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# Influence of iron metabolism on manganese transport and toxicity

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## Abstract

Although manganese (Mn) is critical for proper function of various metabolic enzymes and cofactors, excess Mn in the brain causes neurotoxicity. While the exact transport mechanism of Mn has not been fully understood, several importers and exporters for Mn have been identified over the past decade. In addition to Mn-specific transporters, it has been demonstrated that iron transporters can mediate Mn transport in the brain and peripheral tissues. However, while the expression of iron transporters is regulated by body iron stores, whether or not disorders of iron metabolism modify Mn homeostasis has not been systematically discussed. The present review will provide an update on the role of altered iron status in the transport and toxicity of Mn.

### Keywords

Anemia; divalent metal transporter 1; ferroportin; hepcidin; zinc

## 1. Introduction

Manganese (Mn) is an essential transition metal, which serves a vital role as a cofactor for various metabolic and antioxidant enzymes [1, 2]. In addition, the metal is also involved in the physiological regulation of blood sugar, bone growth, blood clotting, and the immune system [3]. Dietary sources of Mn include nuts, legumes, seafood and tea. Mn deficiency, although rare, is characterized by weight loss, poor bone formation, and reduced fertility [4]. However, more common cases of human pathologies associated with Mn result from excessive exposure to this metal, rather than from Mn deficiency [5, 6], raising a huge concern in public health.

Historically, chronic Mn encephalopathy was first recognized among workers engaged in grinding of Mn ores. These workers displayed symptoms of motor and cognitive deficiencies, tremors, gait disturbances and hallucinations [7]. Over the last few decades, welding, mining, and smelting have been recognized as high-risk occupations for developing

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Mn toxicity [8]. The Bureau of Labor Statistics estimated about 400,000 people to be working in welding-related occupations in the U.S in 2015 [9]. Other subsets of population which can be exposed to high levels of Mn include infants fed formulas based on cow or soy milk [3], and patients receiving prolonged exposure to total parenteral nutrition [10]. The use of methylcyclopentadienyl Mn tricarbonyl (MMT) as an antiknock agent increases Mn exposure in people living in urban, dense traffic zones [11, 12]. Of note, consumption of contaminated water from domestic wells has also been found to be a source of Mn toxicity [13, 14]. Due to the incomplete development of the blood-brain barrier (BBB), neonates are at a higher risk of Mn toxicity compared with adult rats [15–18].

While Mn has many oxidation states, $Mn^{2+}$ and $Mn^{3+}$ are the two common species found in human body [19]. Since $Mn^{2+}$ is chemically more stable than $Mn^{3+}$ in the body, Mn is mainly incorporated into metalloenzymes in the form of $Mn^{2+}$ [20, 21]. However, $Mn^{2+}$ can be oxidized to $Mn^{3+}$ by ceruloplasmin [22] and transported by transferrin in the circulation [23]. Importantly, the redox conversion between $Mn^{2+}$ and $Mn^{3+}$ provides a ‘double-sword’ effect on cellular homeostasis. For example, Mn serves as a cofactor for Mn superoxide dismutase (MnSOD) that catalyzes superoxide ($O_2^{\bullet -}$) to hydrogen peroxide ($H_2O_2$) through the $Mn^{2+}/Mn^{3+}$ cycle and thereby detoxifies free radicals in the mitochondria to prevent oxidative stress. On the other hand, the $Mn^{2+}/Mn^{3+}$ cycle can trigger dopamine auto-oxidation, which is one of proposed mechanisms for Mn-induced neurotoxicity [24]. Together, the Mn redox cycle contributes to both nutritional metabolism and toxic effects on biological function.

Iron (Fe) is another essential metal that provides the redox activity. In particular, iron is adjacent to Mn in the periodic table and shares similar chemical properties with Mn. As a result, iron can interact with Mn in several different physiological processes. First, Mn transport is in part mediated by iron transporters. Since the expression of iron transporters is affected by several conditions, such as iron deficient anemia and iron overload hemochromatosis, altered body iron status modifies Mn transport and consequently Mn-associated neurotoxicity [25–33], as discussed below. In addition, both metals are cofactors for a number of metalloenzymes, which play critical roles in antioxidant defense and neurochemistry in the brain. Due to structural and chemical similarities, it is possible that one metal, if in excess, could substitute the other and thereby modulate enzyme activities. For example, while the structure of the metal binding site of FeSOD is similar to that of MnSOD [34], excess iron could replace Mn in MnSOD. However, the redox potential of iron is incompatible with the function of MnSOD, and thereby the replacement of Mn with iron could deactivate the enzyme [36]. Although some studies using *in vitro* systems have supported such possibility [35, 36], it is yet to be investigated whether imbalance of iron-Mn homeostasis will perturb the redox environment in mammals. Moreover, the interplay of Mn and iron can occur in the neurotransmission system, such as the dopaminergic pathway. For example, both metals support the function of tyrosine hydroxylase (TH) [37, 38], the rate-limiting enzyme for dopamine synthesis. This can allow one metal to compensate for the other in case of deficiency to correct impaired enzyme function. Together, these biochemical similarities between Mn and iron suggest the possible molecular interaction of the two metals in neurological function (Figure 1). Thus, the present review will focus on updates on iron-Mn interaction in the context of transport/toxicokinetics and toxicodynamics of metals.

Readers are encouraged to consult more comprehensive reviews for detailed information on the molecular mechanisms of Mn homeostasis and Mn-induced neurotoxicity [21, 39, 40].

## 2. Absorption, Distribution and Disposal of Mn

As an essential nutrient, Mn is absorbed via different routes and distributes into tissues (Figure 2). These transport processes are critical to maintain Mn homeostasis. While several Mn-specific exporters and regulatory proteins that play an important role in Mn homeostasis have recently been identified, it has long been recognized that Mn is also transported by iron transporters, since Mn possess similar chemical properties to iron. Moreover, several zinc transporters, especially ZIP (Zrt- and Irt-like proteins) family, mediate intracellular Mn uptake. The role of these transporters in Mn transport is reviewed in this section.

### 2.1. Absorption

**2.1.1. Intestinal absorption—**Mn-containing food provides the major source of Mn intake in humans. The bioavailability of ingested Mn is about 3–5% in humans [41]. Mn is absorbed from the intestine by either active transport or facilitated diffusion [42]. While there is no specific Mn transporter identified in the gut, accumulating evidence has indicated that several iron transporters are involved in Mn absorption. The divalent metal transporter 1 (DMT1) plays an important role in intestinal uptake of Mn. DMT1, located in the apical membrane, mediates the uptake of multiple divalent metals into the cell, including iron (Fe), Mn and copper (Cu) [43]. The homozygous Belgrade (*b/b*) rat carries a mutation in DMT1 protein that results in < 1% functional DMT1 [44] and thus has provided a useful tool to investigate the role of DMT1 in Mn transport *in vivo*. Functional studies using the duodenum from *b/b* rats revealed a ~70% reduction in Mn transport activity [45, 46]. Consistently, basal Mn levels are lower in the liver and blood of *b/b* rats compared with control rats [45, 47]. However, Shawki et al. recently reported that specific-knockout of intestinal DMT1 does not affect the absorption or tissue distribution (including spleen, heart, kidney and liver) of Mn in mice [48]. These conflicting results could be attributed to different experimental conditions of Mn transport: closed duodenal loops *in situ* or intestinal brush-border membrane vesicles from *b/b* rats were used along with relatively high concentrations of Mn [45, 46], whereas $^{54}$Mn radiotracer was administered by intragastric gavage to intestine-specific DMT1 knockout mice [48]. Thus, it is possible that intestinal DMT1 significantly participates in Mn absorption when there is a substantial increase in the amount of Mn relative to iron in the gut (i.e. Mn overexposure or iron deficiency) [48].

