

MRI-based detection of the extracellular matrix surrounding the hepatic sinusoid

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Introduction: The goal of this work was to develop a technique to detect structural changes to extracellular macromolecules in the liver *in vivo* using MRI. Changes in macromolecules in the extracellular matrix (ECM) surrounding the hepatic sinusoid (HS) are common in liver fibrosis, a defining characteristic of all chronic liver diseases (1). Non-alcoholic fatty liver disease (NAFLD) is one such disease that has become so pervasive that it now affects approximately 20% of the general population and 13-14% of the pediatric population (2). While non-invasive imaging techniques have been developed to detect late-stage progression of NAFLD, the options for early diagnosis are mostly limited to biopsy. Over-expression of the anionic proteoglycan lumican in the HS is strongly linked to the progression of NAFLD (3). Furthermore, cationic ferritin (CF) has been used as a contrast agent to detect anionic proteoglycans of the kidney ECM with MRI (4), leading us to believe that CF may be a suitable candidate to detect ECM structure in the liver. Here we show that systemic injection of CF yields unique MRI contrast in the liver due to the specific binding of CF to the charged ECM proteoglycans surrounding the HS. These results suggest that CF and MRI may be used as a non-invasive tool for diagnosis of ECM changes in a variety of chronic liver diseases, including NAFLD. **Methods: Preparation:** Male Sprague Dawley rats were given a 5.75 mg/100g dose of either CF (n=3) or NF (n=3) over 3 bolus injections with 1.5 hours between injections. Control rats were given no contrast (n=3). Rats prepared for *ex vivo* imaging (3 CF, 3 NF, and 3 control) were sacrificed via transcardial perfusion with PBS followed by 10% neutral buffered formalin. The livers were resected and stored in a 2% glutaraldehyde, 0.1M cacodylate buffered solution. **MRI:** For *in vivo* imaging of ferritin distributions, rats were anesthetized with isoflurane and imaged at 7T (Bruker) using a gradient echo sequence (TE/TR = 8/60 ms) with a resolution of 234x234x1000 μ m. Perfused *ex vivo* liver sections were imaged in PBS at 7T using a gradient echo sequence (TE/TR = 15/23 ms) at 70x70x1000 μ m. **Histology:** To examine the distributions of ferritin and ECM in the livers of each group, tissue was fixed, dehydrated, and sectioned at 30 μ m. Sections were incubated with rabbit anti-ferritin and chicken anti-fibronectin followed by anti-rabbit Alexa 594 and anti-chicken Alexa 488. Anti-fibronectin was chosen because fibronectin is the most prominent ECM component in Disse's space (5). Cell nuclei were stained with DAPI. **Electron Microscopy:** To confirm the distribution of ferritin in the liver ultrastructure, tissue was sectioned at 65 nm, lightly stained with osmium tetroxide for preservation of membranes, and imaged on a transmission electron microscope (Phillips). **Statistics:** *In vivo* signal intensities were compared between groups using a two-sample student's t-test. **Results and Conclusions:** CF and NF showed distinct patterns of uptake in the rat liver, as seen with immunofluorescence (fig. 1A-I), *in vivo* MRI (fig. 2A-C), and *ex vivo* MRI (fig. 2E-G). Confocal microscopy showed colocalization of CF with the ECM surrounding the HS (fig. 1A-C). In contrast NF was internalized in the macrophages, endothelial cells, and hepatocytes surrounding the HS (fig. 1D-F). Minimal fluorescence was observed on the red (ferritin) channel in unlabeled control livers (fig. 1G-I). The presence of CF in the ECM of the HS and the presence of NF in endothelial cells was confirmed with electron microscopy (fig. 1J, K). *In vivo* MRI reveals unique contrast in the CF-labeled livers compared to NF and unlabeled control livers (fig. 2A-C). Liver signal was compared to surrounding muscle, as muscle signal is assumed to remain constant across all groups, revealing a significant difference in liver contrast between all groups ($p < 0.05$) (fig. 2D). *Ex vivo* MRI (fig. 2E-G) shows hypointense striations exclusive to the CF-labeled livers that are consistent with the distribution of hepatic sinusoids. These striations were discernable in line profiles across the livers (fig. 2H). **We conclude that the specific binding of CF to the anionic ECM proteoglycans of the HS is observable *in vivo* using MRI, raising the possibility to observe ECM changes during development of liver diseases affecting the HS ECM such as NAFLD with MRI.**

