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Article

Intestinal Ferritin H Is Required for an Accurate Control of Iron Absorption

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SUMMARY

To maintain appropriate body iron levels, iron absorption by the proximal duodenum is thought to be controlled by hepcidin, a polypeptide secreted by hepatocytes in response to high serum iron. Hepcidin limits basolateral iron efflux from the duodenal epithelium by binding and downregulating the intestinal iron exporter ferroportin. Here, we found that mice with an intestinal ferritin H gene deletion show increased body iron stores and transferrin saturation. As expected for iron-loaded animals, the ferritin H-deleted mice showed induced liver hepcidin mRNA levels and reduced duodenal expression of DMT1 and DcytB mRNA. In spite of these feedback controls, intestinal ferroportin protein and ^{59}Fe absorption were increased more than 2-fold in the deleted mice. Our results demonstrate that hepcidin-mediated regulation alone is insufficient to restrict iron absorption and that intestinal ferritin H is also required to limit iron efflux from intestinal cells.

INTRODUCTION

Iron absorption by the proximal intestine is strictly controlled in order to maintain body iron homeostasis (Andrews and Schmidt, 2007; Hentze et al., 2004). Iron enters duodenal enterocytes from the intestinal lumen by divalent metal transporter 1 (DMT1)-mediated transport across the apical brush border membrane after reduction to Fe^{2+} by duodenal cytochrome *b* (Dcytb) (Fleming et al., 1997; Gunshin et al., 1997; McKie et al., 2001). Its export at the basolateral membrane is mediated by ferroportin (Donovan et al., 2000; McKie et al., 2000) followed by its reoxidation to Fe^{3+} by hephaestin (Vulpe et al., 1999). The control of iron absorption requires hepcidin, a polypeptide hormone secreted by the liver in response to high body iron levels (Nicolas et al., 2001; Pigeon et al., 2001) and other systemic stimuli (Nicolas et al., 2002; Vecchi et al., 2009). Hepcidin binds to ferroportin, inducing its internalization, which is thought to inactivate cellular iron export (Nemeth et al., 2004). The importance of hepcidin in controlling ferroportin function is supported by the observation that most forms of hereditary hemochromatosis are either linked to a dysfunction of hepcidin itself (Roetto et al., 2003) or to a defect in the signaling pathways that control its expression. These involve HFE (Feder et al., 1996), transferrin receptor 2 (Camaschella et al., 2000), and hemojuvelin (Babitt et al., 2006; Niederkofler et al., 2005; Papanikolaou et al., 2004).

Early investigations proposed that the intestinal ferritin content is involved in the control of intestinal iron absorption by scavenging excess nutritional iron in duodenal cells (Conrad and Crosby, 1963; Granick, 1946; Hahn et al., 1943). Ferritin is a protein polymer of 24 ferritin H and L subunits forming a hollow shell structure that can store up to 4500 Fe^{3+} ions (Harrison and Arosio, 1996). The polymer exhibits variable stoichiometry of the two ferritin subunits in different tissues. Only ferritin H has ferroxidase activity that is indispensable to store iron in the ferritin shell. Initial isotope studies on iron absorption revealed that humans and dogs with chronic iron-deficiency anemia transported 5–15 times more iron across the duodenal mucosa than controls, indicating a physiological barrier to iron absorption in iron-adequate individuals (Hahn et al., 1943). Intestinal iron absorption rates increased with a delay of several days after induction of acute anemia by severe bleeding of dogs, suggesting a slow, probably indirect feedback regulation. In contrast, a single high oral dose of iron sulfate given to anemic animals 1–2 hr before an oral test dose of ^{59}Fe reduced the absorption of subsequent oral doses (Hahn et al., 1943). This "mucosal block" lasted for several days after administration of the blocking dose and correlated with the expression of intestinal ferritin (Granick, 1946). Autoradiography showed that excessively absorbed $^{59}Fe^{3+}$ was retained in the enterocytes at the tip of duodenal villi until they were exfoliated into the lumen (Conrad and Crosby, 1963). Ferritin was considered the most probable candidate protein for such long-term mucosal iron retention. The validity of the "mucosal block" concept (Crosby, 1966) was later questioned, as it was not consistently observed in the context of iron supplementation schemes in developing countries when daily and weekly supplementation were compared (Beard, 1998; Beutler, 2002; Hallberg, 1998). However, the possible role of ferritin in controlling iron absorption was not adequately addressed at the level of gene expression and molecular mechanisms.

