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Assessment of MR-Based R_2^* and Quantitative Susceptibility Mapping for the Quantification of Liver Iron Concentration in a Mouse Model at 7T

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Abstract

Purpose—To assess the feasibility of quantifying liver iron concentration (LIC) using R_2^* and quantitative susceptibility mapping (QSM) at a high field strength of 7T.

Methods—Five different concentrations of Fe-dextran were injected into twelve mice to produce various degrees of liver iron overload. After the mice were sacrificed, blood and liver samples were harvested. Ferritin ELISA assay and inductively coupled plasma mass spectrometry were performed to quantify serum ferritin concentration and LIC. Multi-echo gradient echo MRI was conducted to estimate R_2^* and the magnetic susceptibility of each liver sample through complex non-linear least squares fitting and a morphology enabled dipole inversion method, respectively.

Results—Average estimates of serum ferritin concentration, LIC, R_2^* , and susceptibility all show good linear correlations with injected Fe-dextran concentration; however, the standard deviations in the estimates of R_2^* and susceptibility increase with injected Fe-dextran concentration. Both R_2^* and susceptibility measurements also show good linear correlations with LIC ($R^2=0.78$ and $R^2=0.91$, respectively), and a susceptibility-to-LIC conversion factor of 0.829 ppm/(mg/g wet) is derived.

Conclusion—The feasibility of quantifying LIC using MR-based R_2^* and QSM at a high field strength of 7T is demonstrated. Susceptibility quantification, which is an intrinsic property of tissues and benefits from being field strength independent, is more robust than R_2^* quantification in this ex vivo study. A susceptibility-to-LIC conversion factor is presented that agrees relatively well with previously published QSM derived results obtained at 1.5T and 3T.

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Keywords

liver iron concentration; quantitative susceptibility mapping; R_2^* mapping; liver iron overload; mouse model

Introduction

The liver is the primary organ for iron storage. However, humans do not have physiologically regulated means of excreting excess iron from the body, and therefore, accumulation of iron or iron overload occurs in the liver when the body absorbs more iron than it physiologically needs and naturally excretes. Chronic blood transfusions, which are often necessary for patients with diseases such as sickle cell anemia, thalassemia, and various cancers, are the most common cause of liver iron overload and may lead to high levels of liver iron concentration (LIC) (1–6), and although less common, diseases such as hereditary hemochromatosis, hepatitis C, alcoholic liver disease, and cirrhosis may also lead to high levels of LIC (1,7,8). LIC has been shown to correlate well with total body iron content (1,9,10), but not only does excessive iron in the body lead to adverse effects in the liver, it may also lead to heart complications (1,11,12). Removing excess iron from the body using iron chelation treatments is possible (1,13–15); however, methods to non-invasively monitor these treatments are highly desirable to ensure over- or under-treatment does not occur and healthy iron levels are maintained.

Despite its invasive nature and predisposition to large variations, the current reference standard for the quantification of LIC is liver biopsy (1,7). Many non-invasive medical imaging technologies, such as ultrasound and computed tomography, are either unable to quantify LIC or suffer from the use of ionizing radiation. However, magnetic resonance imaging (MRI) is a non-invasive imaging technique that has proven to be highly sensitive to the presence of iron and doesn't suffer from the same radiation concerns.

Many MR-based methods for quantifying LIC have been proposed, including signal ratio (1,16–18), relaxometry (1,19–25), and susceptometry (1,9,26–34) techniques. Although longitudinal relaxation techniques have been proposed (12,25), the transverse relaxation rate (R_2) and effective transverse relaxation rate (R_2^*) have become the most widely adopted measures of LIC, as they are more sensitive to increases in iron concentration (1,12,25). MRI spin echo protocols used to acquire datasets for R_2 quantification are often very time consuming, and contradictory linear and non-linear relationships between R_2 and LIC have been reported (20,22,24,35–38). This limits the feasibility of quantifying LIC in vivo unless a respiratory motion correction is applied, and the inconsistency among reports is concerning. Advantageously, gradient echo protocols used to quantify R_2^* are generally much shorter, and the relationship between R_2^* and LIC has consistently been shown to be linear (19–23,35). To accurately quantify R_2^* in the liver though, many factors need to be considered and accounted for, such as, the presence of fat and its spectral multi-peak complexity (39–44), phase errors arising due to eddy currents (45), and static field inhomogeneity (43,46–48). Many different methods have been developed to account for

these factors; however, reproducibility across methods, so far, has been poor, as many conflicting R_2^* -to-LIC conversion factors (slope of the best-fit line) have been reported (19–21,23,35). The dependence of R_2^* on the static magnetic field strength also must be considered if a standardized R_2^* -to-LIC conversion factor is to be derived.