References: (1) Bedossa, P et al. J Pathol. 200, 2003 (2) Erickson, S Clin. J. Lipid Res. 50, 2009 (3) Charlton, M et al. Hepatology 49, 2009. (4) Bennett, KM et al. MRM. 60, 2008. (5) Martinez-Hernandez, A FASEB J, 9, 1995

Acknowledgments: Thanks to Joseph Georges for assistance with histology and David Lowry for assistance with TEM.

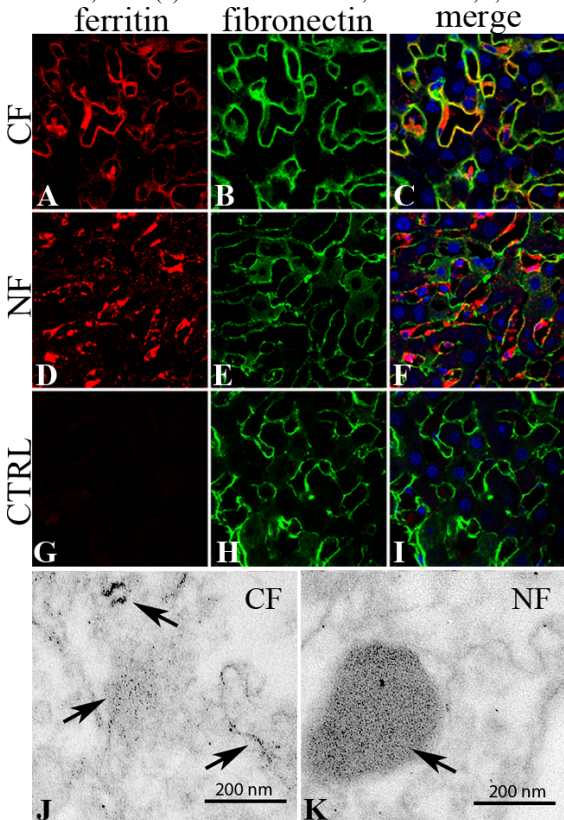


Figure 1 (left) – 63X confocal imaging of CF (A-C), and NF (D-F) and unlabeled control (G-I) livers. Ferritin is shown in red (A, D, and G) and fibronectin is shown in green (B, E, and H). C, F, and I represent the overlay of ferritin and fibronectin with cell nuclei added in blue. A-C show that CF and fibronectin are co-localized, consistent with ECM labeling with CF. No co-localization of NF with fibronectin is observed (D-F); instead NF appears to be taken up in macrophages, endothelial cells, and hepatocytes. Minimal fluorescence was observed on the red (ferritin) channel of the unlabeled control liver (G-I). Electron microscopy reveals CF in the Disse's space (J) and NF in an endothelial vesicle (K).

Figure 2 (below) – Differences in binding between CF (A, n=3), NF (B, n=3), and unlabeled control (C, n=3) groups were distinguishable *in vivo* with MRI (A-C). Liver signal was compared to surrounding muscle, as muscle signal is assumed to remain constant across groups (D). The differences between all groups were statistically significant ($p < 0.05$). *Ex vivo* MRI (E-G) revealed striations of hypointensity in CF-labeled livers (E, n=3), while NF contrasted livers show diffuse hypointensity throughout the entire liver (F, n=3) as compared to unlabeled control livers (G, n=3). Striations in the CF labeled liver are consistent with the distribution of hepatic sinusoids. Line profiles of *ex vivo* livers show discernable signal loss in the hepatic sinusoid of CF-labeled livers as compared to NF and unlabeled control livers (H).

