416434]

25. Swaminathan SV, Tosti CL, Hultman K, Jensen JH, Nunez A, Wu EX, Sheth S, Brittenham GM, Brown TR. Validation of clinically useful fast, multi slice, multi echo T2 quantification sequence. *Proc Intl Soc Mag Reson Med* 2007;15:39.
26. St Pierre TG, Clark PR, Chua-Anusorn W. Single spin-echo proton transverse relaxometry of iron-loaded liver. *NMR Biomed* 2004;17:446–458. [PubMed: 15523601]
27. Henkelman RM. Measurement of signal intensities in the presence of noise in MR images. *Med Phys* 1985;12:232–233. [PubMed: 4000083]
28. McGibney G, Smith MR. An unbiased signal-to-noise ratio measure for magnetic resonance images. *Med Phys* 1993;20:1077–1078. [PubMed: 8413015]
29. Sijbers J, den Dekker AJ, Van Audekerke J, Verhoye M, Van Dyck D. Estimation of the noise in magnitude MR images. *Magn Reson Imaging* 1998;16:87–90. [PubMed: 9436952]
30. Koay CG, Basser PJ. Analytically exact correction scheme for signal extraction from noisy magnitude MR signals. *J Magn Reson* 2006;179:317–322. [PubMed: 16488635]
31. Press, WH.; Teukolsky, SA.; Vetterling, WT.; Flannery, BP. *Numerical recipes in C: the art of scientific computing*. New York: Cambridge University Press; 1992.
32. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood* 1997;89:739–761. [PubMed: 9028304]
33. 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;3:232–235. [PubMed: 6832714]
34. Kim D, Jensen JH, Wu EX, Sheth SS, Brittenham GM. Breathhold multiecho fast spin-echo pulse sequence for accurate R_2 measurement in the heart and liver. *Magn Reson Med* 2009;62:300–306. [PubMed: 19526516]
35. Vymazal J, Urgosik D, Bulte JW. Differentiation between hemosiderin- and ferritin-bound brain iron using nuclear magnetic resonance and magnetic resonance imaging. *Cell Mol Biol (Noisy-le-grand)* 2000;46:835–842. [PubMed: 10875444]
36. Brooks RA, Vymazal J, Goldfarb RB, Bulte JW, Aisen P. Relaxometry and magnetometry of ferritin. *Magn Reson Med* 1998;40:227–235. [PubMed: 9702704]

Fig. 1.

Semilogarithmic graph of representative MSE signal decay data (even echoes only) and fits for phantoms consisting of bottles with agarose gels. The data shown are all from bottles having $C_D = 0.0074$ mg Mn/g. The decay for the bottle with $C_A = 0$ (open symbols) is nearly monoexponential and independent of the interecho time. The decay for the bottle having $C_A = 0.04$ mg Fe/mg (solid symbols) is well fit by the form of Eqs. [1] and [10] with $A = 0.038 \pm 0.001$ ms^{-3/2}, indicating a significant degree of non-monoexponentiality and interecho time dependence.

Fig. 2.

MRI measurements for phantoms as functions of C_T , C_A , and C_D together with linear regression lines. **(a)** $C_{T, MRI}$ found from Eq. [5] with the parameters α_1 , α_2 , α_3 chosen to minimize the residual sum of squares with respect to C_T , resulting in a coefficient of determination of $r^2 = 0.99$. With the same set of calibration parameters, a high correlation is also found between **(b)** $C_{A, MRI}$ and C_A and between **(c)** $C_{D, MRI}$ and C_D , demonstrating the ability of our model to separately quantify the aggregated and dispersed components. In comparison, **(d–f)** R_2 and **(g–i)** R_2^* are significantly less strongly correlated with the known concentrations (with the exception of R_2^* with respect to C_A).

Fig. 3. Semilogarithmic graph of representative MSE signal decay data (even echoes only) and fits for human subjects. The decay for a patient with $C_T = 2.0$ mg Fe/g wet tissue (solid symbols) has a significant degree of non-monoexponentiality and interecho time dependence ($A = 0.036 \pm 0.003 \text{ ms}^{-3/2}$), while the decay for a normal subject (open symbols) is close to monoexponential ($A = 0.0008 \pm 0.0003 \text{ ms}^{-3/2}$).

Fig. 4.

Image and parametric maps for a patient with $C_T = 2.79$ mg Fe/g. The image quality attainable with our pulse sequence is indicated by an unprocessed image with TE = 4 ms (truncated to highlight liver). Breathing artifacts are minimal due to the use of navigator echoes and respiratory gating, although some Gibbs ringing artifacts are apparent. This image quality supports the calculation of parametric maps for A and RR_2 (color overlays), which may be of interest for assessing the spatial uniformity of the liver iron concentration. However, the main results reported in this paper were obtained by first averaging the signal over the entire liver prior to fitting to Eqs. [1] and [10]. The calibration bar for the RR_2 map is in units of s^{-1} , and the calibration bar for the A map is in units of $ms^{-3/2}$.

Fig. 5.

(a) R_2 , (b) R_2^* and (c) $C_{T,MRI}$ as functions of C_T for six iron overload patients and two normal subjects, where C_T was measured with SQUID biosusceptometry for patients and assumed to be 0.3 mg Fe/g for normals. The lines are least squares fit to the data. For all three metrics, a high linear correlation is observed, supporting their utility for quantifying total hepatic iron concentration. Also shown is (d) the ratio $C_{D,MRI}/C_{T,MRI}$ versus C_T . The decrease of this ratio with increasing total iron concentration is qualitatively consistent with prior studies based on liver biopsy samples (33). In (a), (b) and (c), the data points for the normal subjects nearly coincide.