MR Iron Quantification of Soluble (Ferritin-like) and Insoluble (Hemosiderin-like) Iron: A Biochemical Validation in Human Liver Explants

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Introduction: A theoretical MR model has been proposed that separately quantifies dispersed (soluble, ferritin-like) and aggregated (insoluble, hemosiderin-like) iron by distinguishing their effects on R_2 relaxation curves.[1] Recently, this model has been validated in agarose phantoms[2] using $MnCl_2$ and iron oxide microspheres to mimic ferritin and hemosiderin iron, respectively. Here, we directly measure the relationships between tissue concentrations of ferritin and hemosiderin iron determined by biochemical analysis and the MR model parameters measured in human liver explants. We then compare this relationship to one derived in previous studies *in vivo*,[2] where only the total (ferritin + hemosiderin) liver iron was known from biomagnetic susceptometery.[3]

Theory: The signal decay curve of multiple spin echo (MSE) sequences in tissue containing both ferritin-like and hemosiderin-like forms of storage iron has the approximate analytic form[1]:

$$S(t) = S_0 e^{-RR_2 * t} \exp\left(-A^{3/4}\Delta t^{3/4}\left(\mathrm{t} - 2\tau\left[1 - \left(\frac{\tau}{\Delta t}\right)^2\right]\right)^{3/8}\right),$$

where S_0 is the initial signal intensity, 2τ is the first spin echo time, and $2\Delta t$ is the inter-echo time. A series of MSE sequences with different inter-echo times can be used to determine a value for RR_2, the reduced relaxation rate (predominantly influenced by ferritin iron), and A, the aggregation index (predominantly influenced by hemosiderin iron). According to the model, the total iron concentration (C_T) is linearly dependent on RR_2 and A such that $C_T = C_D + C_A = \alpha_1 + \alpha_2 RR_2 + \alpha_3 A$. Here, we use the biochemically determined concentrations of ferritin and hemosiderin iron to determine the calibration parameters α_1, α_2, and α_3.

Methods: We examined 30 samples (1.5 cm thick) from 14 human liver explants obtained at transplantation for hepatic failure. Each sample was placed in a 50 mL plastic tube filled with saline and immersed in a cylindrical water bath. To better replicate conditions *in vivo*, our experiment was conducted at 37°C by use of circulating heated water and fiber optic temperature probes. MR scanning was performed with a 5-channel phased array cardiac coil in a 1.5 T Philips MR scanner. Three MSE sequences were acquired with different inter-echo times [scan duration: 49 s; voxel: 6 x 6 x 10 mm^3; TR: 1000 ms; first TE: 4 ms; inter-echo time: 4, 8, or 16 ms; FA: 90°; NSA: 1; FOV: 384 x 384 mm]. A region of interest (ROI) was centered on each of the samples in the phantom and propagated over all the images in the echo train. The non-monoexponential fitting computation was performed using the Levenberg-Marquardt method. After MRI, the liver sections were biochemically analyzed for ferritin, hemosiderin, and total non-heme iron.[4]

Results: Five livers (N = 12 samples) could not be analyzed because of advanced cirrhosis. In the remaining (N = 18; Figures 1 and 2), the MR model parameters, RR_2 and A, and the corresponding biochemically determined concentrations of ferritin and hemosiderin were closely correlated (R ~ 0.9 for both, P<0.0001). The calibration coefficients from these data are: $\alpha_1 = -(0.39 \pm 0.13)$ (mg Fe/g), $\alpha_2 = (0.037 \pm 0.008)$ (s mg Fe/g), and $\alpha_3 = (50.6 \pm 3.7)$ $ms^{3/2}$ (mg Fe/g). For comparison, the calibration coefficients derived from our earlier *in vivo* study in which only the total (ferritin + hemosiderin) storage iron was known from biomagnetic susceptometry were: $\alpha_1 = -(0.54 \pm 0.61)$ (mg Fe/g), $\alpha_2 = (0.057 \pm 0.02)$ (s mg Fe/g), and $\alpha_3 = (18 \pm 4)$ $ms^{3/2}$ (mg Fe/g).

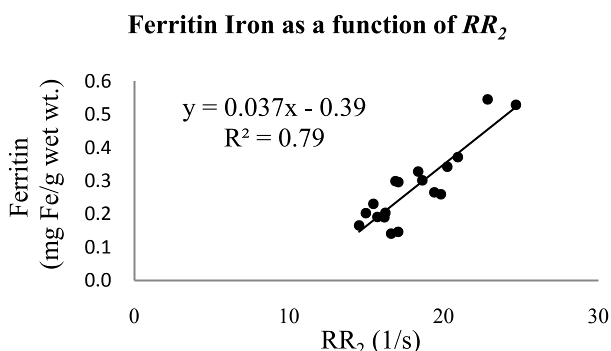

Fig 1. The regression between RR_2 and ferritin iron is used to determine α_1 and α_2.

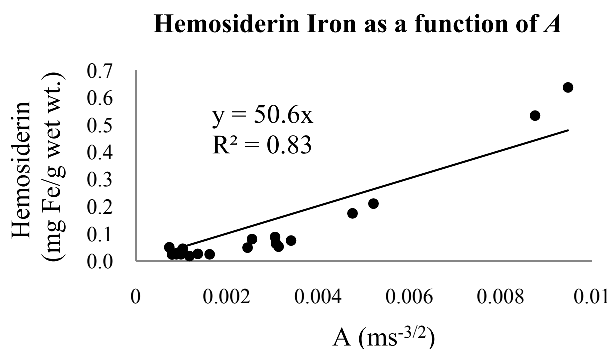

Fig 2. The regression of A and hemosiderin iron is used to determine α_3.

Discussion: These measurements show that the MRI model parameters closely correlate with the concentrations of liver ferritin and hemosiderin iron. Comparison with our earlier *in vivo* study[2] shows reasonable consistency in the calibration coefficients for ferritin iron (α_1 and α_2). Differences in the calibration parameter for hemosiderin (α_3) in the two studies may have resulted from differences in (i) hepatic pathology, (ii) the methods used to determine liver iron concentration, (iii) the range of liver iron concentrations, or (iv) some combination of these factors. This suggests that the method may be very robust at estimating ferritin levels in patients with transfusion iron overload. The method may be able to evaluate the effects of iron-chelating therapy, since cellular ferritin iron is in short-term equilibrium with the potentially toxic cytosolic iron pool[5] that is accessed by iron-chelating agents.

References: **1** Jensen JH et al. Magn Reson Med 2002;47(6):1131-1138. **2** Jensen JH et al. Magn Reson Med 2009; in press. **3** Brittenham GM et al. New Engl J Med 1994;331:567-573. **4** Overmoyer BA et al., Arch Pathol Lab Med 1987;111: 549-555. **5** De Domenico I et al. EMBO J 2006;25 :5396-5404