Lead Article

Metabolic crossroads of iron and copper

James F Collins, Joseph R Prohaska, and Mitchell D Knutson

Interactions between the essential dietary metals, iron and copper, have been known for many years. This review highlights recent advances in iron-copper interactions with a focus on tissues and cell types important for regulating whole-body iron and copper homeostasis. Cells that mediate dietary assimilation (enterocytes) and storage and distribution (hepatocytes) of iron and copper are considered, along with the principal users (erythroid cells) and recyclers of red cell iron (reticuloendothelial macrophages). Interactions between iron and copper in the brain are also discussed. Many unanswered questions regarding the role of these metals and their interactions in health and disease emerge from this synopsis, highlighting extensive future research opportunities.

INTRODUCTION

Optimal nutritional support is critical for mammalian development and health. One global nutrient deficiency, the trace element iron, illustrates the potential consequences of inadequate nutrition on human development. In infancy, for example, lack of sufficient iron intake can result in cognitive limitations that persist into adulthood.[1] It is therefore important to identify and correct iron deficiency as early as possible. Moreover, recent dietary intake data available online from What We Eat In America, NHANES[2] reports daily iron consumption at several mg below the current RDA of 18 mg. Does this pose a threat to pregnant and lactating women who require more iron and may not consume adequate dietary iron or supplemental iron? Copper intake, in contrast, appears to be near or to exceed the RDA of 0.9 mg. The extra copper needed for pregnancy and lactation, however, is often not met by diet alone and many prenatal supplements no longer contain copper. Thus, it seems likely that both iron and copper deficiency can occur in select populations consuming normal diets. In fact, anemia due to copper deficiency has been reported frequently in adults.[3]

The tools available to assess iron status in neonates rely on hemoglobin measurements and serum iron, which are typically low in iron-deficient infants. There may be other factors that result in the same clinical phenotype. Notably, copper deficiency in infants when first detected was associated with low hemoglobin levels and low serum iron.[3] Is this because of dual deficiencies of both copper and iron, or rather an iron deficiency induced by copper limitation? Recently, a study performed in copper-deficient rat pups showed that iron injection (without copper) restored low serum iron and low hemoglobin levels to normal, demonstrating that in early life copper deficiency results in iron deficiency.[4] Conversely, it is well known that iron administration fails to reverse anemia in older copper-deficient rodents.[5,6] Thus, an understanding of the copper-iron interaction is critically important in order to treat the apropos deficiency with the correct nutrient. For example, treating the anemia of copper deficiency with iron is like treating the anemia of B_{12} deficiency with folate. The anemia disappears but the underlying problems of specific nutrient deficiencies do not.

An overview of whole-body iron and copper homeostasis highlights points of interaction between the two

Affiliations: *JF Collins* and *MD Knutson* are with the Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida, USA. *JR Prohaska* is with the Department of Biochemistry and Molecular Biology, University of Minnesota Medical School Duluth, Duluth, Minnesota, USA.

Correspondence: *MD Knutson*, Food Science and Human Nutrition Department, PO Box 110370, University of Florida, Gainesville, FL 32611, USA. E-mail: mknutson@ufl.edu, Phone: +1-352-392-1991 Ext. 204, Fax: +1-352-392-9467.

Key words: anemia, ceruloplasmin, copper, hephaestin, iron

doi:10.1111/j.1753-4887.2010.00271.x

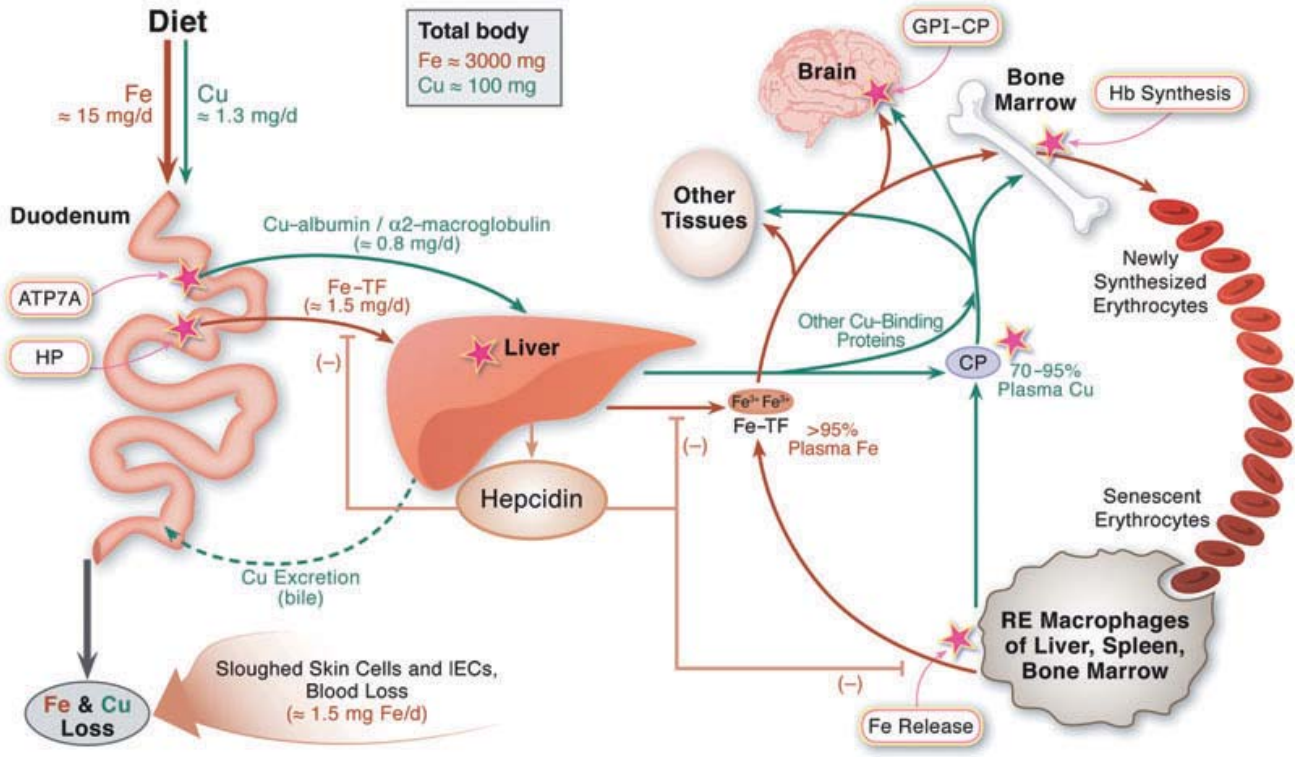

Figure 1 **Overview of whole body iron and copper homeostasis, highlighting points of interaction.** The principal organs controlling body iron and copper homeostasis are shown with points of interaction between the two metals indicated by a star (✶). Dietary iron and copper are absorbed in the duodenum, where two points of interaction occur; namely, the copper-dependence of HP (✶), and the strong induction of ATP7A (✶) during iron deficiency. Absorbed copper is transported by albumin or α_2-macroglobulin in the portal blood to the liver, where it is incorporated into another ferroxidase, CP (✶) or excreted into the bile. Absorbed iron binds to TF (Fe-TF) for transport to the liver. Iron may be stored in hepatocytes bound to ferritin or released into the circulation, where it is distributed by Fe-TF to the cells of the body. Copper is released from the liver principally on CP, which functions to deliver copper to other organs/tissues and which is important for iron release from certain tissues. Copper is efficiently delivered to these other tissues in the absence of CP, so other copper-binding proteins/ligands must exist in the serum. Systemic Fe-TF delivers iron predominantly to the bone marrow for red cell hemoglobin production; iron utilization by these cells is copper dependent (✶). Iron is also taken up by other tissues, including the brain, where iron release is dependent upon GPI-CP (✶). Iron contained within erythrocytes is eventually recycled by RE macrophages, where it can be stored until times of need. The release of iron from RE macrophages is a copper-dependent process, again involving CP and GPI-CP (✶). Iron homeostasis is controlled by the liver-derived peptide hormone hepcidin, which functions to inhibit iron absorption from the diet, and iron release from storage sites in the liver and RE macrophages. There are no active excretory mechanisms for iron, but some iron is lost by sloughing of skin and enterocytes and by blood loss.
Abbreviations: ATP7A, ATPase, Cu^{++} transporting, alpha polypeptide; CP, ceruloplasmin; Fe-TF, iron-transferrin; GPI-CP, glycosylphosphatidylinositol-linked CP; HP, hephaestin; RE, reticuloendothelial; TF, transferrin.

metals (Figure 1). Specific molecular interactions that occur between the two metals within various cell types, along with normal cellular homeostasis of each metal, are detailed in Figure 2. Cell types include the absorptive epithelium of the intestinal tract (enterocytes) and those managing metals in the liver (hepatocytes). Also shown are cells involved in iron recycling and storage (reticuloendothelial, RE, macrophages) and those that utilize the vast majority of iron for hemoglobin production (erythroid cells). Not shown in Figure 2, but discussed in the text, is copper and iron homeostasis in brain cells, which is relevant to many pathological states whereby these metals are deposited in various cell types within the brain causing oxidative stress and associated damage. Also discussed in the text are important observations that have been recently noted regarding the differences and similarities in iron and copper homeostasis among commonly used laboratory rodent models.

