Establishment of an *in vivo* Iron and R_2* Calibration Curve

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Introduction: The inability to regulate iron leading to abnormally elevated levels in brain tissue has been associated with several neurodegenerative disorders including Parkinson's (PD), Huntington (HD) and Alzheimer's disease (AD) (1-4). Iron distribution in tissue acts as a natural contrast agent for magnetic resonance imaging (MRI) because the transverse relaxation rate R_2* (=1/T_2*) is a sensitive MRI parameter for evaluation of tissue iron concentration. This provides a unique natural mechanism for *in vivo* quantitative iron mapping that requires the addition of no other contrast agents (5). The determination of the quantitative relationship between tissue iron concentration and R_2* relaxation would be important in establishing the link between brain iron distributions and the etiology of iron associated neurodegenerative disorders. Here we present data on the *in vivo* relationship between tissue iron measurements with atomic absorption spectrophotometry and R_2* relaxation using a 3,5,5 – Trimethylhexanoyl (TMH) Ferrocene iron loaded mouse model.

Methods: Thirty-six C57BL/6 mice were divided into groups of six. Animals were fed ad libitum a diet composed of 0.1% TMH Ferrocene (6) mixed into Teklad Global 18% protein rodent diet (Harlan Teklad, Indianapolis, Indiana) for a period of 0, 2, 4, 6, or 8 weeks for iron loading. Control animals were free fed a regular iron 18% protein rodent diet for the eight week period and imaged at week zero and eight. Animals were imaged on a 3.0 T Medspec S300 MR imaging-spectrometer (Bruker Biospin, Ettlingen, Germany) using a home-built gradient (9.5 cm aperture and 100 G/cm). Animals were anesthetized and maintained at a 37ºC during scans. T_2 and T_2* measurements were done with a multi spin-echo sequence and mGESEPI (multi Gradient-Echo Slice Excitation Profile) sequence which allows for reliable R_2* measurements without magnetic susceptibility artifacts (5) with a voxel resolution of 179 x 179 x 312 µm. R_2* and R_2 parameter maps were created with a home-developed software dedicated for quantitative MRI analysis. The average R_2* were obtained from regions of the interest (ROIs) in the corpus callosum, sensory and motor cortex, lateral globus pallidus, caudate/putamen and anterior cerebellum. Upon completing the imaging protocol, the mice were transcardially perfused with ice-cold 4% paraformaldehyde, brain tissue excised and fixed overnight at 4ºC. The brain tissue was then stored in 0.1M Phosphate Buffered Saline (PBS) for 48 hours at 4ºC to leech the paraformaldehyde out of the tissue. Each brain was then placed into a tissue matrix and coronal slices were taken at 500 µm. Guided by a mouse brain atlas and the R_2* maps, the selected ROIs were micro-dissected from the slices, weighed and digested in 70% ultra-pure nitric acid for 48 hours at 55ºC. Digested tissue samples were then placed into a graphic furnace atomic absorption spectrophotometer (AAS) and measurements of iron concentration were obtained.

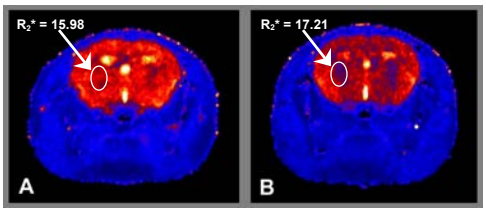

Figure 1: T_2* parameter map of a control mouse (A) and one fed the high iron diet for 8 weeks (B). High iron regions show as dark hypo-intensities on the maps. The two parameter maps were created using the same threshold values. Regions such as the caudate/putamen show statistical differences in R_2* relaxation, which can also be visually seen.

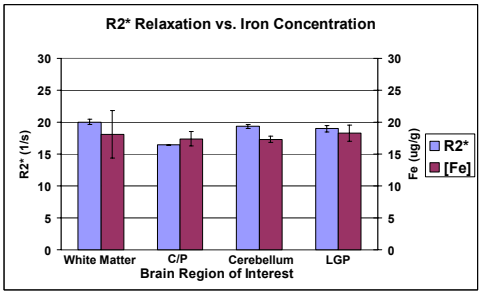

Figure 2: Average R_2* relaxation verses the atomic absorption measured Iron concentration in the week 2 high iron diet group (N=6). The data indicate that there is a relationship between high iron regions and R_2* relaxation as seen in the covariance of the [Fe] and R_2* values.

Results: Figure 1 shows the parameter map of the same slice from a control mouse fed a regular iron diet (a) and a mouse fed the TMH ferrocene diet (b) at for eight weeks. The image shows regions with decreased T_2* corresponding to increased iron content. The hypo-intensive regions in T_2* maps of the mouse overlap with known high iron concentration measured previous by chemical and histological method (7-8). R_2* values in the selected ROIs show a significant difference ($P < 0.05$) between the two groups. For the caudate/putamen, the 8 week group has an R_2* of 17.21 (+/- 0.18) comparing to 15.98 (+/-0.17) for the controls. Figure 2 shows the week 2 high iron diet animals AAS [Fe] verses the corresponding R_2* value in the same brain structures, demonstrating a close correlation between [Fe] and R_2* values. This is further seen in figure 3 with the [Fe] verses R_2* calibration curve. There exists a positive correlation between [Fe] and R_2* values in the animal data with a Pearson's R^2 value of 0.26.

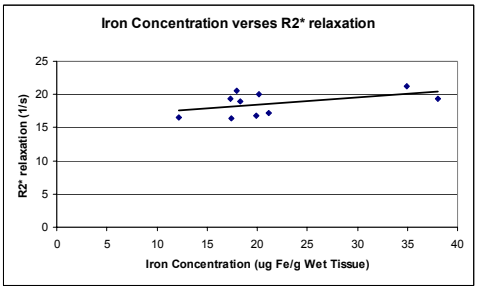

Figure 3: R_2* relaxation verses Iron concentration calibration curve. A positive correlation exists between the R_2* value and the iron concentration with an R_2 value of 0.26.

Discussion: The presented data and method represent the first attempt to establish an in vivo calibration curve of R_2* with brain tissue iron concentration. This is technically challenging because it requires reliable measurements both R_2* and iron concentration from the same brain region. Our results demonstrate the feasibility of acquiring such a relationship and laid a foundation for quantitative iron measurements in vivo with MRI. With the iron loading animal model used here, we show that elevated iron levels in various brain regions can be manipulated to simulate iron abnormalities. The animal model and *in vivo* calibration data provide the necessary tool for scientific clinical investigations on diseases involving abnormal level of iron concentrations.

References:

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