Recently, we developed a mouse model with a ferritin H allele carrying *loxP* sites permitting the conditional deletion of ferritin H by Cre recombinase (Darshan et al., 2009). In the present study, we took advantage of a cross between the floxed ferritin H allele and the transgenic villin-Cre mouse to delete the ferritin H gene specifically in intestinal cells (el Marjou et al., 2004). The deletion

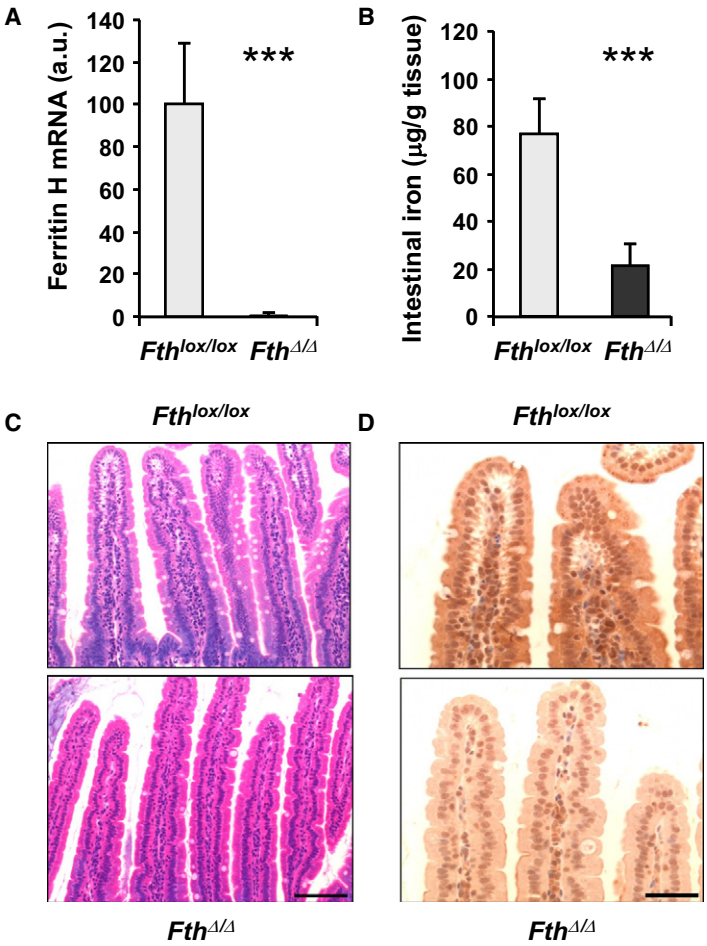

Figure 1. Tissue-Specific Deletion of Ferritin H Mediated by Villin-Cre

(A) Ferritin mRNA expression tested by real-time PCR in total RNA extracts of duodenum in 10- to 13-week-old control $Fth^{lox/lox}$ and experimental $Fth^{\Delta/\Delta}$ mice (average of four animals per group ±SD; ***$p < 0.0005$).

(B) Intestinal iron content of control $Fth^{lox/lox}$ and experimental $Fth^{\Delta/\Delta}$ mice measured by the bathophenanthroline method (average of four animals per group ±SD; ***$p < 0.0005$).

(C) H&E-stained paraffin sections of intestinal villi of control $Fth^{lox/lox}$ and experimental $Fth^{\Delta/\Delta}$ mice.