As seen in Figure 1, dietary iron and copper are absorbed in the upper small bowel. Absorption of both minerals is regulated. Iron absorption, which is controlled by the liver-derived, peptide hormone hepcidin, is

modulated to suit the body's demand for iron, particularly for erythropoiesis. Hepcidin regulates iron absorption by inhibiting iron efflux from enterocytes. Hepcidin also inhibits iron release from important storage depots in the body, including autocrine action on hepatocytes and endocrine action on RE macrophages. Conversely, the precise control of copper transport in the gut is not currently understood, but absorption is modulated in relation to intake levels, with percent absorption being higher with low intakes.[7] Under certain circumstances, a mucosal block to metal absorption may occur via sequestration of iron and copper by ferritin and metallothionein, respectively, with the net effect being the loss of the metals, as enterocytes are sloughed off into the intestinal lumen. Important iron-copper interactions in the gut include the regulation of hephaestin (HP; a multi-copper ferroxidase necessary for iron efflux from enterocytes) by dietary copper levels and the regulation of the Menkes copper ATPase (ATP7A; a protein necessary for copper efflux) expression by iron levels. There are no regulated excretory mechanisms for iron, whereas copper is excreted in the bile (as mediated by the copper exporter, ATP7B) and then passes out of the body with the feces. Iron binds to transferrin (Fe-TF) in the portal blood for delivery to the liver, and copper binds to albumin or α_2-macroglobulin.[8]

After absorption and transport to the liver, iron may be stored in hepatocytes or secreted on transferrin (Fe-TF) for distribution to body tissues. Most copper leaves the liver within the ceruloplasmin (CP) molecule, but other copper-binding proteins must exist in the serum as copper is efficiently distributed in the absence of CP (e.g., during aceruloplasminemia).[9] The absence of CP, however, leads to iron accumulation in the pancreas, retina, and brain, indicating that the ferroxidase activity of circulating CP is critical for normal iron homeostasis.[10] Liver copper levels vary inversely according to iron status for unexplained reasons, exemplifying another important interaction between these two metals.

Iron and copper are delivered from the liver to all organs/tissues/cells of the body, where they are required for cellular metabolism. The ferroxidase activity of a GPI-anchored form of CP (GPI-CP) is necessary for iron release from brain and RE macrophages. This process may thus be affected by body copper levels as CP (and GPI-CP) activity requires copper as a prosthetic group. Most iron is delivered to the bone marrow for hemoglobin production. Interestingly, an unknown aspect of bone marrow iron utilization is copper dependent, for during copper deficiency, hemoglobin production is inefficient, despite normal serum iron levels.[11]

The purpose of this review is to critically review recent data on copper-iron interactions with a focus on overall body homeostasis of these metals. It is hoped that the information presented here will stimulate research to reveal the mechanism for this interaction, which has been known since the 19th century.[12] Many believe the interaction is due to CP, a circulating, copper-dependent enzyme capable of oxidizing ferrous to ferric iron. However, aceruloplasminemia in humans does not result in frank anemia.[13] Others suggest the copper-iron interaction involves HP, a CP homolog expressed in the intestine with similar catalytic properties.[14] An intestinal block in iron uptake could lead to anemia. However, despite a modest elevation in enterocyte iron following copper deficiency, whole body iron is normal, which challenges the concept of anemia being caused by limitations in iron uptake.[11] Clearly, the mechanism(s) for anemia in copper deficiency is not yet known.

ENTEROCYTES

The mechanisms by which dietary iron and copper are absorbed in the upper small intestine have been elucidated in recent years. Several transport proteins and chaperone molecules have been identified by investigations performed principally in mutant rodent models and knockout mice. Dietary non-heme iron (the major source of iron in most human populations) is predominantly in the ferric oxidation state and must be reduced for absorption (Figure 2A). This may occur by cytochrome B reductase 1 (CYBRD1).[15] Cybrd1 is a gene that is strongly induced during iron deficiency in laboratory rodents,[16,17] along with divalent metal transporter 1 (Dmt1), transferrin receptor 1 (TfR1), and other genes with proven roles in iron absorption, which suggests it may have an important physiological role in intestinal iron homeostasis. Cybrd1-null animals, however, were found to not have an iron-deficient phenotype.[18] Although these observations suggest that this reductase is dispensable for intestinal iron absorption, the animals were fed ferrous iron and direct measurements of ferric iron uptake by the intestine were not made; nor was iron deficiency induced in the null animals. Thus, the exact role of Cybrd1 in iron absorption remains unresolved. Other candidate intestinal ferrireductases include cytochrome b (558) ferric/cupric reductase[19] and Steap2.[20] After reduction, ferrous iron (Fe^{2+}) is transported into enterocytes by DMT1,[21] coupled to the electrochemical H^+ gradient from outside to inside cells. The important role of this transporter in intestinal iron uptake is best exemplified by the intestinal-specific Dmt1 knockout mouse, which has a severe iron-deficient phenotype due to impaired vectorial iron transport in the upper small intestine.[22]

Dietary iron can also be in the heme form, obtained principally from meat sources. A recent report described the identification of an apically expressed intestinal heme transporter (heme carrier protein 1; HCP1),[23] but current

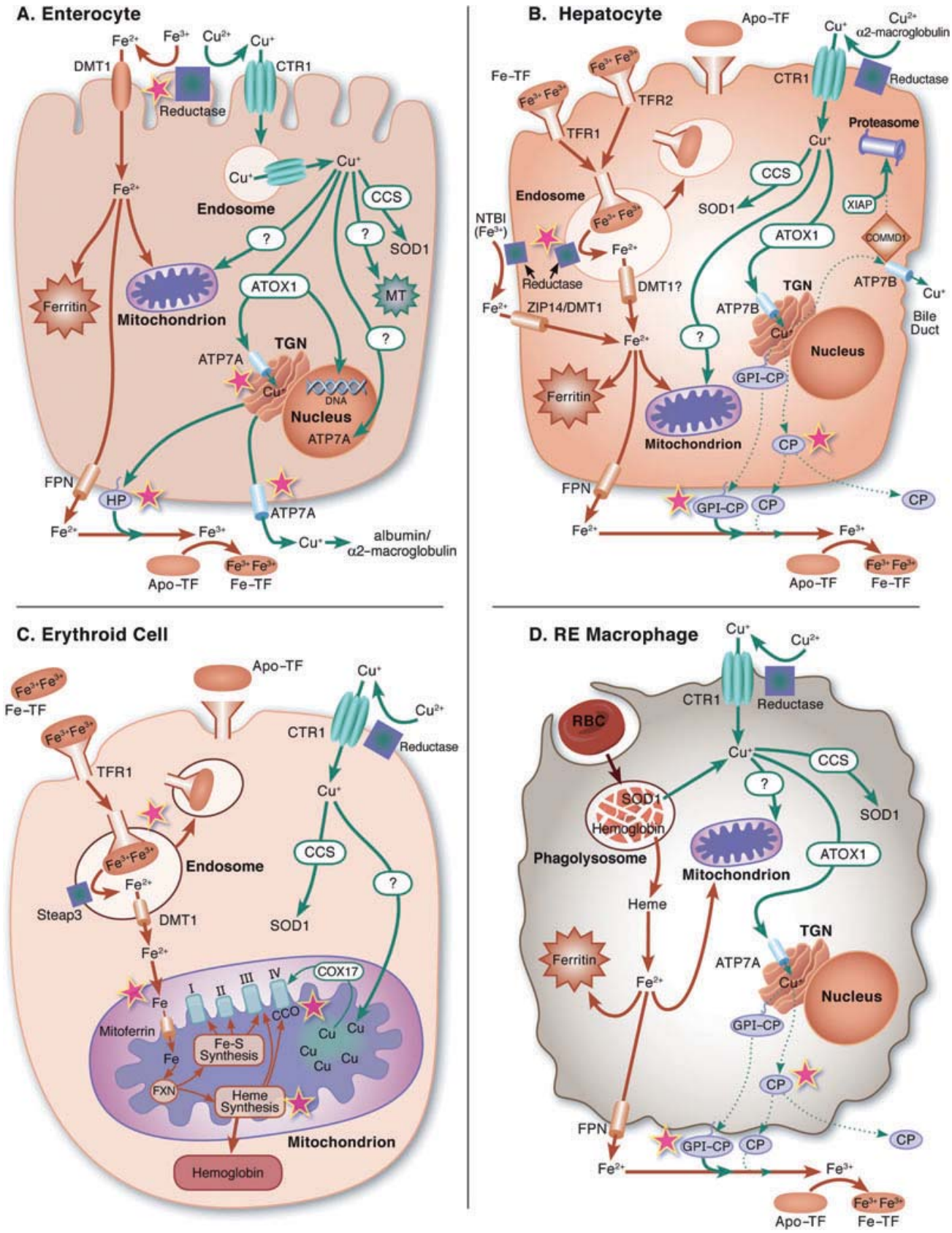
A. Enterocyte
DMT1
CTR1
Reductase
Endosome
CCS
SOD1
Ferritin
Mitochondrion
ATOX1
TGN
ATP7A
Nucleus
DNA
FPN
HP
albumin/
α2-macroglobulin
Apo-TF
Fe-TF
B. Hepatocyte
Apo-TF
α2-macroglobulin
Fe-TF
TFR2
TFR1
Proteasome
XIAP
COMMD1
ATP7B
Bile
Duct
NTBI
(Fe3+)
ZIP14/DMT1
DMT1?
GPI-CP
CP
C. Erythroid Cell
Steap3
COX17
Mitoferrin
Fe-S
Synthesis
FXN
Heme
Synthesis
Hemoglobin
D. RE Macrophage
RBC
Phagolysosome
Hemoglobin
Heme