The lactoferrin receptor is known to mediate the intestinal uptake of iron as well as Mn in multiple species, including humans, rhesus monkeys, mice and rabbits [49–51]. A large portion of Mn in the milk is carried by lactoferrin [52], which can bind to lactoferrin receptors in the intestine and be absorbed into the bloodstream [53]. The lactoferrin-mediated pathway is mainly responsible for intestinal Mn absorption during the lactation period.

Ferroportin (FPN; SLC40A1) is the only known metal exporter involved in the intestinal absorption of iron [54–56]. After taken up by enterocytes via DMT1 and other intestinal importers, iron is released into blood by FPN for circulation. There is growing evidence that FPN transports Mn [57–60]. Although the affinity of FPN to Mn is lower by three orders of

magnitude than that to iron [61], deficiency of FPN in mice results in decreased intestinal uptake of Mn [57, 58], suggesting an important role of FPN in systemic absorption of Mn from diet.

While ZIP14 and ZIP8 were initially discovered as zinc (Zn) transporters [62, 63], *in vitro* studies demonstrated that they also participate in intracellular Mn uptake [64, 65]. Since the transcript levels of ZIP14 are approximately 10 times higher than those of ZIP8 in the intestine [65] and since ZIP14 protein is also highly expressed at the basolateral membrane of the proximal intestine [66], ZIP14 is more likely involved in intestinal uptake of Mn than ZIP8. The proposed transport mechanism of ZIP14 is via endosome-mediated exocytosis [66]. However, the role of ZIP14 in intestinal Mn uptake is questioned by a recent finding that human patients with ZIP14 mutations show elevated Mn levels in blood and brain [67]. In contrast, human patients with loss of function mutations in ZIP8 have reduced Mn in blood along with severe neurological diseases related to Mn deficiency [68, 69]. Combined, these results indicate that ZIP8, not ZIP14, plays a critical role in intestinal Mn absorption. Interestingly, the renal excretion of Mn is increased in individuals with ZIP8 mutation [68]. An *in vitro* study has suggested that ZIP8 could play a significant role in renal reabsorption of Mn [70], and thereby ZIP8 mutation could impair Mn reabsorption in the kidney and contribute to low blood Mn. Pharmacokinetic studies for Mn using ZIP8/14 knockout mice will help to clarify the *in vivo* role of ZIPs in intestinal absorption as well as excretion of Mn.

**2.1.2. Pulmonary absorption—**Pulmonary epithelia provide a direct pathway for airborne Mn transport into the systemic circulation. However, the molecular mechanism of pulmonary transport is still obscure. Although DMT1 is expressed in the airway epithelia [71], it has been suggested that DMT1 is not a major transporter for Mn uptake across the lung: pulmonary absorption of $^{54}$Mn after intratracheal instillation is not different between DMT1-mutant *b/b* and control *+/b* rats [72]. Also, iron deficiency enhances $^{54}$Mn uptake after intratracheal instillation, but the expression of DMT1 is not significantly altered [73].

While DMT1 transports divalent metals (e.g. $Fe^{2+}$ and $Mn^{2+}$), the transferrin-transferrin receptor (Tf-TfR) system is involved in the uptake of trivalent metals (e.g. $Fe^{3+}$ and $Mn^{3+}$) [74–76]. $Mn^{3+}$ is bound to transferrin (Tf) to form a Tf-$Mn^{3+}$ complex, which is internalized into the cell via the transferrin receptor (TfR) and dissociates in the acidic endosomes. $Mn^{3+}$ is reduced to $Mn^{2+}$ and released into the cytosol via DMT1 expressed on the membrane of endosome[75]. Specifically, TfR is expressed in the type II alveolar epithelial cells, alveolar macrophages, bronchial epithelium and bronchus-associated lymphoid tissues [72]. Heilig et al. demonstrated that increased expression of TfR protein in the lung upon iron deficiency is correlated with enhanced intratracheal absorption of $^{54}Mn^{2+}$ [72, 73]. However, iron deficiency does not change Tf concentrations in the lung and bronchoalveolar lavage fluid, and the majority of lung $^{54}Mn^{2+}$ is not bound to Tf [72], suggesting that pulmonary absorption of Mn is Tf-independent. Since the Tf-TfR complex transports $Mn^{3+}$, and not $Mn^{2+}$, future studies using different species of Mn (i.e. $Mn^{2+}$ and $Mn^{3+}$) will help to identify the definitive role of the Tf-TfR system in pulmonary Mn uptake.

The same study demonstrated that L-type $Ca^{2+}$ channels and TRPM7, a member of the transient receptor potential melastatin subfamily, are involved in Mn uptake alveolar epithelial cells *in vitro*. However, the role of these channels in pulmonary Mn transport has not been determined *in vivo*. Although FPN plays a role in intestinal Mn absorption, whether it is also involved in pulmonary Mn uptake is unknown. Interestingly, ZIP8 is abundantly expressed in the lung [65, 77, 78], but the correlation between ZIP8 or ZIP14 expression and pulmonary Mn absorption has not been explored. Further studies are needed to evaluate the impact of other metal transporters on pulmonary absorption of Mn.

**2.1.3. Olfactory absorption—**The olfactory transport pathway provides an efficient route of absorption of airborne Mn into blood due to the absence of hepatic first-pass elimination, thus enhancing Mn bioavailability compared with intestinal absorption of Mn [79, 80]. Furthermore, the nasal-brain pathway allows for direct contact with the brain by circumventing the BBB, increasing access of Mn to the brain, the primary site of metal's toxicity [80–82]. Thus, airborne Mn exposure has long been recognized as a huge concern for Mn neurotoxicity in environmental and occupational health [83], particularly in workers employed in mining and Mn ore processing [84] and agricultural workers exposed to Mn-containing pesticide [85].