Magnetic susceptibility is an inherent property of tissues that, unlike R_2^* , is independent of the magnetic field strength and, similar to R_2^* , increases with increasing LIC.

Superconducting quantum interference devices are able to accurately measure magnetic susceptibility in the liver (9,26,28,29), and LIC quantification using these devices has been shown to correlate well with biopsy LIC measurements (29). However, the operation of these devices is expensive, and their availability is extremely limited. MRI, being widely available and significantly more cost effective, can also be used to quantify magnetic susceptibility through a procedure known as quantitative susceptibility mapping (QSM), which can be performed on the same 3D gradient echo datasets acquired to quantify R_2^* .

Recently, linear relationships between magnetic susceptibility obtained using QSM and LIC measurements have been reported (27,49,50), and a good linear correlation between susceptibility measurements obtained using QSM and a superconducting quantum interference device has been observed (26). However, studies comparing LIC measurements and liver susceptibility measurements obtained using QSM have mostly been performed at low field strengths (e.g. 1.5T and 3T), and the reproducibility of QSM liver susceptibility quantification has not been studied. This raises the question as to whether or not LIC quantification using QSM is reproducible and whether or not a standardized, field independent susceptibility-to-LIC conversion factor can be derived.

In this paper, the feasibility of detecting varying degrees of iron overload and quantifying LIC using R_2^* and QSM at a high field strength of 7T using a mouse model is evaluated and compared to previously published results obtained for human studies performed at lower magnetic field strengths. Previously, only relaxometry methods have been evaluated using a mouse model of liver iron overload (51,52), and to the best of the authors' knowledge, the work presented here is the first to use a mouse model for the assessment of liver iron overload using QSM.

Methods

Animal Preparation and Iron Overloading

All animal experiments were conducted in accordance with the University of Georgia Animal Care and Use Committee guidelines and the NIH Guide for the Care and Use of Laboratory Animals. Six-week-old female Balb/C mice were housed in Innovive Disposable Micro-Isolators in a room maintained at 20 ± 1 °C with 12-hour light and dark cycles. Diet and water were available *ad libitum*. Twelve mice were randomly assigned into six groups (n=2 per group) to receive various degrees of iron overloading based on a dextran model (53,54), which has been shown to cause Kupffer macrophages to be loaded with phagocytized iron dextran (14,55,56). A dextran model was chosen as pharmacological

(parenteral), opposed to dietary (enteral), overload with iron dextran may delay hepcidin activation (53), and parenteral administration is more easily performed in comparison to gene manipulation models (57–59). Mice have also been shown to be able to tolerate high doses of parenterally administered iron dextran (54), and parenteral administered iron dextran is processed similarly to hemoglobin iron, which has been shown not to cause cardiac dysfunction or pathological ventricular remodeling in mice (60).

Groups 1-5 were injected with concentrations of 150, 100, 75, 50, and 25 mg/kg (Fe/mouse mass), respectively, of dextran solutions (200 μ L) on Day 1 using single tail vein injections, while Group 6 received saline. The mice were then started on iron-deficient powder diets *ad libitum* and were monitored for one week to ensure iron overload levels remained constant based on serum ferritin concentration measurements. On day 8, mice were euthanized by CO₂ overdose, and the livers were harvested, rinsed with fresh phosphate-buffered saline, blotted dry with Whatman filter paper, and then weighed. The livers were sectioned into two approximately equal halves. One half was snap-frozen in liquid nitrogen and later used to quantify LIC via inductively coupled plasma mass spectrometry (ICP-MS), and the other half was fixed in neutral buffered formalin and later used for MRI experiments and histology.