Figure 2 **Main pathways of cellular iron and copper transport, highlighting points of interaction.** The principal cells controlling body iron and copper homeostasis are shown with points of interaction between the two metals indicated by a star (✶). (**A**) Duodenal enterocytes absorb dietary iron and copper via transporters located at the apical membrane. Absorption of oxidized forms of the metals requires the action of a cell-surface reductase. In the cytosol, chaperone proteins deliver copper to various proteins and organelles; excess copper is sequestered by MT. How cytosolic iron is transported is less well characterized, but may also involve chaperone proteins, at least for delivery of iron to ferritin. Iron and copper are exported across the basolateral membrane by FPN and ATP7A, respectively. Copper deficiency reduces levels of the copper-containing ferroxidase, HP (✶), required for efficient enterocyte iron efflux and loading onto TF (Fe-TF). Iron deficiency markedly increases levels of basolateral ATP7A (✶), perhaps increasing copper efflux. (**B**) Hepatocytes control iron metabolism by producing the iron-regulatory hormone hepcidin (not shown). These cells also control copper homeostasis, primarily by regulating the secretion of copper into the bile. Hepatocytes can take up Fe-TF via TFR1 or TFR2 cycling through the endocytic pathway. In the endosome, iron carried on Fe-TF is reduced and transported into the cytosol by mechanisms that are not well understood. During iron overload conditions, hepatocytes can also take up NTBI via ZIP14 or perhaps DMT1. Copper taken up via hepatocyte CTR1 is delivered to proteins including ATP7B, which pumps copper into the TGN for incorporation into GPI-CP and CP. These proteins serve a function analogous to HP in the enterocyte. Accordingly, copper deficiency or genetic ablation of CP, including GPI-CP (✶), causes hepatocyte iron accumulation. High copper levels induce the translocation of ATP7B from the TGN to the bile duct, facilitating biliary copper excretion, a process that requires interaction between ATP7B and COMMD1. Proteasomal degradation of ATP7B is regulated by XIAP. (**C**) Developing erythroid cells, the most avid consumers of iron in the body, acquire iron exclusively through the Fe-TF/TFR1 cycle. Endosomal Steap3 probably catalyzes the reduction of iron prior to DMT1-mediated transport into the cytosol. Virtually all the iron in these cells is directed to the mitochondria, where it is transported into the matrix via mitoferrin. The chaperone, FXN, is thought to deliver iron to the sites of Fe-S cluster assembly and heme synthesis. Fe-S and heme are incorporated into proteins comprising complexes I-IV of the electron transport chain. Most copper taken up by erythroid cells is delivered by CCS to SOD1. Copper is also directed to the mitochondrion for incorporation into complex IV (CCO) (✶), in which two copper atoms work in concert with two heme groups to transport electrons. Mitochondrial copper is delivered to CCO by the chaperone COX17. Copper deficiency has been shown to decrease the assimilation of iron from Fe-TF (✶), impair the uptake of iron by mitochondria (✶), and reduce heme synthesis (✶). Molecular details for these iron-copper interactions in erythroid cells remain to be elucidated. (**D**) RE macrophages play a central role in iron metabolism by phagocytosing senescent red blood cells and recycling their iron. Red cell hemoglobin is degraded within the phagolysosome, releasing heme, which is further catabolized to liberate iron. The freed iron is either exported, stored, or utilized by the cell. Although RE macrophages can take up copper via CTR1, they also accrue copper by phagocytosis of red cells, which contain the metal as part of SOD1. Efficient release of iron from RE macrophages, as well as cell-surface expression of FPN, requires GPI-CP/CP (✶).

Abbreviations: Apo-TF, apo-transferrin; ATP7A, ATPase, Cu^{++} transporting, alpha polypeptide; ATP7B, ATPase, Cu^{++} transporting, beta polypeptide; ATOX1, anti-oxidant protein 1; COMMD1, copper metabolism (Murr1) domain containing 1; CCS, copper chaperone for SOD1; COX17, cytochrome c oxidase copper chaperone 17; CP, ceruloplasmin; CTR1, copper transporter 1; DMT1, divalent metal transporter 1; Fe-TF, iron-transferrin; FPN, ferroportin; FXN, frataxin; GPI-CP, glycosylphosphatidylinositol-linked CP; HP, hephaestin; MT, metallothionein; NTBI, non-transferrin-bound iron; RE, reticuloendothelial; SOD1, superoxide dismutase 1; Steap3, six transmembrane epithelial antigen of the prostate 3; TFR1, transferrin receptor 1; TFR2, transferrin receptor 2; TGN, *trans*-Golgi network; ZIP14, Zrt- and Irt-like protein 14; XIAP, X-linked inhibitor of apoptosis protein.

evidence suggests it functions mainly as an intestinal folate transporter (renamed proton-coupled folate transporter; PCFT),[24] because mutation of the gene in humans causes hereditary folate malabsorption. There is, however, recurring debate regarding the possible physiological role of PCFT/HCP1in intestinal heme transport.[25] The scientific community thus awaits the positive identification of the intestinal heme transporter.

Once inside enterocytes, it is not exactly clear how iron is handled. One candidate iron chaperone that delivers iron to ferritin has been identified recently,[26] but its role in the intestinal epithelium is currently unknown. One proposed theory is the "transcytosis" of iron, whereby absorbed iron atoms remain within intracellular vesicular structures, which may fuse with vesicles derived from the basolateral surface for export out of cells or for delivery of iron to other intracellular compartments.[27] Iron may also be bound by cytosolic ferritin, amounting essentially to a mucosal block to iron absorption, as ferritin-bound iron is ultimately lost when enterocytes are sloughed off into the intestinal lumen.

Iron is transported across the basolateral membrane via ferroportin (FPN).[28,29] Once ferrous iron has been effectively pumped out of cells, it is oxidized by HP,[30] a multi-copper ferroxidase that localizes to the basolateral membrane of enterocytes in close proximity to FPN.[31] HP

is required for intestinal iron absorption, as evidenced by the anemic phenotype of the sex-linked anemic (sla) mouse.[30] Once iron is oxidized, it may bind with apotransferrin (Apo-TF) in the lamina propria for transport in the portal blood to the liver. Another circulating, multicopper ferroxidase of hepatic origin, CP, may also participate in intestinal iron transport, particularly during states of hematopoietic stress.[32]

The mechanism by which dietary copper is absorbed is distinct from that of iron, although complementary mechanisms seem plausible given the atomic properties of the two highly reactive and structurally similar transition metals. Dietary copper, like iron, must be reduced (Cu^{2+} to Cu^{+}) for transport across the apical membrane into enterocytes. Candidate proteins that can accomplish this include cytochrome b (558) ferric/cupric reductase,[19] Steap 2,[20] and CYBRD1,[33] which can all reduce copper in addition to iron. Once reduced, the metal is likely transported into the enterocyte by copper transporter 1 (CTR1).[34] The precise mechanism of this transport process is not currently clear, as intestine-specific knockout of Ctr1 leads to increased enterocyte copper levels, with the copper being apparently trapped in an intracellular compartment where it is not biologically available.[34] These mice develop severe systemic copper deficiency, indicating an essential role for Ctr1 in copper absorption. Interestingly, CTR1 has been detected on the basolateral surface of enterocytes as well,[35,36] creating some controversy regarding its precise role in intestinal copper transport. It is also possible that Dmt1 is involved in dietary copper absorption, as in vitro evidence has shown that it can transport copper[37]; however, the precise role of Dmt1 in the absorption of metals besides Fe (and Mn) remains controversial.[38] A potential role for Dmt1 in copper transport certainly seems plausible during dietary iron deprivation, when Dmt1 mRNA and protein levels are very strongly induced in the setting of no competing iron atoms.[17,39,40] Another possible mechanism for copper transport into enterocytes is an ATP-dependent mechanism recently described in Belgrade rats, a Dmt1-deficient rodent model.[41]

Once in cells, copper is bound by one of several chaperone proteins that deliver copper to the mitochondria (possibly COX17; a chaperone for cytochrome c oxidase),[42] to the *trans*-golgi network (ATOX1; a chaperone for the Menkes copper ATPase [ATP7A]),[43] or to the cytosol for Cu, Zn-superoxide dismutase (SOD1) expression (copper chaperone for SOD1 [CCS]).[44] A possible nuclear copper chaperone protein representing an alternatively spliced form of the ATP7A transcript has also been hypothesized.[45] ATOX1 has additionally been described to be a copper-activated transcription factor involved in cell cycle control.[46,47] Whether ATOX1 is involved in enterocyte proliferation and differentiation is not currently known, but it is certainly an intriguing possibility. Excess copper may also be bound in cells by metallothionein (MT),[48] which would presumably lead to copper loss when enterocytes are sloughed off into the lumen. Finally, copper may be transported out of enterocytes by ATP7A, where it is then bound to albumin or α_2-macroglobulin for delivery to the liver via the portal circulation.

The interplay between iron and copper homeostasis in the intestinal epithelium has recently been described at the molecular level. One such example is the multicopper ferroxidases, HP and CP, which both play important roles in intestinal iron transport. HP activity is dependent upon normal copper levels, as exemplified by the fact that decreased HP activity has been documented in the intestine of copper-deficient mice, which correlates with systemic iron deficiency.[14] Additional studies have shown that copper deficiency reduces iron absorption in rats[49] via a mechanism thought to involve decreased activity of duodenal hephaestin.[50] Furthermore, repletion of copper-deficient rats with dietary copper restores intestinal iron absorption and Hp protein levels.[51] These observations support earlier studies that also documented decreased iron transport in copper-deficient swine.[52] Moreover, the same phenomenon was shown in differentiated Caco-2 cells (a widely accepted model for the human intestinal epithelium); copper deficiency led to decreased HP protein and activity levels, presumably resulting in the documented decrease in iron efflux from cells.[53] Another recent investigation confirmed that HP is necessary for iron efflux in colorectal cancer cell lines (HT29 and WiDr), as manipulation of the levels of CDX2, a *trans*-acting factor implicated in HP gene expression, altered not only HP expression but also intracellular iron levels.[54] This study thus revealed a regulatory pathway connecting cellular iron levels to iron export mediated by CDX2-induction of HP expression.

