

## Ferroportin Regulates Cardiac Iron Homeostasis

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**Target:** Researchers interested in iron homeostasis and myocardial tissue characterisation.

**Introduction:** Iron is essential to mammalian life. Both iron overload and deficiency are associated with cardiac pathologies.<sup>1</sup> Effective iron homeostasis is therefore important for cardiac function. Ferroportin (FPN), the regulatory protein of iron homeostasis, is known to manipulate iron levels in the duodenum, spleen and liver.<sup>2</sup> We show here that FPN is expressed in tissues that have no systemic role in iron homeostasis, such as the heart. We generated mice with a cardiomyocyte-specific deletion of *Fpn*, and show by Cine MRI and cardiac T2\* mapping that these animals have both severely reduced cardiac function and substantially increased levels of cardiac iron.

**Methods:** Mouse generation: Cardiac *Fpn* ko mice were generated by a Cre/flox method to provide both ko mice and floxed littermates as a control. MR Protocol: Mice were anaesthetised (2% isoflurane) and scanned on a 7 T preclinical MRI system (Agilent). Cardiac function was determined by a segmented gradient echo cine sequence (30° FA, 4.75 ms TR, 1.21 TE, 8 PE/shot, 128x128 matrix 32x32 mm<sup>2</sup> FOV, 1.2 mm slice, sinc excitation. Cardiac T2\* was measured on a single mid-ventricular axial slice by a multiecho segmented gradient echo sequence gated to diastole (128x128 matrix, 32x32 mm<sup>2</sup> FOV, 1.6 mm slice, sinc excitation, Te=1.8, 2.4, 3.1, 4, 5, 8, 10, 12 ms).

Analysis: Data were regridded and Fourier transformed in Matlab, subject to a threshold based segmentation algorithm and fit to a single exponential model. A manual ROI was drawn around interventricular septum to calculate the mean myocardial T2\*. Cine images were manually segmented and the left and right ventricular ejection fractions (LVEF/RVEF) calculated. Student's *t*-test was used to compare differences between groups. In vitro biochemistry: Hearts were removed under terminal anaesthesia and snap frozen in liquid nitrogen. Cardiac elemental iron concentration was quantified from lysates by mass spectrometry.

**Results:** *Fpn* ko mice showed severe dilated cardiomyopathy, and a significant decrease in myocardial T2\* (Fig. 1). There was a marked and significant increase in the size of the left ventricular volume at both end systole and end diastole. This was accompanied with a 20% decrease in LVEF, and the phenotype worsened with age. Other parameters of cardiac performance were not significantly altered between groups (Fig. 2). Cardiac T2\* was decreased by a factor of three, indicating an increased iron concentration in cardiomyocytes. A 33% increase in cardiac elemental iron was later confirmed by mass spectrometry.

**Discussion:** We have demonstrated that, in addition to its recognised role in iron homeostasis, FPN is important for cardiomyocyte iron homeostasis. The loss of FPN is associated with substantial cardiac impairment in the mouse, and results in an increase in the concentration of cardiomyocyte iron. As expected, relaxometry methods are uniquely placed to non-invasively characterise the myocardial tissue in this mouse, and we appear to have a linear relationship between mean cardiac iron concentration and T2\*. We anticipate further elucidating the vital role that FPN has in cardiac iron homeostasis in future work. Our findings highlight the need to assess carefully the impact of iron chelation, venesection and hepcidin-targeted therapies on FPN as well as the iron load in the heart.

**Conclusion:** Cardiac iron homeostasis is regulated by ferroportin, and removing FPN leads to substantially impaired cardiac function and increased cardiomyocyte iron loading. FPN may therefore be an interesting target for therapeutic opportunities in heart disease associated with iron dysregulation.

**References:** 1 Klip, U., *et al.*, "Iron deficiency in chronic heart failure: an international pooled analysis." *Am Heart J*, 2013. 2 McGregor, J., *et al.* "Impaired iron transport activity of ferroportin 1 in hereditary iron overload", *J. Mem. Biol.*, 2005.

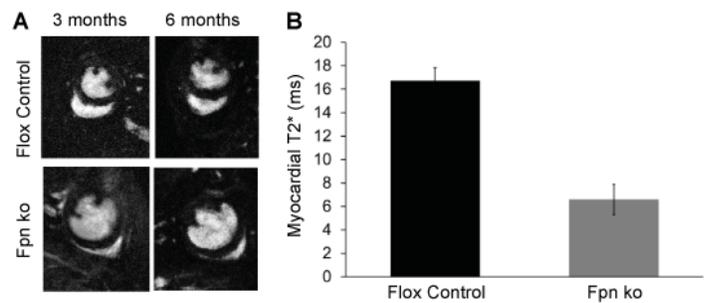


Fig. 1: A: Representative mid-ventricular Cine images showing impaired cardiac function of *Fpn* ko mice. B: Mean myocardial T2\* is significantly shortened in *Fpn* ko mice.

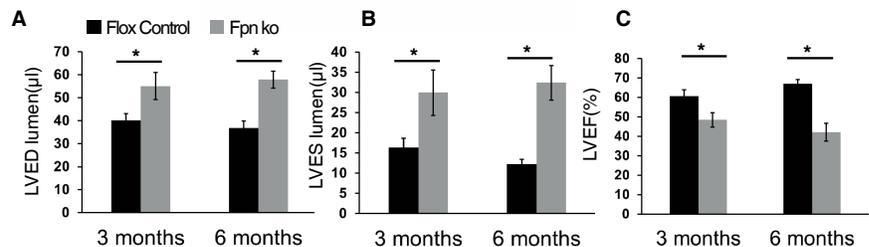


Fig. 2: Left ventricular function is significantly altered in *Fpn* ko mice, with decreases in LV end diastolic lumen volume (LVED lumen, A), LV end systolic lumen (LVES lumen, B) and the ejection fraction (LVEF, C). (\* *p* < 0.05)