Serum Ferritin Concentration, ICP-MS LIC Measurements, and Histology

Blood from each mouse was directly collected via cardiac puncture immediately following CO₂ overdose and added to microcentrifuge tubes to separate out the serum. Final serum ferritin concentration was measured using a colorimetric mouse ferritin ELISA assay (Immunology Consultants Laboratory, Inc.). The absorbance at 450 nm was used to determine serum ferritin concentration according to the manufacturer's instruction.

For ICP-MS measurements, liver samples (0.5 g) were digested in 9 mL of concentrated nitric acid and 3 mL hydrofluoric acid for 15 minutes. More specifically, each liver tissue sample in acid was placed in an inert polymeric microwave vessel and sealed prior to placing it in the laboratory microwave system. The temperature in the vessel was allowed to reach 180 ± 5 °C in approximately 5.5 minutes and was maintained at 180 ± 5 °C for an additional 9.5 minutes to allow for complete digestion of the sample. After cooling, the vessel content was filtered, centrifuged, decanted, and diluted to volume before quantifying iron concentration by ICP-MS. Note that all final tissue samples analyzed by ICP-MS, as well as iron standards used to calibrate (obtained from Sigma-Aldrich), contained 1% HNO₃.

For histopathological evaluation, liver samples were embedded in paraffin, sectioned into 5 μ m slices, stained with Perls' Prussian Blue, and photomicrographed according to standard procedure.

NMR Spectroscopy Experiments

A non-iron overloaded liver sample was harvested using the above described methods and was prepared for NMR spectroscopy using a standard NMR procedure (61). The ¹H NMR spectrum was acquired with a 600 MHz Varian Inova Spectrometer using a standard (rectangular excitation pulse followed by immediate acquisition) water suppressed ¹H NMR

pulse sequence (spectral width $sw = 6600$ Hz, flip angle $FA = 90$ degrees, pulse width $p_w = 6.3$ μ s, repetition time $TR = 2$ s, and 16 acquisitions for averaging). Assuming a 9-peak fat spectral model (39), the relative concentrations of the fat peaks were determined by performing multiplet analysis using MNOVA (Mestrelab Research) to generate a mouse liver fat spectral model to be used in the post-processing field map and R_2^* estimation methods described below.

MRI Experiments

The liver samples harvested for MRI were evenly spaced out in cylindrical tubes (28 mm in diameter, 114 mm in length) filled with agar gelatin. Using a 7T Varian Magnex MRI Scanner, the tubes were scanned with a 38 mm volume coil using a 3D multi-gradient-echo sequence with a unipolar readout gradient ($FA = 5$ degrees, $TR = 25$ ms, initial echo time $TE_i = 1.46$ ms, echo spacing $TE = 0.58$ ms, 12 echoes, field of view $FOV = 85 \times 35 \times 35$ mm, a matrix size of 64^3 , and 6 acquisitions for averaging).

MRI Post-Processing Methods

In gradient echo based chemical shift encoded MRI, the MR signal of a voxel containing water and fat, acquired using a series of n echoes, is given by

$$s(t_n) = (\rho_W + \rho_F) \sum_{m=1}^M \alpha_m e^{i2\pi f_m t_n} e^{i2\pi f_B t_n} e^{-R_2^* t_n} \quad [1]$$

where t_n is the n th echo time, ρ_W and ρ_F are the water and fat signal densities, α_m is the relative concentration and $f_m = \gamma B_0 \sigma_m$ is the frequency shift relative to water of the m th fat peak with γ representing the proton gyromagnetic ratio, B_0 representing the static magnetic field strength, and σ_m representing the chemical shift of the m th fat peak with respect to water (4.7 ppm), and f_B is the frequency shift relative to B_0 that arises due to magnetic field inhomogeneities. Due to the strong paramagnetic nature of iron, f_B is highly dependent on the presence of iron in the local area. A common R_2^* for all chemical species was assumed in this work, as it has been shown that, even for cases of iron overload and high hepatic fat concentrations, accurate measurements can be obtained (44,62–64).

