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EDITORIAL

Ferroxidases and Mammalian Iron Homeostasis: Novel Insight Into a Physiological Phenomenon First Described More Than Half a Century Ago

Intestinal iron absorption is a highly regulated physiological process that determines overall body iron levels, because humans and other mammals cannot efficiently excrete excess iron.[1] Dietary iron is absorbed mainly by duodenal enterocytes. Iron export by these cells is the rate-limiting step in assimilation of dietary iron, which involves ferrous iron export by ferroportin, followed by oxidation to the ferric state. Ferric iron then binds to the iron transport protein transferrin (TF) in the interstitial fluids of the villus lamina propria for distribution to the liver in the portal blood circulation. Iron oxidation is mediated by ferroxidase (FOX) proteins, including hephaestin (HEPH)[2] and possibly ceruloplasmin (CP).[3] The HEPH protein is embedded in the exofacial aspect of the basolateral membrane of duodenal enterocytes, whereas CP is found in serum, and a membrane-anchored form is expressed in various cell types (eg, hepatocytes, macrophages, astrocytes). Intestinal HEPH has been shown to be necessary for optimal intestinal iron transport in mice,[4] but the influence of CP on this process has not been clarified.[5]

The current investigation by Fuqua et al[6] in the current issue of *Cellular and Molecular Gastroenterology and Hepatology* provides novel insight into molecular mechanisms of intestinal iron absorption. The aim of this investigation was to further clarify the physiological roles of the multicopper ferroxidase (MCF) proteins, HEPH and CP, in intestinal iron transport. In this study, the authors tested the hypothesis that iron oxidation by CP complements the FOX activity of HEPH in the upper small intestine. The experimental approach was to cross mice lacking HEPH globally ($Heph^{-/-}$), or only in the intestinal epithelium ($Heph^{int/int}$), with global CP knockout (KO) ($Cp^{-/-}$) mice, thus generating double MCF KO mice. The rationale was that it might not be possible to directly assess CP function in the small intestine in the presence of HEPH because both proteins oxidize ferrous iron.

The authors noted that global KO of both MCF-encoding genes (in $Heph^{-/-}Cp^{-/-}$ mice) led to impaired post-natal growth, splenomegaly and cardiomegaly, iron loading in multiple tissues, depletion of serum iron, and severe hypochromic, microcytic anemia. Some of these physiological perturbations were likely a direct result of the severe anemia (eg, spleen and heart enlargement, impaired growth), whereas others specifically related to a lack of CP FOX activity (eg, iron loading in liver and some other tissues).[7] Moreover, although no overt defect in intestinal iron transport was noted in the absence of these FOXs, evidence suggested that iron absorption in the double KO mice was inappropriately low given the degree of iron deficiency. Also, stainable iron was detected in duodenal enterocytes, indicating abnormal iron retention (or efflux). The combined activity of these MCFs is thus required for optimal intestinal iron transport. It was also noted that distribution of dietary iron was altered in mice with HEPH and CP ablation, with abnormal iron loading being detected in numerous peripheral tissues. This was most prominently exemplified by higher retention of the orally administered, radiolabeled iron dose in the livers of the double KO mice and the impairment of iron delivery to the bone marrow to support erythropoiesis. In contrast, intestine-specific HEPH KO in CP KO mice ($Heph^{int/int}Cp^{-/-}$) had a much less severe phenotype that was essentially indistinguishable from the phenotype of $Cp^{-/-}$ mice.

On the basis of these observations, the authors postulated that lack of the MCFs prevents a fraction of iron exported from the intestine from binding to TF in the interstitial fluids. In this scenario, unbound ferrous iron (ie, non–transferrin-bound iron [NTBI]) appears in the portal circulation and is then taken up by hepatocytes in an unregulated, non-specific fashion (because the liver normally acquires dietary iron from diferric TF). The authors further postulate that HEPH and CP may be important in a variety of cell types to ensure that exported iron is bound to TF, enabling the released iron to be targeted to developing erythroid cells rather than being taken up by other tissues non-specifically as NTBI.

In summary, this investigation has demonstrated that the MCFs HEPH and CP function in tandem to maintain intestinal and systemic iron homeostasis. Both proteins are required to provide the necessary oxidizing equivalents to permit efficient ferrous iron oxidation in the duodenal epithelium. In their absence, as hypothesized by these authors, NTBI appears in the portal circulation, and because hepatocytes have evolved to specifically uptake iron from diferric TF, hepatic iron homeostasis is perturbed. This investigative team further postulates that HEPH has important extraintestinal functions that facilitate iron delivery to the erythroid marrow. Overall, this study has advanced our collective knowledge of the critical process of intestinal iron absorption.

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Conflicts of interest

The author discloses no conflicts.

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