While olfactory absorption of Mn is rapid and efficient, the transport mechanisms remain largely unexplored. Only a few metal transporters have been found to be involved in olfactory Mn uptake. Earlier, Pautler et al. demonstrated that olfactory Mn transport can be inhibited by a calcium channel blocker and a microtubule disturbing compound, suggesting that Mn can enter the olfactory neurons via calcium channels, followed by a microtube-dependent transport system [86]. DMT1 is also involved in olfactory transport of Mn, based on the finding that *b/b* rats showed decreased $^{54}$Mn uptake into blood after intranasal instillation of $^{54}MnCl_2$ [87]. While some studies indicated that other metal transporters, including FPN, ZIP14 and ZIP8, are expressed in the olfactory epithelium or olfactory bulb [79, 88], it remains to be tested whether these transporters contribute to olfactory uptake of Mn *in vivo*.

## 2.2. Distribution

**2.2.1. Tissue uptake—**The Tf-TfR system transports both Fe and Mn into the cell, as reviewed elsewhere [40, 89]. Mice with decreased circulating Tf display reduced $^{54}$Mn uptake as well as reduced steady-state levels of Mn in the liver [90, 91], indicating that the Tf-TfR system is important for tissue distribution of Mn. Tf-mediated transport of Mn requires normal function of DMT1, as evidenced by the finding that *b/b* rats have impaired uptake of Tf-bound Mn, but not free Mn in serum [45]. However, the contribution of DMT1 to Mn distribution appears to be limited to the uptake process, and steady-state levels of Mn in the tissue could be more dependent on the export process. For example, *b/b* rats has lower or unchanged basal Mn levels in the liver compared with control rats [45, 47], whereas the distribution of intravenously injected $^{54}$Mn into the liver is greater in *b/b* rats [45]. Since the liver transfers Mn into the bile for excretion [92], it is possible that Mn excretion is enhanced in *b/b* rats. Collectively, the Tf-TfR1 system and DMT1 work together to contribute, at least partially, to systemic Mn distribution.

Whether the Tf-TfR1 system mediates Mn uptake into the brain is not completely understood. Mice deficient in Tf showed no change in brain Mn levels after intravenous or subcutaneous $^{54}$Mn administration compared with wild-type mice [90, 93]. Likewise, conflicting results exist about the role of DMT1 in Mn uptake into the brain. DMT1 is expressed in ependymal cells, neurons, astrocytes and vascular endothelial cells throughout the brain [94, 95]. Crossgrove et al. demonstrated that dysfunction of DMT1 does not affect the transport of free $^{54}Mn^{2+}$ in serum or Tf-$^{54}$Mn into the brain, as assessed by both *in vivo* and *in situ* brain perfusion experiments [96], which is different from $^{59}$Fe transport [97]. Han et al. revealed that *b/b* rats have normal levels of Mn in the brain [47]. These studies suggest that DMT1 is not a primary transporter responsible for Mn uptake into the brain. However, it should be noted that gene knockout in the whole body could trigger compensatory response that activates the expression and/or function of other transporters or regulators, thereby masking the effects of single gene on brain Mn homeostasis. Although mice with hippocampal specific-knockout of DMT1 showed reduced iron levels in the hippocampus, Mn levels were not determined [98]. Future studies utilizing tissue-specific gene knockout mice will increase our understanding of the role of these transporters in brain metal homeostasis.

Families of zinc transporters, especially ZIP14 and ZIP8, have been implicated in Mn uptake into cells. The expression levels of ZIP14 and ZIP8 are positively correlated with Mn uptake *in vitro* [99, 100]. However, the tissue distribution patterns of ZIP14 and ZIP8 are different. ZIP14 is mostly expressed in the liver, pancreas, heart and duodenum, while ZIP8 is more abundant in the lung, testis and kidney [101]. Therefore, it is likely that the role of ZIP14 and ZIP8 in Mn uptake is tissue-specific. However, the exact mechanism of ZIPs in Mn distribution and metabolism using *in vivo* experiments is yet to be characterized.

In the brain both active and facilitated transport mechanisms exist, including transport of a Mn-citrate complex by an organic anion transporter or a monocarboxylate transporter, $Ca^{2+}$ channels (voltage-gated, store-operated, glutamatergic ionotropic), and choline transporters, as reviewed in elsewhere [21].

**2.2.2. Mn export—**Although an *ex vivo* study from Yokel et al. suggested that Mn efflux from the brain could be non-carrier-mediated [102], there has been increased understanding about the molecular mechanism of Mn export during the past decade. Several recent clinical studies have reported that human subjects with Solute Carrier Family 30 Member 10 (SLC30A10; ZnT10) deficiency/mutation display higher Mn accumulation in the liver [67] and blood [103, 104], as well as 10 times higher Mn in the basal ganglia, which is associated with dystonia, a typical phenotype of Mn neurotoxicity [105–107]. SLC30A10 was thought to be a zinc exporter, but its amino acid structure is different from other zinc transporters in this family [108], and it was later suggested that SLC30A10 may not be involved in zinc transport [109]. In contrast, the expression of SLC30A10 is inversely correlated with intracellular Mn accumulation in several *in vitro* systems, such as Hela cells, chicken DT40 cells and SH-SY5Y cells [100, 110, 111]. SLC30A10 is localized the cell surface as well as intracellular compartments of secretory pathways [103]. Both transmembrane and C-terminal domains of cell membrane-associated SLC30A10 are structurally critical for Mn export activity [112]. Mutated SLC30A10 is unable to traffic to the cell surface and thereby decreases

Mn efflux activity [110]. Another study demonstrated that SLC30A10 forms heterodimers with several ZnTs in TfR-positive endosomes [113]. However, whether the heterodimer is also critical for Mn transport and whether this mechanism also exists *in vivo* is not well understood. Although SLC30A10 is expressed in the intestine, whether it is involved in intestinal Mn absorption, like FPN, is yet to be investigated [114].

The secretory pathway $Ca^{2+}$-ATPase isoform 1 (SPCA1) is another transporter involved in the Mn secretory pathway out of the cell. SPCA1 is a $Ca^{2+}$-ATPase that can pump the cytoplasmic $Mn^{2+}$ into the Golgi apparatus for secretion [115]. Enhanced function of SPCA1 increases $^{54}$Mn levels in the Golgi apparatus and contributes to Mn detoxification [116], whereas deletion of SPCA1 reduces cell viability upon Mn exposure [117]. In addition, ATP13A2, a P-type ATPase [118], reduces intracellular Mn accumulation in HEK293 and N2a cells and protects cells against Mn-induced toxicity [119]. The contribution of these transporters to Mn homeostasis *in vivo* remains to be evaluated.