First, an initial estimate of f_B was obtained using a 3D graphcut method (48,65) to perform spatially regularized complex non-linear least squares (NLLS) fitting of the signal. After the field map component was removed from the signal, an initial estimate of R_2^* was obtained on a voxel-by-voxel basis from the remaining signal using variable projection to perform complex NLLS fitting (47,66). The final estimates of f_B and R_2^* were obtained by using the initial estimates and a Levenberg-Marquardt algorithm (Matlab, The Mathworks, Matwick, MA) to perform complex NLLS fitting of the signal on a voxel-by-voxel basis (27), as complex NLLS fitting of the signal has been shown to outperform other methods in the

presence of noise (42,62). Note that the methods described here assume α_m and f_m in the signal model (Eq. [1]) to be known as *a priori* information, and ρ_W and ρ_F were estimated simultaneously along with the final estimates of R_2^* and f_B .

Using the final f_B estimate, QSM was then performed, which consists of two main steps: 1) removal of the background magnetic field and 2) calculation of the susceptibility from the remaining local field through dipole inversion (67). The background field is generally assumed to be harmonic and can, therefore, be removed by solving Laplace's equation (67,68). However, the solution to Laplacian's equation loses uniqueness at the boundaries of the region of interest (68). The boundaries for each MR dataset were defined by manually drawing masks around the cylindrical tubes containing the liver samples that excluded background air. Then, by assuming simple boundary value conditions, the background field was removed from the final f_B estimate by solving Laplace's and Poisson's equations using a Laplacian boundary value method (68) to solve the partial differential equations using a full multigrid finite difference algorithm (69). After removing the background field, the susceptibility can be determined from the remaining local field by deconvolving the local field with a unit dipole field; however, the inversion problem is ill-posed (67,70,71). In this work, the ill-posed problem was solved using a morphology enabled dipole inversion method (71) to construct a L_1 -norm minimization problem that incorporates structural information from the magnitude images. Final susceptibility estimates were obtained by finding the solution to the L_1 -norm problem using an iterative conjugate gradient method (72) with the solution to the deconvolution without the L_1 -regularization term serving as the initial guess. A flowchart depicting the above-described MRI post-processing procedure is presented in Figure 1.

Using the NMR derived mouse liver fat spectral model and the above-described post-processing methods, R_2^* and susceptibility maps were generated for each MR dataset. For each liver sample, the average and standard deviation of measurements obtained throughout a region-of-interest that covered the full volume of the liver sample, while avoiding voxels with partial volume effects, was determined. The susceptibility measurements were determined in reference to the average susceptibility of the agar gelatin background on a slice-by-slice basis.

Results

Serum Ferritin Concentration, ICP-MS LIC Measurements, and Histology

Measured serum ferritin concentrations and ICP-MS LIC measurements are compared to the injected Fe-dextran concentrations using linear regression analysis (Fig 2a,b). Both measurements increase linearly with increasing injected Fe-dextran concentration and show good linear correlations ($R^2=0.95$ and $R^2=0.91$, respectively). The injected Fe-dextran concentration-to-LIC conversion factor was determined to be 0.0386 (mg/g wet)/(mg/kg) (Fig. 2b). A good linear correlation is also observed between the serum ferritin concentration and ICP-MS LIC measurements ($R^2=0.88$ and serum ferritin concentration-to-LIC conversion factor = 8006 (ng/ml)/(mg/g wet)) (Fig 2c).

It is also observed in the Prussian blue stained liver sections (Fig. 3) that iron accumulates in Kupffer macrophages as expected, and that this accumulation occurs non-homogeneously throughout the liver tissue.

NMR Spectroscopy Experiment

The water suppressed ^1H NMR spectrum for a non-iron overloaded mouse liver is shown in Figure 4, which displays the chemical shifts (σ) in reference to water (4.7 ppm) and the measured relative concentrations (α) of each of the 9 fat resonance peaks. The resonance peak associated with total choline concentration, which includes choline and its derivatives phosphocholine and glycerophosphocholine, is also labeled (73–75). The 9-peak mouse liver fat spectral model derived here differs from previously published human liver models (39,76–78). When estimating R_2^* and f_B from MRI data, this mouse model (α_m and $f_m = \gamma B_0 \sigma_m$) was used in the post-processing methods as *a priori* information.

