Age dependent elevation of liver R2 in h-ferritin over expressing transgenic mice

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Introduction:
Iron is a paramagnetic reactive metal, which is essential for the functionality and viability of cells; it participates in many metabolic pathways and is required for proper body homeostasis. Iron deposition and accumulation occurs with aging and was implicated in multiple pathologies. Increase of iron cellular content can be used for enhancement of MR contrast (1-3). The increase in iron content requires the increase of iron cellular uptake and storage. Ferritin, an iron storage protein serves to protect cells from iron toxicity by sequestration of free iron. Ferritin generates unique and particularly large R_2 relaxivity at low iron loading. Therefore overexpression of Ferritin was proposed as a possible reporter gene for MRI (2-5). We generated transgenic mice that over-express HA-tagged ferritin and EGFP in a tissue specific and tetracycline inducible manner. As expected, these mice showed elevated R_2 for endothelial cells expression. However, young mice showed reduced R_2 upon acute expression of h-ferritin in liver hepatocytes (5). Here we report the change in R_2 upon long-term overexpression of h-ferritin in liver hepatocytes in aging mice.

Materials and Methods:
LAP:tTA x Tet:EGFP-HAferritin double transgenic mice: Homozygous Tet:EGFP-HAferritin mice were mated with heterozygous LAP:tTA (liver activator protein) (6), double transgenic offspring (liver-hfer) have overexpression of h-ferritin in the liver. Wild type CB6F1 mice (WT) were used as control. These mice were grown for 2 years and were scanned by MRI at least once in two months.
MRI studies: horizontal 4.7T and 9.4T Biospec Bruker spectrometers were used. R_2 relaxation was measured using multi echo spin echo sequence with 8 echo times (TR= 2000 ms, TE= 11-88 ms, 2 averages, FOV 6X6 cm, slice thickness 1 mm, matrix 128 X 128, SW=50,000Hz).
MRI was applied for dynamic follow up of 2 groups of mice: WT mice and liver-hfer mice (with constitutive transgene expression in hepatocytes). Analysis of R_2 was applied according to regions of interest (ROI) using Matlab. Following MRI analysis, livers were retrieved for histological evaluation.

Results and Conclusions:
While young liver-hfer mice showed reduced R_2 upon acute expression of h-ferritin in liver hepatocytes and no effect on R_2 with prolonged expression of h-ferritin, old age liver-hfer mice (2 years) with prolonged expression of h-ferritin showed significant increase in liver R_2 values compared to WT ($p<0.05$, 2 tailed unpaired Ttest). As expected for ferritin, the change in R_2 was strongly enhanced at high magnetic field. Histological analysis of liver-hfer and WT mice confirmed the increased iron content in the livers of transgenic mice.

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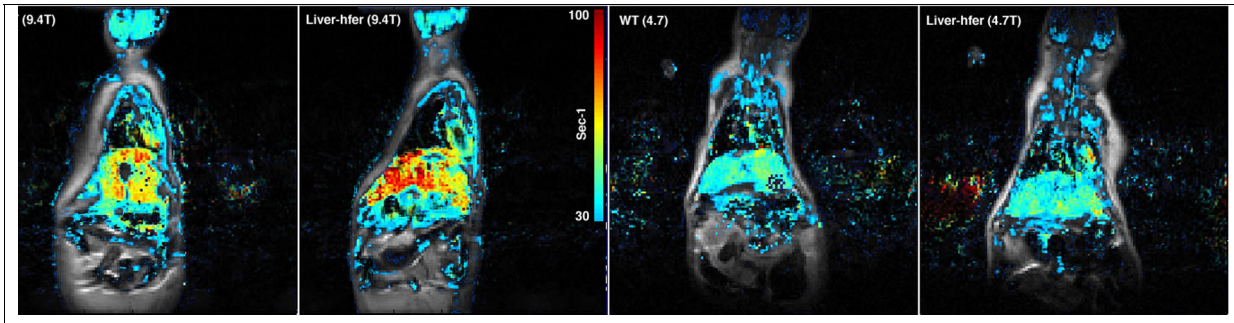

Figure 1: overlay of R_2 maps on a gray scale image of WT mice and liver-hfer mice (age 2 years) with constitutive transgene expression (no tetracycline). These mice were scanned on 2 magnetic fields: 4.7T and 9.4T.

Refernces:1.Fenzi, A., Bortolazzi, M., Marzola, P., and Colombari, R. In vivo investigation of hepatic iron overload in rats using T2 maps: quantification at high intensity field (4.7-T). J Magn Reson Imaging., *13:* 392-396., 2001. 2.Genove, G., DeMarco, U., Xu, H., Goins, W. F., and Ahrens, E. T. A new transgene reporter for in vivo magnetic resonance imaging. Nat Med., *11:* 450-454. Epub 2005 Mar 2020., 2005. 3.Deans, A. E., Wadghiri, Y. Z., Bernas, L. M., Yu, X., Rutt, B. K., and Turnbull, D. H. Cellular MRI contrast via coexpression of transferrin receptor and ferritin. Magn Reson Med., *56:* 51-59., 2006. 4.Cohen, B., Dafni, H., Meir, G., Harmelin, A., and Neeman, M. Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors. Neoplasia., *7:* 109-117., 2005. 5.Cohen, B., Ziv, K., Plaks, V., Israely, T., Kalchenko, V., Harmelin, A., Benjamin, L., and Neeman, M. MRI detection of transcriptional regulation of gene expression in. Nat Med., *13:* 498-503. Epub 2007 Mar 2011., 2007. 6.Kistner, A., Gossen, M., Zimmermann, F., Jerecic, J., Ullmer, C., Lubbert, H., and Bujard, H. Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. Proc Natl Acad Sci U S A., *93:* 10933-10938., 1996.