On the other hand, CP, a circulating multi-copper ferroxidase of hepatic origin, has not historically been strongly associated with basolateral iron flux. A recent study, however, demonstrated that Cp plays a redundant role in iron absorption to Hp during times of stress.[32] These studies, carried out on $Cp^{-/-}$ mice, revealed a marked impairment of iron absorption in acutely bled Cp-null mice, suggesting that Cp plays an important physiological role in iron transport for which Hp apparently cannot compensate. Moreover, Cp was found to relocalize from the duodenal epithelium into the lamina propria when wild-type mice were bled. These observations suggest that the role of Cp in basolateral iron efflux needs to be reconsidered and investigated further.

Interestingly, in vivo studies have demonstrated that there might be changes in the absorption of one of these trace minerals by perturbations in the homeostasis of the

other. For example, copper absorption may be increased during states of iron deficiency, as suggested by increased metallothionein (MT) mRNA expression (>20-fold)[17,55] and ATP7A mRNA and protein expression in the duodenal epithelium,[17,39] as well as increased copper levels in the intestinal mucosa and liver.[39,55] These observations may be indicative of enhanced copper transport across the apical membrane of enterocytes, although the precise mechanism by which this could occur has not been delineated. Another potential iron-copper interaction in the gut relates to the proposed role of ATOX1 in control of cell proliferation and differentiation. ATOX1 translocation to the nucleus and its *trans*-activating capabilities are stimulated by copper in endothelial and cancer cells.[47] As mentioned, copper accumulates in enterocytes during iron deficiency.[39] Moreover, there are documented morphological changes in the intestinal epithelium during iron deficiency, including increased villus height and width, crypt depth and overall mucosal thickness, and increased numbers of mitotic cells in the crypts.[56,57] It was also shown that enterocytes in the lower half of the villus absorbed iron in iron-deficient rats as compared to those higher up the villus in controls.[57] These observations are consistent overall with alterations in duodenal cell proliferation and differentiation during iron deficiency. It is thus conceivable that ATOX1 is involved in the adaptive response of the intestinal epithelium to iron deficiency, perhaps further linking iron and copper metabolism.

Of additional interest is the recent description of the regulation of the iron transport-related genes Dmt1 and Cybrd1 by the hypoxia inducible factor, Hif-2α.[40,58] This observation is of particular importance because the HIF factors are stabilized by copper, resulting in enhanced expression of HIF-responsive genes (e.g., Cp in the liver by Hif-1α).[59,60] This then raises the possibility that increased copper levels in enterocytes,[39] as well as in the liver and in the body fluids,[56,61] may play a role in enhancing HIF activity, which in turn regulates genes related to intestinal (and body) iron homeostasis.

Finally, studies in the Caco-2 cell model have shown that depletion of iron or copper led to increased uptake of both metals into cells[62] and that depletion of either metal led to enhanced iron flux from the apical to the basolateral cell culture chamber. It was also noted that copper supplementation enhanced expression of iron transport-related genes in Caco-2 cells. The authors of this study suggested that copper repletion induced intracellular iron depletion, ultimately resulting in increased transepithelial iron flux.[63] Although the precise molecular mechanisms underlying these observations are not known, they further exemplify the intimate connections between iron and copper in the intestinal epithelium and demonstrate the need for additional investigations in this area.

HEPATOCYTES

After absorption by the duodenum, iron (Fe) in portal blood is bound to transferrin (Fe-TF) and transported to the liver (Figure 1). Fe-TF is taken up by hepatocytes (Figure 2B) in proportion to the amount of transferrin receptor 1 (TFR1).[64] The liver may also take up Fe-TF via TFR2, a TFR1 homologue that has been shown to enhance Fe-TF uptake by cells.[65] After endocytosis of the Fe-TF/TFR complex, vesicular acidification causes Fe-TF to release its iron, which is reduced and transported into the cytosol. It is presumed that DMT1 mediates the transfer of iron from the endosome to cytosol,[66] but $Dmt1^{-/-}$ mice are able to assimilate iron into the liver (including hepatocytes), indicating that this protein is dispensable for hepatic iron uptake. In iron overload conditions, the carrying capacity of plasma TF becomes exceeded, giving rise to non-TF-bound iron (NTBI), which is rapidly cleared, mainly by the liver. Uptake of NTBI into hepatocytes is mediated, at least in part, by ZIP14,[67,68] and perhaps DMT1.[69] Iron taken up by the hepatocyte is either used for synthesis of iron-requiring proteins, stored in ferritin, or exported via FPN.

Absorbed copper is bound to α_2-macroglobulin or albumin and transported to the liver (Figure 1). Studies in the human hepatoma cell line, HepG2, provide evidence that α_2-macroglobulin delivers copper primarily to CTR1, whereas albumin donates the metal to a different, yet undefined, uptake mechanism.[70] Assimilation of Cu^{2+} from either protein requires a reduction step because CTR1 transports only Cu^{+}.[71] Targeted deletion of hepatocyte Ctr1 in mice demonstrated the importance of this protein in hepatic copper uptake in vivo.[72] Once inside the cell, copper is bound to chaperones and distributed to copper-requiring proteins (Figure 2B). ATP7B pumps copper into the TGN where it is incorporated into CP[73] and other cuproproteins. Excess copper stimulates the translocation of ATP7B from the TGN to the canalicular membrane of the hepatocyte, facilitating the secretion of copper into the bile.[74] Biliary secretion of copper appears to require the interaction between ATP7B and COMMD1 (copper metabolism MURR1 domain), although the mechanism for this effect remains poorly understood.[75] COMMD1 levels are regulated by the ubiquitin ligase XIAP (X-linked inhibitor of apoptosis).[76]

Hepatocyte iron and copper metabolism are linked through CP, a copper-containing enzyme that catalyzes the oxidation of Fe^{2+} to Fe^{3+}, the form of iron that binds to Apo-TF. CP exists as a soluble secreted form and a glycosylphosphatidylinositol (GPI)-anchored form (GPI-CP).[77] Efficient iron release from hepatocytes requires CP, as formally demonstrated by hepatocyte iron accumulation of $Cp^{-/-}$ mice.[78] Copper-deficient rodents also

accumulate iron in hepatocytes,[79] most likely because of reduced Cp activity.

The Steap (six-transmembrane epithelial antigen of the prostate) family of proteins has recently emerged as another potential link between iron and copper homeostasis.[80] Steap proteins share distant, though significant, sequence homology with yeast FRE (Fe^{3+} reductase) proteins. Yeast use FRE proteins not only for the uptake of iron, but also for the reduction and uptake of copper. Similarly, three of the four known mammalian Steap proteins (Steap2, Steap3, and Steap4) have been shown to reduce both iron and copper, enhancing their cellular uptake.[20] Epitope-tagged Steap proteins have been localized to endosomes and the plasma membrane.[20,81] Analysis of Steap3$^{-/-}$ mice demonstrated that this reductase was required for efficient uptake of Fe-TF by erythroid cells (discussed below).[81] Steap3 is most abundantly expressed in liver (and fetal liver), suggesting it may serve as a reductase in this organ as well. If so, one would predict lower hepatic copper/iron concentrations in mice lacking Steap3. Interestingly, mice harboring a mutation in Steap3 that decreases ferrireductase activity were found to have not lower but higher concentrations of hepatic copper and iron.[82] Although this study does not clarify the function of Steap3 in the liver, it does suggest a role for this metalloreductase in hepatic copper/iron metabolism. Future studies need to evaluate tissue iron and copper status in Steap3-null animals.

In addition to its cupric/ferric reductase activity, Steap3 appears to play an essential role in the secretion of exosomes.[83] First discovered as membrane vesicles that extruded TFR1 from maturing reticulocytes, exosomes are now recognized as important cellular and physiologic mediators.[84] Indeed, defective exosome formation leading to deregulation of TfR1 shedding in Steap3-null mice has been proposed as an alternative explanation for the anemia of these animals.[83] A number of iron/copper-related proteins have been shown to undergo exosomal secretion, including TFR2,[85] GPI-CP,[86] ATP7A, and ATP7B.[87] The contribution of these secreted proteins to the regulation of cellular/systemic iron and copper homeostasis remains to be determined.

Since the 1960s, numerous studies have documented that hepatic copper concentrations vary inversely with iron status. Iron-deficient rodents have elevated hepatic copper levels,[39,56,88–92] whereas iron-loaded animals have decreased levels.[93] The molecular basis for this relationship remains unknown. In iron deficiency, it is possible that elevated hepatic iron concentrations result from increased absorption of dietary copper. This possibility is supported by the observation that iron deficiency causes dramatic increases in intestinal expression of the basolateral copper exporter Atp7a and Cybrd1,[17,39] which can function as a cupric reductase.[33] Studies in rats, however, found no increase in copper absorption after the induction of iron deficiency by diet or bleeding.[94] Alternatively, it is possible that iron deficiency decreases biliary secretion of copper, incorporation of copper into secreted copper-containing proteins (e.g., CP), or the mobilization of copper from the liver.