MR-Based LIC Quantification

Quantitative measurements of R_2^* and susceptibility for all liver samples are displayed in Figure 5. The average R_2^* values measured throughout each liver appear to increase linearly with injected Fe-dextran concentration up to a concentration of 50 mg/kg (Fig. 5a); a slight loss in linearity is then observed for injected concentrations between 50–100 mg/kg. A continuous, positive, linear dependency on the injected Fe-dextran concentration, however, is observed in the average susceptibility measurements (Fig. 5b). The standard deviations of the R_2^* and susceptibility measurements obtained throughout each liver also tend to increase with increasing injected Fe-dextran concentration.

The changes in the averages and standard deviations of the measurements can be more easily seen by examining their distributions (Fig. 5c,d). For injected concentrations of 50–100 mg/kg, the R_2^* distributions significantly overlap with each other, while clear shifts between other concentrations are observed. The susceptibility distributions, conversely, show clear shifts between all injected Fe-dextran concentrations. It is also observed that the distributions of both R_2^* and susceptibility become more spread out for higher injected concentrations, indicating larger standard deviations.

LIC measurements obtained using ICP-MS are compared to the R_2^* and susceptibility measurements using linear regression analysis (Fig. 6a,b). Good correlations between ICP-MS LIC measurements and the average R_2^* and susceptibility measurements are observed ($R^2=0.78$ and $R^2=0.91$, respectively), and R_2^* -to-LIC and susceptibility-to-LIC conversion factors of $372.2 \text{ s}^{-1}/(\text{mg/g wet})$ and $0.829 \text{ ppm}/(\text{mg/g wet})$ are derived. A good linear correlation is also observed between the average R_2^* and susceptibility measurements ($R^2=0.90$ and susceptibility-to- R_2^* conversion factor = $0.0020 \text{ ppm/s}^{-1}$) (Fig. 6c).

The overall average susceptibility of the agar gelatin background, in which liver susceptibility measurements were made in reference to on a slice-by-slice basis, was found to be -0.050 ppm, and the average measured proton density fat fractions of the liver samples varied within the range of 4-18%.

An MRI magnitude image and its corresponding R_2^* and quantitative susceptibility maps for a representative slice containing two excised livers from mice that were injected with differing Fe-dextran concentrations are shown in Figure 7. Clear differences in signal intensity, R_2^* , and susceptibility can be seen between the two livers. It is also observed that the spatial distributions of R_2^* and susceptibility are non-homogeneous.

Discussion and Conclusion

The observed increases in the average R_2^* and susceptibility measurements suggest that iron accumulates approximately linearly in the liver with increasing injected Fe-dextran concentration, which is confirmed with ICP-MS LIC measurements (Fig. 2b). This demonstrates the feasibility of the proposed methodology to create a controlled iron overload in the mouse liver, and the injected Fe-dextran concentration-to-LIC conversion factor can further be used to generate desired approximate LICs in future studies. However, the observed increases in the standard deviations of the R_2^* and susceptibility measurements and their observed spatial distributions suggest that the accumulation of iron throughout the liver is non-homogeneous and that this inhomogeneity increases with higher injected Fe-dextran concentration, which is confirmed by histology (Fig. 3). These observed increases and spatial distributions may also be due in part to noise more strongly influencing the estimates for livers with higher measured LICs, as the MR signal decays more rapidly leading to reduced SNR of the acquired data, especially for later echoes.

The good linear relationships between serum ferritin measurements and injected Fe-dextran concentration and ICP-MS measurements (Fig. 2a,c) suggest that serum ferritin measurements correlate well with LIC, which has previously been reported (1,79). Although serum ferritin concentration assays are commonly used to screen for iron overload in clinical settings, excessive alcohol use, viral hepatitis, and other systemic inflammatory disorders can injure hepatocytes and elevate serum ferritin levels (1,9). Therefore, these measurements may be misleading and are not always good quantitative measures of LIC.