FPN mediates the export of Mn as well as iron. Induction of FPN expression in SH-SY5Y, HEK293T cells and oocytes increases Mn efflux and reduces Mn accumulation [57, 59, 60]. Contradictory findings have been also reported. Mitchell et al. did not observe enhanced $^{54}$Mn efflux in oocytes overexpressing FPN [61]. In animal studies, Seo et al. reported decreased Mn levels in several organs in flatiron mice that carry a mutation in FPN, but they did not differentiate the absorption and distribution processes of Mn into peripheral tissues [58]. The same group also reported that $^{54}$Mn in blood is unchanged in the flatiron mice after intravenous injection of $^{54}$Mn, suggesting that FPN may not be responsible for systemic Mn clearance [57]. It is possible that mutation in FPN and/or subsequent changes in iron status [58], as seen in the flatiron mice, could up-regulate other Mn exporters (e.g. SLC30A10) to control the excretion of Mn. FPN could also be involved in Mn export from the brain. The levels of Mn are elevated in the olfactory bulb and brain from FPN-deficient mice, especially in male mice, despite lower Mn levels in the peripheral tissues [58]. In parallel, increased expression of FPN is correlated with reduced Mn accumulation in the brain [31]. However, no studies have directly determined Mn export out of the brain, and pharmacokinetic studies after intracerebral injection of $^{54}$Mn using mice with FPN deficiency or overexpression will directly address the role of FPN in brain Mn export.

### 2.3. Disposal

The liver plays a major role in Mn excretion, and impairment of liver function results in excessive retention of Mn [120, 121]. Mn is excreted from the liver through the bile into the feces [92]. It has been suggested that $Mn^{2+}$ cations may be secreted from blood into bile to form complexes with bile acids [122]. Biliary excretion of Mn could be transporter-dependent [123, 124]. Interestingly, FPN is expressed at the sinusoidal borders of the hepatocytes [125]. However, there is no direct evidence that FPN is involved in biliary Mn excretion. Of note, SLC30A10 is highly expressed in the liver and the epithelium of bile ducts, and the expression of defective SLC30A10 leads to high levels of systemic Mn [103], suggesting that SLC30A10 could play a critical role in systemic excretion of Mn through bile. Tuschl et al. reported that mutations in ZIP14 increase blood Mn without affecting liver

Mn, suggesting that ZIP14 is involved in Mn transport into the liver, followed by biliary excretion of Mn by SLC30A10 [67].

## 3. Effect of iron status on Mn homeostasis

Factors that change the expression of metal transporters involved in Mn transport perturb Mn homeostasis. Specifically, imbalance in body iron status alters the expression of several key iron transporters that also mediate Mn transport. Both animal and human studies showed a 'U-shape' correlation between iron levels and Mn status in the body, likely due to the complex mechanisms in both causes and consequences of dysregulation of iron metabolism (i.e. genetic, nutritional and environmental), as discussed below.

### 3.1. Systemic Mn homeostasis in iron deficiency

Iron deficiency is the most prevalent nutritional disorder, affecting more than 2 billion people worldwide [126]. The effects of iron deficiency on Mn transport have been well-documented. The transcripts of many iron transporters, including DMT1, FPN and TfR1, contain the iron-responsive element (IRE) [127]. The iron-responsive proteins (IRP) bind to the IRE sequence and regulate transporter expression at the levels of transcription and post-transcription [128]. Through this mechanism, the expression of iron transporters changes in response to body iron status. For example, dietary iron deficiency up-regulates the expression DMT1 in the intestine [43, 129, 130], resulting in increased basal levels of Mn in various tissues, including brain, heart, kidney, testis, femoral muscle and tibia [131, 132]. Elevated blood Mn levels have been observed in human subjects with iron deficiency in children, infants and women [133–135]. In addition, Mena et al. reported that anemic patients have a 2.5-fold greater intestinal $^{54}$Mn absorption compared with normal individuals [136], likely due to increased expression levels of duodenal iron transporters [137]. In parallel, blood ferritin concentrations are inversely correlated with $^{54}$Mn absorption, suggesting that body iron stores could negatively influence intestinal Mn absorption [138, 139].

Many animal studies have characterized the effect of non-heme iron on Mn homeostasis, but little is known about the role of heme iron in Mn metabolism, although heme iron is the major form of iron source in non-vegetarian diet. Davis et al. examined the effect of different forms of iron (i.e. heme and non-heme) on serum Mn levels in human subjects provided with typical western diet and found that serum Mn levels are affected by the intake of non-heme iron, but not heme iron [140]. More studies are warranted to better understand the exact role of heme iron in Mn transport using relevant animal models.

### 3.2. Brain homeostasis upon iron deficiency

Iron deficiency is associated with increased accumulation of Mn in the brain in a region-specific manner; elevated Mn levels observed with iron deficiency are more pronounced in the caudate putamen and globus pallidus [26]. Iron deficiency also increases extracellular Mn concentrations in the striatum in rodents [141]. Relatively less information is known about the Mn level in the brain of iron deficient/anemic patients. In a preliminary clinical study with a small sample size, it was reported that iron deficiency is correlated with increased Mn deposition in the basal ganglia [142]. In contrast, Mn levels in the globus pallidus are

minimally affected in anemic humans [143], which is different from animal studies [26] and other cases of Mn intoxication [144, 145]. The reasons for these different results remain unclear.

The mechanism by which iron deficiency enhances Mn accumulation in the brain is not clearly understood. Although mixed results exist, it is believed that DMT1 and the Tf-TfR system may play a role in Mn retention upon iron deficiency. *In vitro* studies have shown that deferoxamine (DFO), an iron chelator, up-regulates the expression of DMT1 in several types of cells, including SH-SY5Y cells [25] and rat primary astrocytes [146], but this is not correlated with TfR expression [146]. Similarly, Siddappa et al. demonstrated that the expression of DMT1 is up-regulated in the hippocampus and cerebral cortex of iron-deficient rats during the perinatal period [147]. However, post-weaning iron deficiency by diet does not alter DMT1 levels in the brain [148]. These results suggest that the regulation of brain DMT1 in response to iron stores could be more sensitive during early development. In addition to DMT1, the expression of the protein, and not the mRNA, of TfR1 is elevated in the brain of rats exposed to iron-deficient diet during either prenatal or postnatal period [147–149]. These findings are different from the *in vitro* results that showed no change in Tf expression upon removal of iron [146]. This discrepancy could result from a difference in the Tf-TfR1 system between *in vitro* and *in vivo* conditions. For example, the function of the Tf-TfR1 system is affected by several membrane-associated and/or soluble factors (such as hepcidin as discussed below) *in vivo*, but this mechanism could be lacking in cell culture conditions. Together, these studies suggest that enhanced Mn accumulation *in vivo* upon iron deficiency could be attributed to altered expression of transporters in an iron-responsive manner.

Thompson et al. demonstrated that dietary iron deficiency increases the expression of DMT1 in the rat olfactory epithelium, resulting in significantly enhanced blood Mn after a single dose of intranasal instillation of $^{54}$Mn isotope [87]. However, iron deficiency does not alter $^{54}$Mn levels in the olfactory bulb and basal ganglia after 2 weeks post-instillation [87]. Although both uptake and clearance affect brain Mn homeostasis, scarce information is known about the clearance from the brain. Chua et al. reported that iron deficiency does not affect blood Mn clearance after intravenous injection [131], but efflux kinetics of Mn from the brain was not investigated. Direct administration of Mn (e.g. intracerebral injection of $^{54}$Mn) will address this question by separating the absorption and elimination process of Mn transport under different body iron status. Moreover, Mn deposition in the brain is heterogeneous after intravenous $MnCl_2$ injection upon iron deficiency; Mn distribution is increased in the brain stem, striatum and cortex, but decreased in the hippocampus [150]. Whether or not the heterogeneous pattern of Mn distribution is correlated with the expression of transporters has not been evaluated. The influence of iron deficiency on Mn-specific exporters (e.g. SLC30A10 and SPCA1) has not yet been examined.

