

**Title:** The Role of Hepcidin in Monitoring Inflammation by Magnetic Resonance Imaging

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**Structured Abstract:**

**Introduction:** Magnetic resonance imaging (MRI) is a non-invasive tool to track cellular activities in the body using iron-based contrast agents [1, 2]. This suggests that a particular cell's iron handling mechanism may influence the detection of magnetic resonance (MR) contrast [3]. For instance, inflammation involves downregulation of iron export in monocytes and macrophages by the hormone hepcidin [4], due to degradation of the iron export protein, ferroportin (Fpn) [5]. We therefore examined the effect of hepcidin on transverse relaxation rates in multipotent P19 cells, which provide a convenient model of molecular activities present during inflammation owing to their high iron import and export activities, similar to macrophage [6].

**Methods:** Iron-exporting P19 cells were cultured in iron-supplemented medium (+Fe) containing 25  $\mu$ M ferric nitrate for 7 days prior to removal of extracellular supplement and culture for an additional 1, 2, 4 and 24 hours. To examine interruption in iron export, hepcidin (200 ng/ml) was added to the medium. Cells were harvested; mounted in gelatin phantoms; and scanned at 3 Tesla. Image-based measurements of total transverse relaxation rate ( $R2^* = 1/T2^*$ ) and the irreversible component ( $R2 = 1/T2$ ) were performed [7] and the reversible component,  $R2'$  (defined as the difference between  $R2^*$  and  $R2$ ) was calculated. Total cellular iron content was measured by inductively-coupled plasma mass spectrometry (ICP-MS). Expression of Fpn was examined by Western blot. Analysis of variance (ANOVA) was performed to compare the groups. Linear regression modelling was conducted to determine the correlation between relaxation rate, as the dependent variable, and total cellular iron content, as the independent variable. Independent Student's t-test was performed to compare the linear regression slopes. The threshold of statistical significance was set at  $p < 0.05$ .

**Results:** Hepcidin caused ferroportin degradation in P19 cells. At each time point, hepcidin did not significantly alter the magnitude of transverse relaxation rates or total cellular iron in P19 cells. While the correlation between total cellular iron and either  $R2$  or  $R2^*$  was significant in both treatment groups, the slope of this linear regression relationship in hepcidin-treated cells was significantly higher, approximately 3-4-fold, than in non-hepcidin treated samples ( $p < 0.05$ ).

**Discussion:** These results demonstrate how hepcidin-dependent alteration of iron export might influence the MR signal. This study indicates the potential for non-invasively monitoring such inflammation-related changes using MRI.

**References:** [1]Li et al (2013) *Theranostics* 3, 595, [2]Goldhawk et al (2012) *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 4, 378, [3]Goldhawk et al (2015) *Magnetic Resonance Insights* 8, 9, [4]Theurl et al (2008) *Blood* 111, 2392, [5]Nemeth et al (2004) *Science* 306, 2090, [6]Liu (2015) *MSc., Western Univeristy*, [7]Sengupta et al (2014) *Frontiers in Microbiology* 5, 29