However, in this work, MR-based R_2^* and susceptibility techniques are shown to be good quantitative measures of LIC, and the feasibility of quantifying LIC at a high field strength of 7T is demonstrated. Approximately linear correlations between the estimates of these parameters and ICP-MS LIC measurements are observed (Fig. 6a,b), which agrees with previously published results from experiments performed at lower field strengths (e.g. 1.5T and 3T) (19–23,27,35,49,50). However, a better linear correlation with LIC is observed for susceptibility ($R_2=0.91$) than for R_2^* ($R_2=0.78$), which is likely due to the slight loss of linearity in the R_2^* measurements for injected Fe-dextran concentrations of 50-100 mg/kg

(Fig. 5a). The susceptibility measurements also tend to have a smaller average standard deviation to slope ratio than the R_2^* measurements indicating that smaller changes in LIC can be measured without significant overlap in the distributions of the measurements. This suggests that R_2^* quantification may be more sensitive due to factors such as, background field perturbations, static field inhomogeneities, the choice of quantification method, and acquisition parameter selection (1), while susceptibility quantification through QSM is more robust.

Due to the high sensitivity of R_2^* quantification and the fact that R_2^* is dependent on field strength, the development of a standardized R_2^* -to-LIC conversion factor is difficult to obtain. Many studies have demonstrated good reproducibility of R_2^* estimations using various acquisition parameters (1,80–84), and estimates of R_2^* obtained at 1.5T and 3T have been shown to differ by a factor of two when using consistent acquisition parameters and quantification methods (85). However, agreement across studies that have used different quantification methods has been poor, as many different R_2^* -to-LIC conversion factors have been reported (19–21,23,35), even for studies performed at the same field strengths. Although this limits the ability to make direct comparisons of R_2^* -to-LIC conversion factors across studies, these differences can be corrected through the use of method specific calibration curves (86).

Good linear correlations between R_2^* and susceptibility have also been reported (26,27,87), but with various susceptibility-to- R_2^* conversion factors. Factors obtained at 1.5T and 3T using the same quantification method have also been shown to differ by a factor of two (27), but susceptibility-to- R_2^* conversion factors also suffer from the sensitivity and poor reproducibility of R_2^* across quantification methods.

Susceptibility is an intrinsic property of tissues that is independent of field strength, and this ex vivo study suggests that its quantification may be more robust than R_2^* quantification. Therefore, a standardized susceptibility-to-LIC conversion factor should be feasible to obtain, and the direct comparison of derived conversion factors across studies should be possible without the need for method specific calibration curves. Langkammer et al. (88) derived a factor of 0.89 ppm/(mg/g wet) by performing QSM on MR datasets acquired at 3T and comparing it to ICP-MS measurements in post mortem human brains. Using this factor, Sharma et al. (27) converted measured susceptibility values in the human liver obtained using QSM to LIC values. Once the susceptibility values were converted to LIC values, linear regression analysis was performed between the converted QSM-LIC values and acquired Ferriscan-LIC measurements. Using the same conversion factor by Langkammer et al. (88), the slopes from Sharma et al.'s (27) linear regression analysis can be converted to susceptibility-to-LIC conversion factors, and upon making this conversion, results of 0.703 ppm/(mg/g wet) for 1.5T and 0.730 ppm/(mg/g wet) for 3T are obtained. The susceptibility-

to-LIC conversion factor derived in this work of 0.829 ppm/(mg/g wet) (Fig. 6b) is in relatively good agreement with the factors derived by Sharma et al. (27) and Langkammer et al. (88). The slight differences observed between the susceptibility-to-LIC conversion factors may be due to differences in the accuracies of the chosen QSM techniques, which have been shown to lead to different degrees of underestimation (67), and/or due to differences in the spatial resolution of the acquired data, which has been shown to effect QSM estimates (89,90). Two recent studies using QSM have similarly reported good linear correlations between susceptibility and LIC (49,50); however, they did not present susceptibility-to-LIC conversion factors.