## 3.3. Effect of iron overload on Mn homeostasis

### 3.3.1. Iron overload and systemic Mn transport

**Dietary iron overload:** Compared with iron deficiency, the effects of iron overload on the expression of iron/Mn transporters are less understood. Iron overload suppresses the expression of DMT1 in enterocytes by an iron-responsive manner, which has been

consistently reported by different researchers [151, 152]. However, the effects of iron loading on DMT1 expression in other types of cells or tissues are mixed. Dietary iron overload increases hepatic DMT1 expression as well as DMT1 expression in rat primary astrocytes, resulting in increased Mn uptake [146, 152]. However, Hansen et al. demonstrated that a high-iron diet decreases hepatic and duodenal Mn levels in pigs, along with decreased mRNA expression of DMT1, but the protein levels of DMT1 or FPN are not significantly altered [153]. More mechanistic experiments are required to understand the effect of dietary iron overload on systemic Mn accumulation.

**Genetic iron overload:** Mutations in iron regulatory genes lead to genetic iron overload diseases. In particular, the HFE (High Fe or hyperferremia) protein is essential for proper control of iron transport [154–157], and mutations in the HFE gene are the major cause of hereditary hemochromatosis (HH), a most common genetic iron overload disorder among the North American Caucasian population [158–160]. The C282Y mutation of the HFE gene is primarily involved in systemic iron loading, such as liver and heart [154], which predisposes to liver cirrhosis and cardiomyopathy. Importantly, the H63D variant of the HFE gene (22.9% gene variants worldwide) [161] receives increased attention due to a greater susceptibility to elevated iron and the development of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis [154, 162].

It is generally accepted that systemic iron overload in HFE dysfunction is due to the altered expression of iron transporters, although there are some controversies. Several studies reported up-regulation of DMT1 and FPN protein expression in the intestine upon hemochromatosis [137]. Elevated activity of intestinal FPN and DMT1 in HFE mutations is believed to be caused by the suppression of hepcidin, the master iron regulatory peptide produced in the liver and released into blood; hepcidin induces the degradation of FPN and DMT1 via a ubiquitination-dependent pathway [163, 164], while loss of HFE function results in hepcidin deficiency [165–167], which thereby up-regulates these iron transporters in several tissues.

The effects of HFE dysfunction on systemic Mn transport are mixed. On the one hand, Kim et al. found that blood levels of $^{54}Mn$ are increased via gavage but unchanged after intravenous injection of $^{54}MnCl_2$ [79]. On the other hand, the steady-state levels of Mn are decreased in both HFE-knockout mice and humans with HFE variants [168]. Similarly, Jouihan et al. found that the mitochondrial Mn levels in the liver are lower in HFE-deficient mice [169]. Together, these results suggest that HFE dysfunction could increase both absorption and elimination of Mn, and that the effect of HFE on elimination could outweigh that on absorption. It is unknown whether iron overload or HFE function modifies SLC30A10 and other Mn-specific transporters.

### 3.3.2. Iron overload and brain Mn transport

**Dietary iron overload:** Although it is conceivable that iron loading would decrease brain Mn transport due to the competition for the metal transporters, multiple studies reported different results [131, 150]. Similar to iron deficiency, iron supplementation increased Mn deposition in the brain stem, striatum and cortex after intravenous $MnCl_2$ injection, whereas

hippocampal Mn accumulation was decreased [150]. Iron loading throughout both pre-weaning and post-weaning periods also increases Mn accumulation in the brain via drinking water [131]. In contrast, Fitsanakis et al. found no difference in brain Mn accumulation between iron-deficient and iron-loaded rats after intravenous injection of Mn [170]. A recent study also indicated no change in striatal Mn accumulation after dietary iron treatment [31]. Collectively, these results suggest that dietary iron loading may influence Mn transport in different brain regions by non-competitive mechanisms in time- and dose-dependent manners.

**Genetic iron overload:** In the brain, HFE mutation is associated with increased DMT1, but reduced Tf expression [171], and this implies that HFE dysfunction could alter Mn accumulation in the brain. Kim et al. reported that a single intranasal instillation of $^{54}Mn$ increases $^{54}Mn$ levels in the brain, but not in blood, with the expression of DMT1 and FPN unchanged in the olfactory bulb of Hfe-deficient mice [79]. Interestingly, after repeated intranasal instillations of $MnCl_2$, Hfe-knockout mice do not alter Mn levels in most brain regions except the cerebellum, in which Mn levels are decreased [33]. These studies suggest that loss of HFE function could increase intranasal uptake of Mn into the brain as well as the clearance of the metal from the brain. A recent study by Ye et al. [31] demonstrated that mice carrying HFE H67D mutation, a mouse model with elevated brain iron [171], display decreased Mn levels in blood, liver and brain, especially in the striatum, after intranasal instillations of Mn for 3 days. The attenuated striatal Mn accumulation in H67D mutation is associated with increased transcript levels of FPN. Although the protein levels of FPN should be determined, this study underscores the effect of HFE-related hemochromatosis on regional expression of metal transporters and clearance of Mn from the brain after olfactory exposure [31]. With respect to oral absorption of Mn, Alsulimani et al. reported that Mn accumulation is not different in the liver, but decreased in the brain in HFE-deficient mice after subchronic exposure to Mn via drinking water [172]. Together, these studies suggest an important role of HFE function and iron overload in Mn homeostasis in the brain.

## 4. Effect of iron homeostasis on Mn-associated neurotoxicity

Excess Mn in the brain is neurotoxic and results in a number of neurobehavioral impairments known as manganism, including memory deficits, decreased motor skills and psychotic behavior resembling Parkinson's disease [173]. High levels of Mn are found in the striatum, globus pallidus and substantia nigra [174–177]. While the exact mechanism of Mn-induced neurotoxicity is unclear, multiple mechanisms have been proposed, including oxidative stress, dysregulation of neurological signaling and apoptosis [178, 179]. Several recent review papers have extensively discussed mechanisms of Mn-induced neurotoxicity in detail [21, 179–185].

Since Mn transport is closely associated with body iron stores, it is likely that altered iron status would influence Mn homeostasis and Mn-associated biological function. Moreover, excess iron is known to cause neurological damage similar to Mn intoxication, which could exacerbate Mn-induced neurotoxicity. Specifically, both iron and Mn can accelerate dopamine auto-oxidation to form cytotoxic quinones and produce ROS [24, 186, 187]. In addition, Mn induces hyperphosphorylation of tau protein [188], and iron promotes the

aggregation of hyperphosphorylated tau protein[189]. It is thus plausible that high levels of both metals could synergistically lead to the accumulation of neurofibrillary tangles, a hall mark of Alzheimer's diseases. Combined, altered iron status could modify Mn-induced neurotoxicity and result in abnormal behavioral consequences. In this section, we will review the impact of iron homeostasis on neurotoxic effects associated with Mn exposure.