ERYTHROID CELLS

Erythroid cells are the most avid consumers of iron in the body. Each day, erythroid precursors of the bone marrow take up 20–25 mg of iron as they mature into erythrocytes. Unlike hepatocytes or enterocytes, which can rapidly assimilate NTBI, erythroid cells can acquire iron only from Fe-TF (Figure 2C). At the cell surface, TFR1 binds to circulating Fe-TF, leading to internalization of the Fe-TF/TFR1 complex into endosomes. The acquired ferric iron is then reduced to Fe^{2+} by Steap3 and transported into the cytosol by DMT1. That Steap3 serves as the endosomal ferrireductase in erythroid cells was revealed by studies of Steap3-mutant and Steap3-null animals.[19] The essential role of DMT1 in iron assimilation by erythroid cells was demonstrated by mice engineered to lack Dmt1 in hematopoietic cells,[22] as well as by studies of reticulocytes from anemic Belgrade rats, which express a poorly functional mutant form of the protein.[95]

Nearly all the iron taken up by developing erythroid cells is rapidly directed to mitochondria, where it is used for heme biosynthesis and Fe-S cluster protein assembly.[96] Mitochondrial iron assimilation requires mitoferrin, an iron transporter located on the inner mitochondrial membrane.[97] The iron chaperone, frataxin, is thought to deliver iron to the site of Fe-S formation and heme synthesis.[98] In the developing erythroid cell, the overwhelming majority of newly synthesized heme is destined for hemoglobin in mature erythrocytes, ultimately accounting for 70% of total body iron.

Human erythrocytes contain approximately 2.5 μg Cu/g.[99] As with other cell types, it is probable that erythroid cells obtain their copper via CTR1. Reduction of Cu^{2+} at the plasma membrane may be facilitated by the metalloreductases Steap2, Steap3 or Steap4, which are all expressed in erythroid cells.[20,81] The contribution of Steap3 to copper uptake can (and should) be readily assessed by measuring copper concentrations in erythrocytes of Steap3-null mice. An estimated 60% of the copper in mature erythrocytes is associated with SOD1.[100] Copper is delivered to SOD1 in the cytosol by the copper chaperone CCS. Levels of CCS in erythrocytes have proved to be a reliable indicator of copper deficiency in various species.[101,102] Copper also traffics to the mitochondrial matrix where it becomes part of a bioactive pool of copper that is used for the metallation of proteins such as cytochrome c oxidase (CCO). Also known as complex IV

of the electron transport chain, CCO contains two copper atoms as well as two heme molecules. The delivery of copper to CCO requires COX17.[103]

In terms of iron-copper interactions, no cell type has been studied more than the erythroid cell. Indeed, the demonstration by Hart et al. in 1928[104] that copper has an important function in hemoglobin synthesis is acknowledged as the beginning of the iron/copper field.[12] In their classic studies, Hart, Elvehjem, and colleagues showed that rats fed an all-milk diet developed an anemia that could not be corrected by supplementation with iron salts.[104] The anemia was, however, readily corrected by supplementation with copper, revealing an essential role for copper in hemoglobin formation. Why does copper deficiency lead to anemia? It is frequently cited that copper-deficiency anemia results from diminished activities of CP and HP, the two copper-containing proteins that are required for efficient iron release from stores and for iron absorption.[5,14] According to this model, anemia ensues because sufficient iron cannot be mobilized from stores or absorbed from the diet. However, several studies have documented that iron administration – including intravenous iron injection, which bypasses any defect in iron absorption or release from stores – fails to correct the anemia in copper-deficient animals.[5,105] Moreover, copper-deficient mice can become anemic despite having normal plasma iron concentrations,[11] indicating that the anemia does not result from insufficient iron availability to developing erythroid cells of the bone marrow. These studies rather indicate that copper deficiency affects iron utilization by erythroid cells. Defective erythroid iron utilization in copper deficiency may reflect a defect in any one or a combination of these processes: cellular uptake, intracellular mobilization, uptake into mitochondria, or heme/hemoglobin synthesis. Studies of copper-deficient rat reticulocytes have documented that iron accumulates in ferritin in intracellular vesicular bodies, but its uptake into mitochondria is impaired, even under conditions of iron loading.[106] Other studies of copper-deficient swine reticulocytes found that the uptake of iron from Fe-TF was 50% lower than normal, with a 67% reduction in heme synthesis.[107]

RE MACROPHAGES

Because of its key role in iron recycling (Figure 1), iron handling by RE macrophages has been thoroughly studied.[108] Although RE macrophages can take up iron from Fe-TF, in vivo these cells receive most of their iron by phagocytosis of senescent erythrocytes (Figure 2D). The ingested erythrocyte and its hemoglobin are degraded in the phagolysosome, releasing heme. Heme oxygenase cleaves the protoporphyrin ring to liberate iron, which is either utilized by the cell, stored in ferritin, or released from the cell via FPN. Efficient recycling of erythrocyte iron in vivo has recently been shown to involve Nramp1 (Natural resistance-associated macrophage protein 1), a divalent metal ion transporter that localizes to the late phagolysosome.[109] RE macrophages additionally serve as a large reservoir of storage iron, normally holding approximately half of the body's total iron reserve.[108]

In contrast to our understanding of RE macrophage iron metabolism, comparatively little is known about how these cells handle copper. A small, and perhaps significant, amount of copper is obtained via phagocytosis of red cells, which contain the metal as part of SOD1. RE macrophages may also take up copper via CTR1. A recent study in RAW264.7 cells, a macrophage-like cell line, found that hypoxia increased cellular uptake of copper and the levels of Ctr1.[110] The influx of copper resulted in the following: an increase in Atp7a and its translocation from the TGN to cytoplasmic vesicles near the plasma membrane; a decrease in the levels of CCS, SOD1, and CCO; and an increase in the abundance and activity of Cp secreted into the culture medium. These observations suggest that, during hypoxia, macrophages divert the extra copper preferentially into Cp. In vivo, hypoxia has been shown to markedly increase Cp mRNA levels in mouse liver.[60] Cell culture studies revealed that the induction of CP expression by hypoxia (or iron deficiency) requires HIF-1α and HIF-1β.[111] Because the erythropoietic demand for iron is increased in both hypoxia and iron deficiency, the increase in CP resulting from either condition likely serves to enhance the release of iron from stores for mobilization to the bone marrow. Moreover, Atp7a has recently been shown to be involved in bacterial killing in a macrophage cell line,[112] implicating copper in macrophage function.

As in the hepatocyte, copper and iron metabolism in the RE macrophage are linked via CP. Cp deficiency, resulting from dietary copper deprivation or genetic ablation, causes iron to accumulate in the RE cells of the spleen and liver.[78,79] The iron accrues secondarily to impaired cellular iron efflux via FPN. De Domenico et al.[113] demonstrated that the GPI-anchored form of Cp was required for cell-surface FPN expression and iron release from cells. They also showed that murine bone marrow macrophages express GPI-Cp, and that copper depletion prevented cell-surface localization of GPI-Cp and Fpn. These data suggest that GPI-linked, cell-bound Cp is more critical than is circulating Cp for iron release from RE cells. Selective deletion of the GPI-Cp variant will be required to better define its role in RE cells, as well as in other cell types, in vivo.

BRAIN

Brain metal homeostasis is carefully regulated by an elegant series of influx and efflux transporters because,

while essential, excess copper and iron lead to neuropathology.[114,115] These transporters are especially critical in the brain, where metal flux requires passage through multiple membranes of the neurovascular unit, often called the blood-brain-barrier (BBB). There is another cellular barrier separating cerebral spinal fluid (CSF) from blood lined by polarized cells of the choroid plexus, often called the blood-CSF-barrier (BCB). Both copper and iron transporters are enriched in cells lining these barriers compared to brain parenchyma.[116,117]

Current working models suggest copper is taken up from blood by CTR1 and transported across the BBB by ATP7A.[118] Likewise, copper is taken up from CSF by CTR1, which is enriched on the apical surface of the choroid plexus.[119] Choroid plexus Ctr1 location and abundance appear to be enhanced following copper restriction.[119,120] Transport of copper across the BCB is greater than across the BBB.[117] Brain also contains ATP7B, but it is not known if this is involved in copper transport or metallation of GPI-CP in astrocytes in parallel to its role in hepatocytes.

Transport of iron from blood to brain is somewhat controversial regarding the role of Fe-TF versus NTBI, particularly with respect to the presence of DMT1 in capillary endothelial cells.[116,121–123] There is some consensus that neurons acquire their iron via TFR1 and glia via DMT1. Ferritin seems more abundant in glia than neurons. Recent data suggest plasma ferritin may be a source of brain iron.[124]

Iron flux from CSF to blood across the BCB appears to involve DMT1, whereas movement from blood to CSF requires TFR1 and FPN.[125] Similar to copper restriction, iron deprivation upregulates the iron transport machinery of the brain.[126]

It is not clear if copper modulates iron flux or iron modulates copper flux. It is clear that proper regulation of both copper and iron transport are critical to avoid neurological disturbances associated with metal imbalance including Alzheimer's disease, Parkinson's disease, Huntington's disease, and diseases specifically associated with copper (Menkes and Wilson's disease) or iron (Friedreich's ataxia and aceruloplasminemia).[115] As noted earlier, copper deficiency results in elevated iron levels in the liver, due in part to diminished CP activity. In contrast, in the brain (at least in rats), iron concentration is lower following dietary copper deficiency.[127] This seems to be due to the accompanying lower serum iron because injection of copper-deficient rats with iron reverses the serum and brain iron deficit.[4] Brains of copper-deficient rats behave as though iron deficient, with robust enhancement of TfR1 expression.[127]

Brains of young rats subject to iron deficiency have elevated copper levels.[128] It is tempting to speculate that this may be due to higher Dmt1 expression, which is known to accompany iron deficiency, since some believe that Dmt1 can transport copper.[29] However, in a parallel study, brain iron deficiency was induced by high-level dietary manganese supplementation resulting in lower brain iron and higher Dmt1.[129] Interestingly, brain copper was not augmented, suggesting that iron deficiency induced by diet rather than by Dmt1 overexpression is responsible for copper augmentation.