Iron in the body is primarily found in the storage complexes hemosiderin and ferritin, which based on the Curie-Weiss law, have reported theoretical susceptibility concentrations in the range of 1.1-1.6 ppm/(mg/g wet) (22,88,91,92), which are larger than the susceptibility-to-LIC conversion factors discussed in this work. Recent work quantifying susceptibility in the human liver has also reported smaller measurements obtained using QSM in comparison to measurements obtained using a superconducting quantum interference device (26). This suggests that the above discussed susceptibility-to-LIC conversion factors derived using QSM may be underestimated. Sharma et al. (26) suggested that the use of a relatively large slice thickness in comparison to the in-plane resolution may have contributed to the QSM underestimation observed in their work. All three susceptibility-to-LIC conversion factors discussed above were estimated from acquired data with slice thicknesses that were at least 2.4 times greater than the in-plane resolution. Therefore, this may be a contributing factor to the underestimations.

Although the presence and spectral complexity of fat, as well as the static field inhomogeneity, was accounted for in this work, confounding factors related to phase errors (45) were not addressed. Upon examination of the phase within all samples and within the agar gelatin, no significant deviations from linear behavior were observed, which suggests that no significant phase errors were arising due to eddy currents.

The work presented here does suffer from a few limitations though, including a small sample size and relatively small LIC concentrations. Quantitative measurements were only performed on twelve mice (six groups of $n=2$), all of which had measured LICs of less than 0.8 mg/g of wet tissue. Other studies conducted on human liver tissues have reported significantly higher LIC measurements, with a maximum around 7 mg/g of wet tissue (19–22,24,27,37,38). Therefore, in order to more accurately develop a standardized susceptibility-to-LIC conversion factor, a larger sample size covering a wider range of LIC values should be evaluated. Nevertheless, the results presented in this work demonstrate the feasibility of accurately detecting very small changes in LIC in ex vivo mouse livers using a high field strength of 7T.

LIC quantification, especially for cases of severe iron overload, would presumably be less challenging at lower clinical field strengths (e.g. 1.5T and 3T) due to slower signal decay (smaller R_2^*) in comparison to that at a higher field of 7T. However, high field iron quantification is becoming increasingly important as it allows for pre-clinical animal studies, for which biopsy validation is possible, and for human neural imaging, as certain

neurodegenerative diseases and micro-bleeding caused by traumatic brain injuries can lead to relatively small iron deposits or changes in iron concentration that can be more easily measured using higher fields. However, the study presented here only demonstrates the high field (e.g. 7T) feasibility of quantifying small differences in iron concentration in ex vivo mouse livers. In vivo LIC quantification at high fields will likely be more challenging due to faster signal decay (larger R_2^*) caused by additional factors such as blood flow and respiratory motion. Although this study suggests that susceptibility quantification using QSM may be more robust than R_2^* quantification, these additional factors, as well as changes in the geometry of the iron deposits, may lead to greater challenges for in vivo QSM than for R_2^* . Therefore, further in vivo validation studies are necessary.

In this work, twelve echoes of MR data was acquired resulting in a scan time of about four minutes per acquisition. However, in vivo human studies seek to minimize scan times so that data can be acquired in single breath holds. Using Cramer-Rao bound analysis, Hernando et al. (42) found that the theoretical noise performance of R_2^* estimation doesn't significantly improve beyond six echoes, except for cases of low R_2^* . Since the non-iron overloaded livers (Group 6) had average measured R_2^* values of approximately 40 s^{-1} and highly accurate quantification was desired for all R_2^* and susceptibility estimations, all twelve acquired echoes were used for MRI post-processing procedures. Future work will seek to use the proposed methodology to quantify LIC using an in vivo mouse model and explore the feasibility of monitoring various iron chelation treatments. These studies will likely require reduced scan times, and therefore, a reduced number of echoes. However, confounding factors such as blood flow and respiratory motion should compromise R_2^* values with a reduced number of echoes.

In conclusion, the work presented here demonstrates the feasibility of quantifying LIC using MR-based R_2^* and QSM techniques at a high field strength of 7T. Susceptibility quantification, which is an intrinsic property of tissues and benefits from being field strength independent, is found to be more robust and more comparable across studies than R_2^* quantification, and a susceptibility-to-LIC conversion factor is presented that agrees relatively well with previously published QSM derived results obtained at 1.5T and 3T (27,88).

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Figure 1.