### 4.1. Iron deficiency and Mn-induced toxicity

It is deduced that iron deficiency exacerbates neurological dysfunction caused by Mn exposure due to enhanced Mn accumulation in the brain (Figure 3). Alternatively, iron also serves as a cofactor for several antioxidant enzymes, such as catalase [190], and iron deficiency could reduce catalase activity and thereby decrease the protective capacity against Mn-induced oxidative stress. Co-incubation of DFO, an iron chelator, worsens the toxicity in rat pheochromocytoma cells, including cell apoptosis and mitochondrial dysfunction [191]. In addition, DFO incubation additively increases markers for endoplasmic reticulum stress in human neuroblastoma cells [25]. Consistent results have been reported by Seo et al. that dietary iron deficiency potentiates Mn-induced apoptosis in the olfactory bulb in rats after intranasal Mn instillation [25].

Iron deficiency modifies Mn-induced neurochemical alterations. Mn via drinking water decreases extracellular concentrations of norepinephrine in the caudate putamen, which is enhanced by iron deficiency [26]. There is a synergistic effect of oral Mn exposure and iron deficiency on increased gene expression, but not protein expression, of the γ-aminobutyric acid (GABA) transporter [141]. Nevertheless, iron deficiency does not exacerbate the inhibition of striatal synaptosome uptake of GABA by Mn [192]. Furthermore, Amos-Kroohs et al. [27] found that iron deficiency exacerbates Mn-induced psychiatric behavior, including anxiety, acoustic startle response and sociability, after oral administration of Mn. In addition, a combination of iron deficiency and Mn exposure increases the hippocampal levels of serotonin and norepinephrine, whereas Mn alone only moderately increases or does not affect the levels of these two monoamines [27]. However, such synergistic effects of iron deficiency and Mn exposure are not observed on the levels of dopamine and its metabolites in the same study [27]. Interestingly, it is possible that iron deficiency could counteract the effect of Mn intoxication on key proteins responsible for dopamine turnover. For example, iron is a cofactor for TH, and iron deficiency is associated with impaired function of TH [37]. Mn can also support TH activity [38], and acute oral exposure of Mn increases the activity of TH [193]. Mn supplementation could compensate for impaired TH in iron deficiency, as evidenced by a finding that olfactory Mn exposure corrects impaired dopamine release and motor dysfunction caused by iron deficiency [194].

### 4.2. Iron overload and Mn-induced toxicity

Compared with iron deficiency, the information about the effects of iron overload on Mn-induced neurotoxicity is scarce. On the one hand, since both excess iron and Mn could induce oxidative stress [195–200], they could enhance metal-associated toxicity. On the other hand, iron overload facilitates Mn disposal [31] such that Mn-related toxicity could be mitigated. Lines of evidence have suggested that co-exposure to Mn and iron appears to counteract oxidative stress each other. Sziraki et al. demonstrated that a co-treatment of

$MnCl_2$ with iron attenuates dopamine depletion, lipid peroxidation and neuronal degeneration induced by iron in the brain [28–30]. One potential mechanism is that Mn may act as an anti-oxidant against iron-induced oxidative stress since Mn is an indispensable cofactor for MnSOD, a crucial antioxidant exclusively expressed in the mitochondria [29]. Recent studies also demonstrated that olfactory or gavage exposure to Mn induces impulsivity, recognition memory impairment and motor dysfunction, and these abnormal behaviors are decreased in genetic iron loading caused by HFE dysfunction [32, 33, 172]. The protective effect of genetic iron overload against Mn-induced behavioral toxicity is associated with attenuated oxidative stress, enhanced antioxidant capacity and restored function of monoaminergic proteins [31–33]. On the other hand, Alsulimani et al. found that Hfe deficiency exacerbates impaired spatial memory capacity induced by Mn exposure via drinking water [172]. Combined, these studies indicate that iron loading alters the physiological function of Mn in the brain, and further modifies Mn-induced neurological dysfunction. However, the effect of iron loading could vary depending on several factors, including routes of Mn exposure, exposure window, severity of iron loading, physiological factors (e.g. age, sex) and types of behavior examined.

## 5. Conclusions

Mn plays an important role in maintaining proper organ function by serving as a cofactor of several critical enzymes. However, Mn in excess causes a variety of neurotoxic effects. Therefore, the body develops multiple regulatory systems for Mn transport, which provide adaptive physiological responses and homeostasis. Besides Mn-specific transporters, iron transporters have been shown to mediate both import and export of Mn. Changes in iron status result in altered expression of iron transporters, which consequently modifies Mn transport and associated neurotoxicity. While Zn transporters also contribute to Mn transport and altered Zn levels affect Mn accumulation *in vitro* and *vivo* [201–203], it remains to be evaluated whether changes in Zn homeostasis impact Mn-associated neurotoxicity and behavioral deficits. Although molecular mechanisms of Mn transport and neurotoxicity are not completely understood, a better understanding about the interaction effects of these essential metals would provide an important insight into the role of nutrient metals in Mn deficiency and intoxication.

Several factors have been identified that can alter iron metabolism and transport by modulating the production of hepcidin; these include inflammation, erythropoiesis, alcohol and estrogen [204–207]. Thus, it is possible that these factors may also affect Mn homeostasis and regulate Mn-induced neurotoxicity. For example, a pro-inflammatory cytokine IL-6 and erythropoietin alter the expression of iron transporters (TfR, DMT1 and FPN), and it has been shown that inflammation and erythropoiesis increase cellular Mn uptake *in vitro* [100, 208]. Interestingly, it is reported that alcohol exposure worsens Mn-associated neurobehavioral problems [209]. Further studies are required to determine if this is due to increased Mn uptake into the brain upon alcohol consumption and/or alcohol's neurotoxic effects. In addition, Claro et al. reported that the transcript and protein levels of SLC30A10 are significantly up-regulated in subjects who receive vitamin D treatment [114], but Mn levels from these subjects are yet to be quantified. Both *in vitro* and *in vivo* transport studies along

with exposure-response relationships will improve our understanding of Mn toxicity associated with these factors.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **BBB** | blood-brain barrier |
| **DFO** | deferoxamine |
| **DMT1** | divalent metal transporter 1 |
| **FPN** | ferroportin |
| **GABA** | γ-aminobutyric acid |
| **HFE** | high Fe or hyperferremia |
| **HH** | hereditary hemochromatosis |
| **IRE** | iron-responsive element |
| **IRP** | iron-responsive protein |
| **ROS** | reactive oxygen species |
| **SLC30A10** | Solute Carrier Family 30 Member 10 |
| **SPCA1** | secretory pathway $Ca^{2+}$-ATPase isoform 1 |
| **SOD** | superoxide dismutase |
| **Tf** | transferrin |
| **TfR** | transferrin receptor |
| **TH** | tyrosine hydroxylase |
| **ZIP** | Zrt- and Irt-like protein |