Copper-iron interactions in the brain may involve CP. In fact, the brain, like the liver, accumulates excess iron when CP is missing, for both humans with aceruloplasminemia and $Cp^{-/-}$ mice exhibit high levels of brain iron as adults.[13,130] Thus, adequate dietary copper is necessary to ensure proper transport of iron from the diet to ferric iron TF loading and for metallation of brain CP to perform its ferroxidase function in astrocytes.

The impact of copper and iron loading in rats has also been evaluated. Following a lactational challenge with high dietary copper administered to dams, weanling rats had markedly higher liver copper but no significant changes in plasma or brain copper.[131] The same study demonstrated a reduction in liver iron, higher plasma iron, but no alteration in brain iron following copper loading. Similar pups from iron-loaded dams had no change in liver copper but lower plasma and brain copper. Iron loading resulted in higher liver and plasma iron levels but no change in brain iron concentration. Thus, the only impact on brain was when iron loading lowered both plasma and brain copper. Mechanisms for this observation are unknown.

VARIABLES

Copper-iron interactions are influenced by age and species. Space does not permit an exhaustive review but select examples will be given to stimulate thinking and further research to confirm, refute, or extend current dogma.

Since an animal's needs for copper and iron depend on stage of development, it is not surprising that homeostatic mechanisms differ between infants and adults. For example, iron uptake in the intestine of nursing mammals (mice and humans) may depend on lactoferrin rather than DMT1.[132] Thus, even if DMT1 transports copper, it may not be operative during lactation. Uptake and retention of brain copper and iron are both greater in suckling rodents than adults.[127,133] This is likely regulated by enhanced uptake transporters in the developing brain. Enhanced brain copper was not observed when iron was withheld post-weaning in iron-deficient pups following lactational deprivation.[134] However, the elevated hepatic copper phenotype was observed in both paradigms. Thus, the timing of iron deficiency can impact copper in different organs. Moreover, copper-deficient rodents are born

iron deficient, which is likely due to impaired iron transport across the placenta.[135,136]

The response to copper limitation in mice appears different than in rats in some aspects of iron homeostasis. For example, copper-deficient rats and mice both develop severe anemia but a reduction in plasma iron is only seen in rats.[11] This fact likely explains why these young copper-deficient mice do not have lower brain iron whereas copper-deficient rat pups do. The differences in plasma iron between copper-deficient rats and mice with a common severe hypohemoglobinemia suggest strongly that lack of iron in plasma cannot explain the anemia of copper deficiency.

CONCLUSION

The numerous iron-copper interactions described in this review, many of which have been discovered within the last few years, exemplify the strong continued interest of metal biologists in this area of research. It is clear that there are many unanswered questions regarding the interactions of these two metals that provide ample opportunity for future investigations. Following are a few examples of questions that remain to be answered.

Is the decrease in intestinal iron absorption in copper deficiency solely due to a decrease in HP activity? CP was shown to be important during iron depletion, but does it have an important, unappreciated role in intestinal iron homeostasis under normal circumstances?

What is the effect of enhanced copper uptake into enterocytes during iron deficiency? Copper can alter the activity of various factors involved in regulating iron homeostasis, including the hypoxia inducible factors that are increasingly being recognized as important in mediating intestinal metal ion homeostasis. Moreover, the copper-dependent chaperone ATOX1 has recently been identified as a transcription factor involved in cell proliferation. Could ATOX1 play a role in the apparent cellular proliferation in the intestine of iron-deficient animals?

What are the roles of Steap proteins in iron/copper metabolism? Steap2, Steap3, and Steap4 have been shown to reduce both iron and copper. Steap3 clearly plays a role in iron uptake by erythroid cells, but what is its role in the liver, where it is most abundantly expressed? Why do Steap3-null mice accumulate liver iron and copper? Does intestinal Steap2 serve as an apical cupric/ferric reductase?

Why do hepatic copper levels increase in iron deficiency and decrease during iron overload?

Why do copper-deficient mice become anemic? The anemia does not result from insufficient supply of iron to the bone marrow, but rather from a defect in erythroid iron utilization. Hopefully new research on erythropoiesis following copper deficiency will clarify this most important copper-iron interaction.

What is the role of GPI-CP in iron metabolism Is GPI-CP required for cell-surface expression of FPN in vivo?

Why is brain iron concentration decreased by dietary copper deficiency in contrast to what occurs in the liver, where iron levels increase under these circumstances? Moreover, why does copper deficiency have the opposite effect in young rats, where brain iron levels are elevated during copper deficiency? These documented copper-iron interactions in brain are at least partially mediated by CP; in the absence of Cp, brain iron levels are increased.

It becomes increasingly apparent that there are numerous copper-iron interactions in all organs (and in cell types within those organs) that are primarily responsible for managing overall body metal levels. In numerous cases, these interactions are of proven (or hypothesized) physiological relevance. Many of these interactions have been reported within the last 4–5 years, reflecting the continued interest by the scientific community in this area of research. It is our hope that this review will encourage new areas of experimental pursuit and will further stimulate interest in this field. There is no question that the next 5–10 years hold great promise as scientists and clinicians with interests in metal biology seek to further understand the complex interactions between dietary metals and how these are important in human health and disease.

Acknowledgments

Funding. The authors of this manuscript are supported by the following NIH grants: DK074867 (JFC), HD039708 and HD055423 (JRP), and DK080706 (MDK).

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. Beard JL. Why iron deficiency is important in infant development. J Nutr. 2008;138:2534–2536.
2. What We Eat In America (WWEIA), NHANES: Overview. http://www.ars.usda.gov/Services/docs.htm?docid=13793. Accessed 29 September 2009.
3. Brubaker C, Sturgeon P. Copper deficiency in infants; a syndrome characterized by hypocupremia, iron deficiency anemia, and hypoproteinemia. AMA J Dis Child. 1956;92:254–265.
4. Pyatskowit JW, Prohaska JR. Iron injection restores brain iron and hemoglobin deficits in perinatal copper-deficient rats. J Nutr. 2008;138:1880–1886.
5. Reeves PG, DeMars LC. Signs of iron deficiency in copper-deficient rats are not affected by iron supplements administered by diet or by injection. J Nutr Biochem. 2006;17:635–642.