(D) Immunostaining with rabbit anti-ferritin H antibody and goat anti-rabbit IgG-HRP of the same paraffin sections. Scale bar = 50 µm.

is constitutive at birth in Cre-expressing mice, but absent in Cre-negative littermates. We have compared the iron metabolism in these two sets of mice at 10–35 weeks of age and show that the intestine-specific deletion of ferritin H triggers loss of mucosal iron storage along with increasing serum iron and body iron load. Although expression of duodenal apical transporters was repressed and liver hepcidin mRNA was induced in correlation with increased serum iron, these changes were insufficient to control iron absorption. Ferritin H-deleted animals showed increased ferroportin expression and enhanced ^{59}Fe absorption over 7 days after gavage. In consequence, we propose a revised model for the regulation of iron absorption, in which mucosal ferritin participates actively and complements the hepcidin-mediated control of duodenal ferroportin.

RESULTS

Mice with an intestine-specific ferritin H gene deletion were generated by crossing $Fth^{lox/lox}$ mice (Darshan et al., 2009) with villin-Cre mice (el Marjou et al., 2004) in a predominantly C57BL/6J genetic background. Twelve-week old $Fth^{\Delta/\Delta}$ mice expressed less than 0.5% ferritin H mRNA in the intestinal mucosa compared to nondeleted control $Fth^{lox/lox}$ littermates (Figure 1A). No morphological changes were observed in duodenal villi (Figure 1C). Ferritin H protein expression was consistently reduced in immunological staining of frozen sections from the duodenum of $Fth^{\Delta/\Delta}$ mice (Figure 1D), and tissue iron content diminished due to the absence of iron storage capacity (Figure 1B).

At 10–13 weeks of age, $Fth^{\Delta/\Delta}$ mice showed a significant increase in serum iron concentration (Figure 2A) and transferrin saturation (Figure 2B). Moreover, hepatic iron accumulation increased on average 1.9 times as compared to control $Fth^{lox/lox}$ littermates (Figure 2C). This was not observed in the spleen (Figure 2D). These parameters showed no difference between females and males. In agreement with hepatic iron overload, $Fth^{\Delta/\Delta}$ mice showed on average a 2.4-fold increase in hepcidin mRNA (Figure 2E). It is noteworthy that hepcidin mRNA levels were 1.8-fold higher in females than in males, independent of the ferritin H deletion status. Increased hepcidin mRNA levels correlated positively both with the serum iron concentration and liver iron content (Figure 2F).

Iron accumulated over time in both liver and spleen, with a more pronounced accumulation in females (Figure 3). This was observed both in $Fth^{lox/lox}$ and in $Fth^{\Delta/\Delta}$ mice. The ratio of enhanced liver iron accumulation in $Fth^{\Delta/\Delta}$ versus $Fth^{lox/lox}$ mice was 1.5- to 1.8-fold at all ages (Figure 3A). Splenic iron content was normal in young animals but increased to a 1.8-fold higher level in 35-week old $Fth^{\Delta/\Delta}$ animals, except for 2 out of 6 male $Fth^{\Delta/\Delta}$ mice that remained normal (Figure 3B). Hepcidin mRNA in the liver was consistently higher in $Fth^{\Delta/\Delta}$ versus $Fth^{lox/lox}$ mice at all age groups (Figure 3C), though values in female $Fth^{\Delta/\Delta}$ mice were about 1.8-fold higher than in male $Fth^{\Delta/\Delta}$ mice (Figure 3C). However, in contrast to the hepatic iron content, hepcidin mRNA did not increase with age. Serum iron (Figure 3D) and transferrin saturation (Figure 3E) were always higher in $Fth^{\Delta/\Delta}$ versus $Fth^{lox/lox}$ mice, but did not change significantly with increasing age. These results suggest that over time hepcidin mRNA levels correlated directly to serum iron concentrations and transferrin saturation as has been previously proposed (Lin et al., 2007; Wilkins et al., 2006).

Taken together, these results indicate that mice with the intestinal ferritin H deletion were unable to maintain their iron homeostasis. We therefore measured whether increased hepcidin expression correlated with expected changes in iron transport protein expression. $Fth^{\Delta/\Delta}$ mice showed a highly significant