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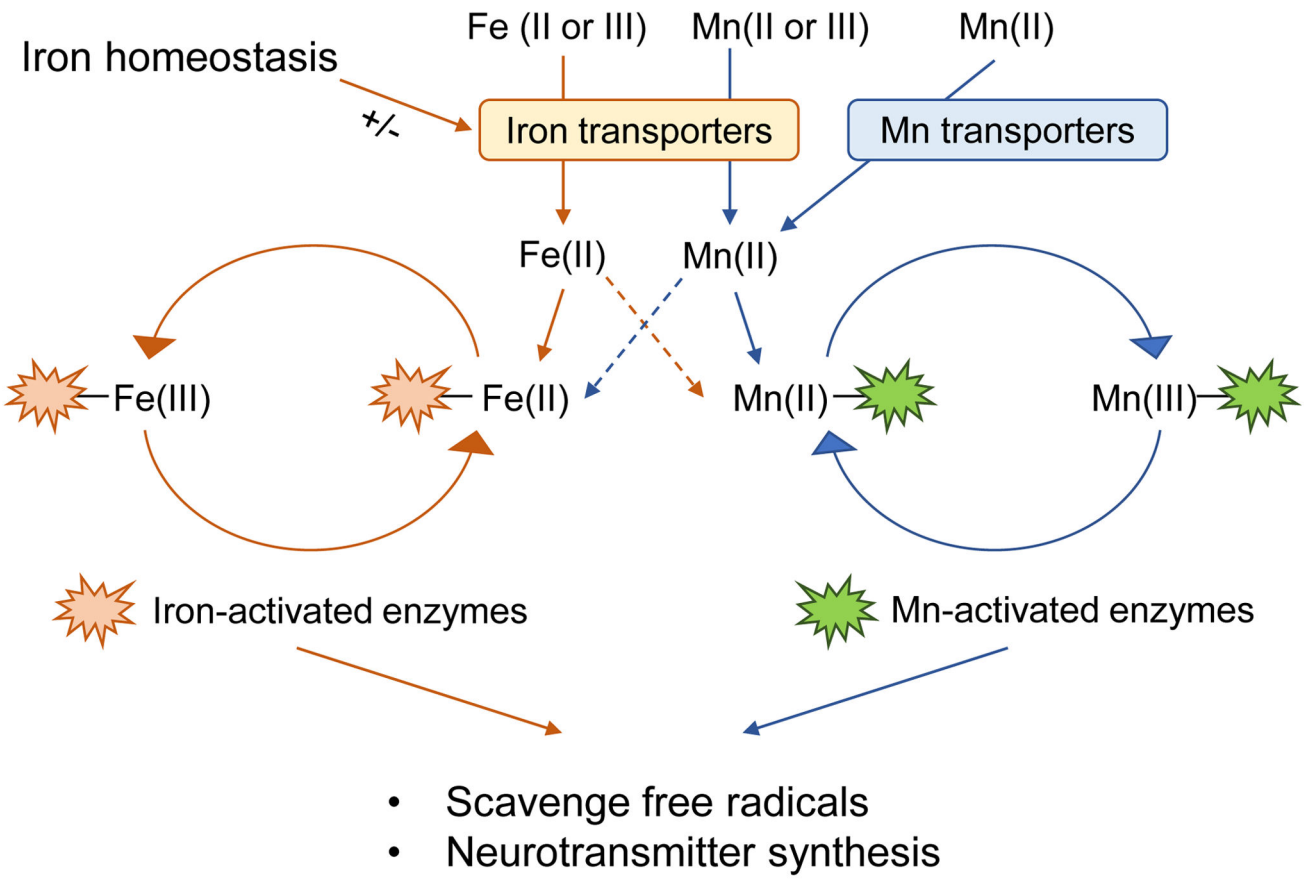


**Figure 1. Proposed mechanisms of Mn-iron interaction**

The structural and chemical similarities between Mn and iron allow them to interact with each other in biological systems. Both Mn and iron can be transported as divalent forms by several divalent metal transporters (e.g. DMT1 and FPN) or as trivalent forms by the Tf/TfR system. It has been known that iron status can alter the expression of these transporters, thereby modifying Mn levels in the body. In addition, both Mn and iron serve as cofactors for several metalloproteins that play critical roles in antioxidant defense and neurological function. Since many of these enzymes have binding affinities for both metals, it is possible that they can substitute each other under certain conditions (dotted arrows), thereby alter the activity of these enzymes.