6. Williams DM, Kennedy FS, Green BG. Hepatic iron accumulation in copper-deficient rats. Br J Nutr. 1983;50:653–660.
7. Turnlund JR, Keyes WR, Peiffer GL, Scott KC. Copper absorption, excretion, and retention by young men consuming low dietary copper determined by using the stable isotope 65Cu. Am J Clin Nutr. 1998;67:1219–1225.
8. Liu N, Lo LS, Askary SH, et al. Transcuprein is a macroglobulin regulated by copper and iron availability. J Nutr Biochem. 2007;18:597–608.
9. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. Annu Rev Nutr. 2002;22:439–458.
10. Xu X, Pin S, Gathinji M, Fuchs R, Harris ZL. Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. Ann N Y Acad Sci. 2004;1012:299–305.
11. Pyatskowit JW, Prohaska JR. Copper deficient rats and mice both develop anemia but only rats have lower plasma and brain iron levels. Comp Biochem Physiol C. 2008;147:316–323.
12. Fox PL. The copper-iron chronicles: the story of an intimate relationship. Biometals. 2003;16:9–40.
13. Harris ZL, Klomp LW, Gitlin JD. Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. Am J Clin Nutr. 1998;67(Suppl):S972–S977.
14. Chen H, Huang G, Su T, et al. Decreased hephaestin activity in the intestine of copper-deficient mice causes systemic iron deficiency. J Nutr. 2006;136:1236–1241.
15. McKie AT, Barrow D, Latunde-Dada GO, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291:1755–1759.
16. Collins JF. Gene chip analyses reveal differential genetic responses to iron deficiency in rat duodenum and jejunum. Biol Res. 2006;39:25–37.
17. Collins JF, Franck CA, Kowdley KV, Ghishan FK. Identification of differentially expressed genes in response to dietary iron deprivation in rat duodenum. Am J Physiol Gastrointest Liver Physiol. 2005;288:G964–G971.
18. Gunshin H, Starr CN, Direnzo C, et al. Cybrd1 (duodenal cytochrome b) is not necessary for dietary iron absorption in mice. Blood. 2005;106:2879–2883.
19. Knopfel M, Solioz M. Characterization of a cytochrome b(558) ferric/cupric reductase from rabbit duodenal brush border membranes. Biochem Biophys Res Commun. 2002;291:220–225.
20. Ohgami RS, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. Blood. 2006;108:1388–1394.
21. Gunshin H, Mackenzie B, Berger UV, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997;388:482–488.
22. Gunshin H, Fujiwara Y, Custodio AO, Direnzo C, Robine S, Andrews NC. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. J Clin Invest. 2005;115:1258–1266.
23. Shayeghi M, Latunde-Dada GO, Oakhill JS, et al. Identification of an intestinal heme transporter. Cell. 2005;122:789–801.
24. Qiu A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell. 2006;127:917–928.
25. Laftah AH, Latunde-Dada GO, Fakih S, Hider RC, Simpson RJ, McKie AT. Haem and folate transport by proton-coupled folate transporter/haem carrier protein 1 (SLC46A1). Br J Nutr. 2009;101:1150–1156.
26. Shi H, Bencze KZ, Stemmler TL, Philpott CC. A cytosolic iron chaperone that delivers iron to ferritin. Science. 2008;320:1207–1210.
27. Ma Y, Specian RD, Yeh KY, Yeh M, Rodriguez-Paris J, Glass J. The transcytosis of divalent metal transporter 1 and apotransferrin during iron uptake in intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2002;283:G965–G974.
28. McKie AT, Marciani P, Rolfs A, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell. 2000;5:299–309.
29. Donovan A, Lima CA, Pinkus JL, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1:191–200.
30. Vulpe CD, Kuo YM, Murphy TL, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet. 1999;21:195–199.
31. Han O, Kim EY. Colocalization of ferroportin-1 with hephaestin on the basolateral membrane of human intestinal absorptive cells. J Cell Biochem. 2007;101:1000–1010.
32. Cherukuri S, Potla R, Sarkar J, Nurko S, Harris ZL, Fox PL. Unexpected role of ceruloplasmin in intestinal iron absorption. Cell Metab. 2005;2:309–319.
33. Wyman S, Simpson RJ, McKie AT, Sharp PA. Dcytb (Cybrd1) functions as both a ferric and a cupric reductase in vitro. FEBS Letters. 2008;582:1901–1906.
34. Nose Y, Kim BE, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab. 2006;4:235–244.
35. Zimnicka AM, Maryon EB, Kaplan JH. Human copper transporter hCTR1 mediates basolateral uptake of copper into enterocytes: implications for copper homeostasis. J Biol Chem. 2007;282:26471–26480.
36. Kaplan JH, Lutsenko S. Copper transport in mammalian cells: special care for a metal with special needs. J Biol Chem. 2009;284:25461–25465.
37. Arredondo M, Munoz P, Mura CV, Nunez MT. DMT1, a physiologically relevant apical Cu1+ transporter of intestinal cells. Am J Physiol Cell Physiol. 2003;284:C1525–C1530.
38. Mackenzie B, Garrick MD. Iron imports. II. Iron uptake at the apical membrane in the intestine. Am J Physiol Gastrointest Liver Physiol. 2005;289:G981–G986.
39. Ravia JJ, Stephen RM, Ghishan FK, Collins JF. Menkes copper ATPase (Atp7a) is a novel metal-responsive gene in rat duodenum, and immunoreactive protein is present on brush-border and basolateral membrane domains. J Biol Chem. 2005;280:36221–36227.
40. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab. 2009;9:152–164.
41. Knopfel M, Smith C, Solioz M. ATP-driven copper transport across the intestinal brush border membrane. Biochem Biophys Res Commun. 2005;330:645–652.
42. Palumaa P, Kangur L, Voronova A, Sillard R. Metal-binding mechanism of Cox17, a copper chaperone for cytochrome c oxidase. Biochem J. 2004;382:307–314.
43. Larin D, Mekios C, Das K, Ross B, Yang AS, Gilliam TC. Characterization of the interaction between the Wilson and Menkes disease proteins and the cytoplasmic copper chaperone, HAH1p. J Biol Chem. 1999;274:28497–28504.

44. Casareno RL, Waggoner D, Gitlin JD. The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. J Biol Chem. 1998;273:23625–23628.
45. Reddy MC, Majumdar S, Harris ED. Evidence for a Menkes-like protein with a nuclear targeting sequence. Biochem J. 2000;350:855–863.
46. Itoh S, Ozumi K, Kim HW, et al. Novel mechanism for regulation of extracellular SOD transcription and activity by copper: role of antioxidant-1. Free Radic Biol Med. 2009;46:95–104.
47. Itoh S, Kim HW, Nakagawa O, et al. Novel role of antioxidant-1 (Atox1) as a copper-dependent transcription factor involved in cell proliferation. J Biol Chem. 2008;283:9157–9167.
48. Kelly EJ, Palmiter RD. A murine model of Menkes disease reveals a physiological function of metallothionein. Nat Genet. 1996;13:219–222.
49. Reeves PG, DeMars LC. Copper deficiency reduces iron absorption and biological half-life in male rats. J Nutr. 2004;134:1953–1957.
50. Reeves PG, Demars LC, Johnson WT, Lukaski HC. Dietary copper deficiency reduces iron absorption and duodenal enterocyte hephaestin protein in male and female rats. J Nutr. 2005;135:92–98.
51. Reeves PG, Demars LC. Repletion of copper-deficient rats with dietary copper restores duodenal hephaestin protein and iron absorption. Exp Biol Med (Maywood). 2005;230:320–325.
52. Lee GR, Nacht S, Lukens JN, Cartwright GE. Iron metabolism in copper-deficient swine. J Clin Invest. 1968;47:2058–2069.
53. Chen H, Attieh ZK, Dang T, Huang G, van der Hee RM, Vulpe C. Decreased hephaestin expression and activity leads to decreased iron efflux from differentiated Caco2 cells. J Cell Biochem. 2009;107:803–808.
54. Hinoi T, Gesina G, Akyol A, et al. CDX2-regulated expression of iron transport protein hephaestin in intestinal and colonic epithelium. Gastroenterology. 2005;128:946–961.
55. Collins JF, Hua P, Lu Y, Ranganathan PN. Alternative splicing of the Menkes copper ATPase (Atp7a) transcript in the rat intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2009;297:G695–G707.
56. Collins JF, Hu Z, Ranganathan PN, et al. Induction of arachidonate 12-lipoxygenase (Alox15) in intestine of iron-deficient rats correlates with the production of biologically active lipid mediators. Am J Physiol Gastrointest Liver Physiol. 2008;294:G948–G962.
57. Smith MW, Debnam ES, Dashwood MR, Srai SK. Structural and cellular adaptation of duodenal iron uptake in rats maintained on an iron-deficient diet. Pflugers Arch. 2000;439:449–454.
58. Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaux C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. J Clin Invest. 2009;119:1159–1166.
59. Feng W, Ye F, Xue W, Zhou Z, Kang YJ. Copper regulation of hypoxia-inducible factor-1 activity. Mol Pharmacol. 2009;75:174–182.
60. Martin F, Linden T, Katschinski DM, et al. Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation. Blood. 2005;105:4613–4619.
61. Ece A, Uyanik BS, Iscan A, Ertan P, Yigitoglu MR. Increased serum copper and decreased serum zinc levels in children with iron deficiency anemia. Biol Trace Elem Res. 1997;59:31–39.
62. Linder MC, Zerounian NR, Moriya M, Malpe R. Iron and copper homeostasis and intestinal absorption using the Caco2 cell model. Biometals. 2003;16:145–160.
63. Han O, Wessling-Resnick M. Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells. Am J Physiol Gastrointest Liver Physiol. 2002;282:G527–G533.
64. Morgan EH, Smith GD, Peters TJ. Uptake and subcellular processing of 59Fe-125I-labelled transferrin by rat liver. Biochem J. 1986;237:163–173.
65. Kawabata H, Yang R, Hirama T, et al. Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. J Biol Chem. 1999;274:20826–20832.
66. Graham RM, Chua AC, Herbison CE, Olynyk JK, Trinder D. Liver iron transport. World J Gastroenterol. 2007;13:4725–4736.
67. Gao J, Zhao N, Knutson MD, Enns CA. The hereditary hemochromatosis protein, HFE, inhibits iron uptake via down-regulation of Zip14 in HepG2 cells. J Biol Chem. 2008;283:21462–21468.
68. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci USA. 2006;103:13612–13617.
69. Chua AC, Olynyk JK, Leedman PJ, Trinder D. Nontransferrin-bound iron uptake by hepatocytes is increased in the Hfe knockout mouse model of hereditary hemochromatosis. Blood. 2004;104:1519–1525.
70. Moriya M, Ho YH, Grana A, et al. Copper is taken up efficiently from albumin and alpha2-macroglobulin by cultured human cells by more than one mechanism. Am J Physiol. 2008;295:C708–C721.
71. Nose Y, Rees EM, Thiele DJ. Structure of the Ctr1 copper trans'PORE'ter reveals novel architecture. Trends Biochem Sci. 2006;31:604–607.
72. Kim H, Son HY, Bailey SM, Lee J. Deletion of hepatic Ctr1 reveals its function in copper acquisition and compensatory mechanisms for copper homeostasis. Am J Physiol Gastrointest Liver Physiol. 2009;296:G356–G364.
73. Terada K, Nakako T, Yang XL, et al. Restoration of holoceruloplasmin synthesis in LEC rat after infusion of recombinant adenovirus bearing WND cDNA. J Biol Chem. 1998;273:1815–1820.
74. Roelofsen H, Wolters H, Van Luyn MJ, Miura N, Kuipers F, Vonk RJ. Copper-induced apical trafficking of ATP7B in polarized hepatoma cells provides a mechanism for biliary copper excretion. Gastroenterology. 2000;119:782–793.
75. Maine GN, Burstein E. COMMD proteins: COMMing to the scene. Cell Mol Life Sci. 2007;64:1997–2005.
76. Maine GN, Mao X, Muller PA, Komarck CM, Klomp LW, Burstein E. COMMD1 expression is controlled by critical residues that determine XIAP binding. Biochem J. 2009;417:601–609.
77. Patel BN, David S. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. J Biol Chem. 1997;272:20185–20190.
78. Harris ZL, Durley AP, Man TK, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. Proc Natl Acad Sci USA. 1999;96:10812–10817.
79. Welch KD, Hall JO, Davis TZ, Aust SD. The effect of copper deficiency on the formation of hemosiderin in Sprague-Dawley rats. Biometals. 2007;20:829–839.
80. Knutson MD. Steap proteins: implications for iron and copper metabolism. Nutr Rev. 2007;65:335–340.