A flowchart depicting the MRI post-processing procedure used in this work. Using complex multi-gradient echo data, estimates of the field map (f_B), R_2^* map, and water and fat signal densities (ρ_W and ρ_F) were first estimated using a series of algorithms (graphcut, variable projection, and Levenberg-Marquardt) to perform complex non-linear least squares fitting of the MR signal. Using the field map and manually drawn boundary masks, the local field contribution of the field map was then determined using a Laplacian boundary value method

(LBV). Finally, susceptibility (χ) maps were determined using the local field map, the magnitude images, and a morphology enabled dipole inversion method (MEDI).

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Figure 2.

Linear regression plots for injected Fe-dextran concentration (six groups, $n=2$ per group) and serum ferritin measurements (a) and ICP-MS LIC measurements (b). Both serum ferritin concentration and ICP-MS LIC increase linearly with injected Fe-dextran concentration and show good linear correlations ($R^2=0.95$ and $R^2=0.91$, respectively). A good linear correlation is also observed between the serum ferritin concentration and ICP-MS LIC measurements (c).

Figure 3.

Photomicrographs of histological liver sections stained with Prussian blue at 40 (**a-c**, scale bar equals 200 μm) and 400 (**d-f**, scale bar equals 20 μm) times original magnification obtained from mice injected with 0 (**a,d**), 25 (**b,e**), and 150 mg/kg (**c,f**) concentrations (Fe/mouse mass) of saline-dextran solutions (200 μL). Many scattered iron-containing Kupffer macrophages are observed in the mice injected with non-zero concentrations, and the degree of iron overloading increases with concentration. No iron deposits are observed in the mouse injected with a concentration of zero.

Figure 4.

Acquired NMR water suppressed ^1H spectrum for a non-iron overloaded mouse liver. The locations of the 9 fat resonance peaks (ppm relative to water) and their measured relative concentrations (%) of the total fat concentration are provided below and above each peak, respectively. A larger non-fat peak located between -0.50 and -1.95 ppm is also observed and is likely associated with the total choline (tCho) concentration, which includes choline and its derivatives phosphocholine and glycerophosphocholine.

Figure 5.

Quantitative R_2^* (**a and c**) and susceptibility (**b and d**) measurements obtained from MR acquired data. Average R_2^* and susceptibility measurements both increase relatively linearly with the experimentally injected Fe-dextran concentration, and the standard deviations also tend to increase as the injected concentration increases. The increases in the averages of R_2^* and susceptibility suggest that larger amounts of iron is accumulating in the liver with higher concentrations of injected Fe-dextran, which is confirmed by ICP-MS measurements (Fig. 2b), and the larger standard deviations suggest that iron is accumulating non-homogeneously (which can be seen in a representative slice in Fig. 7), which is confirmed by histology (Fig. 3).

Figure 6.

Linear regression plots for measured ICP-MS LIC and average measured R_2^* (**a**) and susceptibility (**b**) values. Both R_2^* and susceptibility measurements correlate well with ICP-MS LIC ($R^2=0.78$ and $R^2=0.91$, respectively), which demonstrates the feasibility of quantifying LIC using MR-based quantification methods. The susceptibility measurements, however, correlate more strongly and also have a smaller average standard deviation to slope ratio (0.073 ± 0.045 mg/g wet) than the R_2^* measurements (0.128 ± 0.0735 mg/g wet)

indicating that smaller changes in LIC can be measured without significant overlap in the distributions of the measurements. This suggests that susceptibility may be a better measure for quantifying LIC than R_2^* . A good linear correlation is also observed between the average R_2^* and susceptibility measurements (c).

Figure 7.

MRI magnitude image (a), R_2^* map (b), and quantitative susceptibility map (c) for a representative slice containing two excised livers from mice injected with different Fe-dextran concentrations (left: 100 mg/kg and right: 25 mg/kg). The liver from the mouse injected with a concentration of 100 mg/kg shows lower signal intensities and higher R_2^* and susceptibility values than the liver from the mouse injected with 25 mg/kg. The spatial distributions of R_2^* and susceptibility throughout each liver also appear to be non-homogeneous; suggesting that iron accumulation is non-homogeneous, which is confirmed by histology (Fig. 3).