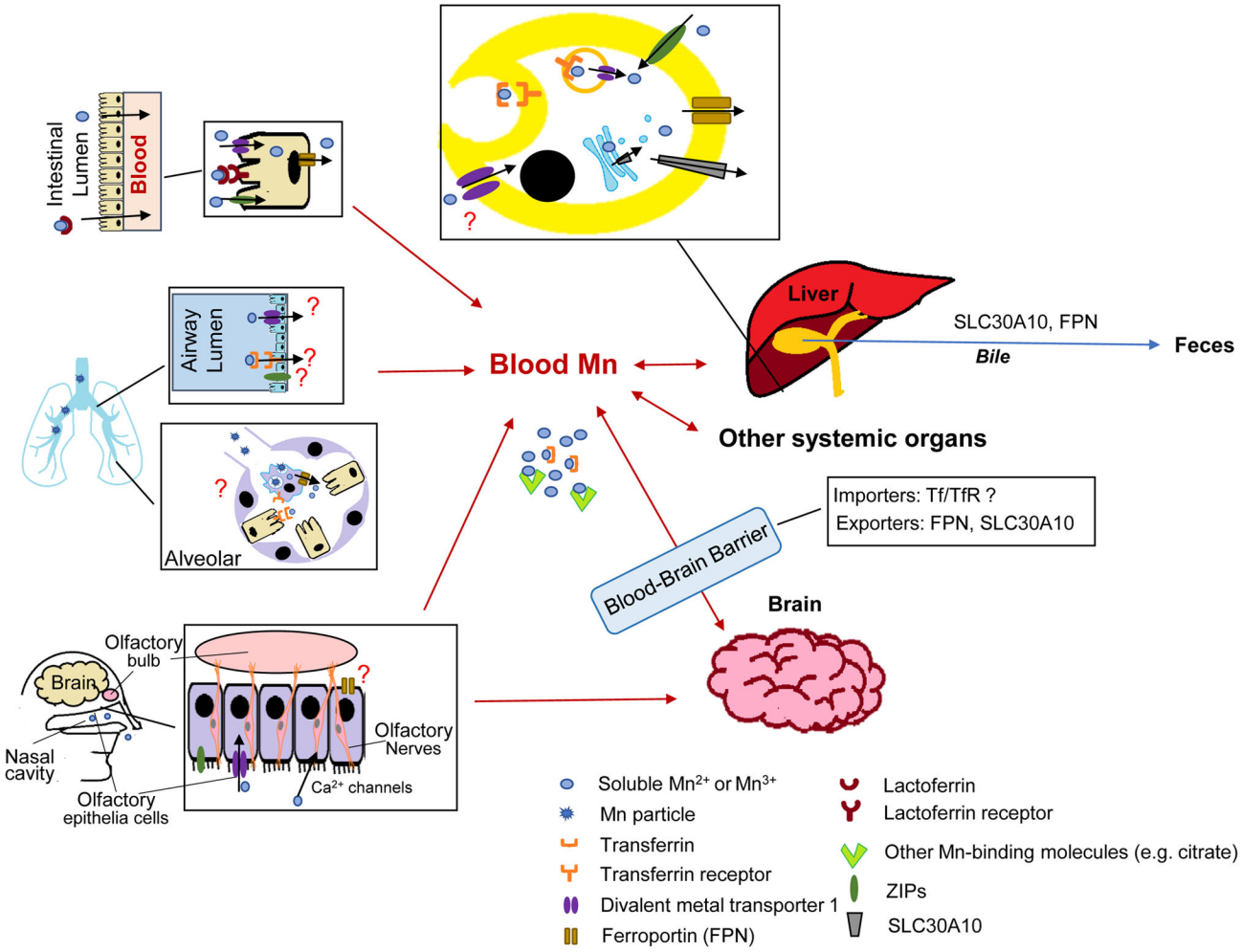


**Figure 2. Absorption, distribution and disposal of Mn**

Mn is absorbed via intestinal, pulmonary and olfactory transport. At the intestine lumen, free Mn or lactoferrin-bound Mn can be taken into the enterocytes via divalent metal transporter 1 (DMT1), ZIP8 and lactoferrin receptor. The free Mn inside the enterocytes is released into blood for systemic circulation. Airborne Mn, especially Mn particles, is absorbed through the lung by inhalation. While both DMT1 and transferrin receptor (TfR) are expressed at the epithelial cells and transferrin (Tf) is found in bronchoalveolar fluid, it is unclear whether they are directly involved in pulmonary Mn transport. Ferroprotein (FPN) is expressed at the alveolar macrophages, where it could contribute to dissolution of Mn-containing particles and thereby absorption of soluble Mn. DMT1 is involved in the olfactory uptake of Mn into blood and brain. The nasal route also expresses several metal transporters, including FPN and ZIPs, but their roles in olfactory Mn transport have not been evaluated. Mn can be directly taken up into the brain by calcium channels expressed at the terminal of olfactory nerves. After absorption, Mn distributes into the tissues by several importers. Intracellular Mn is released into blood or excreted out of the body by metal exporters, such as FPN and SLC30A10. The liver is the major organ for Mn disposal, and Mn is mainly excreted by the bile into the feces. However, the exact mechanism of biliary Mn secretion is unknown. The

mechanism of Mn uptake/export in the brain is incompletely understood and results are controversial.

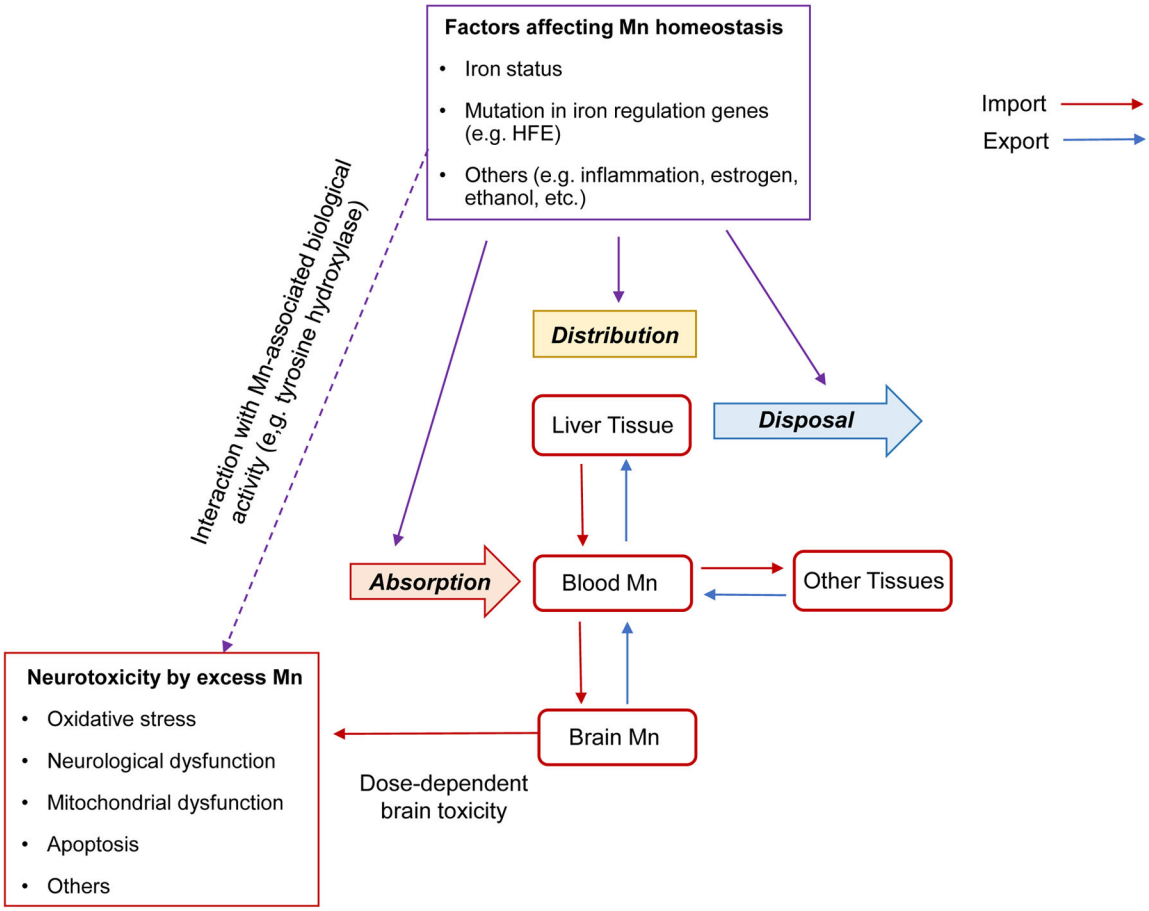


**Figure 3. Impact of iron status on Mn-associated neurotoxicity**

Changes in iron homeostasis or gene mutations in iron transporters and regulatory proteins can alter the expression of metal transporters that mediate the import and export of Mn, which thereby influences the absorption, distribution and disposal of Mn. Consequently, Mn homeostasis in the brain could be perturbed, and the neurotoxicity associated with Mn accumulation could be altered.