81. Ohgami RS, Campagna DR, Greer EL, et al. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet. 2005;37:1264–1269.
82. Lambe T, Simpson RJ, Dawson S, et al. Identification of a Steap3 endosomal targeting motif essential for normal iron metabolism. Blood. 2009;113:1805–1808.
83. Lespagnol A, Duflaut D, Beekman C, et al. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. Cell Death Differ. 2008;15:1723–1733.
84. Johnstone RM. Revisiting the road to the discovery of exosomes. Blood Cells Mol Dis. 2005;34:214–219.
85. Calzolari A, Raggi C, Deaglio S, et al. TfR2 localizes in lipid raft domains and is released in exosomes to activate signal transduction along the MAPK pathway. J Cell Sci. 2006;119:4486–4498.
86. Faure J, Lachenal G, Court M, et al. Exosomes are released by cultured cortical neurones. Mol Cell Neurosci. 2006;31:642–648.
87. Safaei R, Larson BJ, Cheng TC, et al. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Mol Cancer Ther. 2005;4:1595–1604.
88. Owen CA Jr. Effects of iron on copper metabolism and copper on iron metabolism in rats. Am J Physiol. 1973;224:514–518.
89. Sherman AR, Guthrie HA, Wolinsky I. Interrelationships between dietary iron and tissue zinc and copper levels and serum lipids in rats. Proc Soc Exp Biol Med. 1977;156:396–401.
90. Sherman AR, Moran PE. Copper metabolism in iron-deficient maternal and neonatal rats. J Nutr. 1984;114:298–306.
91. Sherman AR, Tissue NT. Tissue iron, copper and zinc levels in offspring of iron-sufficient and iron-deficient rats. J Nutr. 1981;111:266–275.
92. Sourkes TL, Lloyd K, Birnbaum H. Inverse relationship of heptic copper and iron concentrations in rats fed deficient diets. Can J Biochem. 1968;46:267–271.
93. Klevay LM. Iron overload can induce mild copper deficiency. J Trace Elem Med Biol. 2001;14:237–240.
94. Pollack S, George JN, Reba RC, Kaufman RM, Crosby WH. The absorption of nonferrous metals in iron deficiency. J Clin Invest. 1965;44:1470–1473.
95. Garrick MD, Gniecko K, Liu Y, Cohan DS, Garrick LM. Transferrin and the transferrin cycle in Belgrade rat reticulocytes. J Biol Chem. 1993;268:14867–14874.
96. Ponka P, Sheftel AD, Zhang AS. Iron targeting to mitochondria in erythroid cells. Biochem Soc Trans. 2002;30:735–738.
97. Shaw GC, Cope JJ, Li L, et al. Mitoferrin is essential for erythroid iron assimilation. Nature. 2006;440:96–100.
98. Bencze KZ, Yoon T, Millan-Pacheco C, et al. Human frataxin: iron and ferrochelatase binding surface. Chem Commun (Camb). 2007;18:1798–1800.
99. Hatano S, Nishi Y, Usui T. Copper levels in plasma and erythrocytes in healthy Japanese children and adults. Am J Clin Nutr. 1982;35:120–126.
100. Evans GW. Copper homeostasis in the mammalian system. Physiol Rev. 1973;53:535–570.
101. Hepburn JJ, Arthington JD, Hansen SL, Spears JW, Knutson MD. Technical note: copper chaperone for Cu, Zn superoxide dismutase: a potential biomarker for copper status in cattle. J Anim Sci. 2009;87:4161–4166.
102. West EC, Prohaska JR. Cu,Zn-superoxide dismutase is lower and copper chaperone CCS is higher in erythrocytes of copper-deficient rats and mice. Exp Biol Med (Maywood). 2004;229:756–764.
103. Cobine PA, Pierrel F, Winge DR. Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. Biochim Biophys Acta. 2006;1763:759–772.
104. Hart EB, Steenbock H, Waddell J, Elvehjem CA. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. J Biol Chem. 1928;77:797–812.
105. Prohaska J, Bailey W, Cox D. Failure of iron injection to reverse copper-dependent anemia in mice. In: Mills CF, Bremner I, Chesters JK, eds. *Trace Elements in Man and Animals, Vol. TEMA 5: Commonwealth Agricultural Bureaux, Farnham Royal*. 1985:27–32.
106. Goodman JR, Dalman PR. Role of copper in iron localization in developing erythrocytes. Blood. 1969;34:747–753.
107. Williams DM, Loukopoulos D, Lee GR, Cartwright GE. Role of copper in mitochondrial iron metabolism. Blood. 1976;48:77–85.
108. Knutson M, Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. Crit Rev Biochem Mol Biol. 2003;38:61–88.
109. Soe-Lin S, Apte SS, Andriopoulos B, et al. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. Proc Natl Acad Sci USA. 2009;106:5960–5965.
110. White C, Kambe T, Fulcher YG, et al. Copper transport into the secretory pathway is regulated by oxygen in macrophages. J Cell Sci. 2009;122:1315–1321.
111. Mukhopadhyay CK, Mazumder B, Fox PL. Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. J Biol Chem. 2000;275:21048–21054.
112. White C, Lee J, Kambe T, Fritsche K, Petris MJ. A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. J Biol Chem. 2009;284:33949–33956.
113. De Domenico I, Ward DM, di Patti MC, et al. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J. 2007;26:2823–2831.
114. Bressler JP, Olivi L, Cheong JH, Kim Y, Maerten A, Bannon D. Metal transporters in intestine and brain: their involvement in metal-associated neurotoxicities. Hum Exp Toxicol. 2007;26:221–229.
115. Madsen E, Gitlin JD. Copper and iron disorders of the brain. Annu Rev Neurosci. 2007;30:317–337.
116. Burdo JR, Simpson IA, Menzies S, Beard J, Connor JR. Regulation of the profile of iron-management proteins in brain microvasculature. J Cereb Blood Flow Metab. 2004;24:67–74.
117. Choi BS, Zheng W. Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. Brain Res. 2009;1248:14–21.
118. Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proc Natl Acad Sci USA. 2001;98:6842–6847.
119. Kuo YM, Gybina AA, Pyatskowit JW, Gitschier J, Prohaska JR. Copper transport protein (Ctr1) levels in mice are tissue specific and dependent on copper status. J Nutr. 2006;136:21–26.
120. Gybina AA, Prohaska JR. Variable response of selected cuproproteins in rat choroid plexus and cerebellum following perinatal copper deficiency. Genes Nutr. 2006;1:51–60.

121. Garrick MD, Garrick LM. Cellular iron transport. Biochim Biophys Acta. 2009;1790:309–325.
122. Moos T, Skjoerringe T, Gosk S, Morgan EH. Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. J Neurochem. 2006; 98:1946–1958.
123. Rouault TA. Systemic iron metabolism: a review and implications for brain iron metabolism. Pediatr Neurol. 2001;25:130–137.
124. Fisher J, Devraj K, Ingram J, et al. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. Am J Physiol Cell Physiol. 2007;293:C641–C649.
125. Wang X, Li GJ, Zheng W. Efflux of iron from the cerebrospinal fluid to the blood at the blood-CSF barrier: effect of manganese exposure. Exp Biol Med. 2008;233:1561–1571.
126. Siddappa AJ, Rao RB, Wobken JD, et al. Iron deficiency alters iron regulatory protein and iron transport protein expression in the perinatal rat brain. Pediatr Res. 2003;53:800–807.
127. Prohaska JR, Gybina AA. Rat brain iron concentration is lower following perinatal copper deficiency. J Neurochem. 2005;93:698–705.
128. Garcia SJ, Gellein K, Syversen T, Aschner M. Iron deficient and manganese supplemented diets alter metals and transporters in the developing rat brain. Toxicol Sci. 2007;95:205–214.
129. Garcia SJ, Gellein K, Syversen T, Aschner M. A manganese-enhanced diet alters brain metals and transporters in the developing rat. Toxicol Sci. 2006;92:516–525.
130. Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. J Neurosci. 2002;22:6578–6586.
131. Crowe A, Morgan EH. Iron and copper interact during their uptake and deposition in the brain and other organs of developing rats exposed to dietary excess of the two metals. J Nutr. 1996;126:183–194.
132. Lopez V, Suzuki YA, Lonnerdal B. Ontogenic changes in lactoferrin receptor and DMT1 in mouse small intestine: implications for iron absorption during early life. Biochem Cell Biol. 2006;84:337–344.
133. Roskams AJ, Connor JR. Iron, transferrin, and ferritin in the rat brain during development and aging. J Neurochem. 1994;63:709–716.
134. Yokoi K, Kimura M, Itokawa Y. Effect of dietary iron deficiency on mineral levels in tissues of rats. Biol Trace Elem Res. 1991;29:257–265.
135. Andersen HS, Gambling L, Holtrop G, McArdle HJ. Effect of dietary copper deficiency on iron metabolism in the pregnant rat. Br J Nutr. 2007;97:239–246.
136. Pyatskowit JW, Prohaska JR. Multiple mechanisms account for lower plasma iron in young copper deficient rats. Biometals. 2008;21:343–352.