

# Iron and Brain Disorders

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## Abstract

*Iron is the most important element in the body, essential for almost all types of cells, including brain cells. The role of iron in the brain has been known for years. Iron deficiency and iron excess have been associated with pathophysiology of different brain disorders. Iron deficiency has been reported to have a role in brain development and the pathophysiology of restless legs syndrome. Iron accumulation has been related to some neurologic disorders such as Alzheimer disease, Parkinson disease, type I neurodegeneration with brain iron accumulation, and other disorders. Despite years of investigation, the reason for iron imbalance in the brain is not known. It also is not known whether the accumulation of iron in the brain is primary or secondary to development of neurodegenerative disorders. This review summarizes the present knowledge on the role of iron in human brain disorders.*

Iron is the second most prevalent metal on the earth and probably existed 4.5 billion years ago, after the earth had cooled and condensed. Photo-oxidation of ferrous ions caused by UV light might have had a role in the evolution of life on earth.<sup>1</sup> Iron is a vital element to microbial, plant, animal, and human life. Almost all organisms ingest iron in daily life.<sup>2</sup>

The total body iron content in men is approximately 4 g; women typically have smaller amounts than men. Most iron is in the form of heme iron that is found in hemoglobin, myoglobin, and iron-containing enzymes (such as catalase and the cytochromes).<sup>3</sup> The rest of the total body iron exists as a nonheme iron, which consists of plasma iron, iron bound to transferrin, and stored iron in ferritin and hemosiderin.<sup>3</sup>

Most iron is absorbed in the duodenum, but some also may be absorbed in the stomach, ileum, and colon. The epithelial cells of the duodenal mucosa are responsible for regulating transport of iron from the intestinal lumen to the circulation.<sup>4</sup> During digestion, only 15% of ingested iron is absorbed, depending on the source of iron and the levels of body iron store. Typically, two thirds of absorbed iron comes from heme iron derived from hemoglobin, myoglobin, and other animal proteins.<sup>3</sup> Hemoglobin and myoglobin are cleaved by gastric acids and pancreatic enzyme, and then heme is released and taken up directly by the mucosal cells.<sup>5</sup> Nonheme iron is solubilized and chelated by mucin and other compounds and enters the duodenum.

At the surface of the duodenal mucosal cell, iron binds to an integrin-like molecule that facilitates its passage across the cell membrane, possibly by an iron transporter.<sup>3</sup> The presence of reducing substances such as ascorbic acid, lactic acid, citric acid, malic acid, and cysteine glutathione increases iron absorption.<sup>3,6</sup> However, compounds such as phytates, polyphenols, casein, calcium, phosphates, soybean, and ovalbumin reduce iron absorption.<sup>3,4</sup>

After absorption, usually a fraction of the iron within the cells is delivered rapidly to plasma transferrin, and some of the iron is stored in ferritin, depending on the body's iron requirements.<sup>7</sup> When the body has enough iron, the formation of ferritin within the mucosal cells is maximal. In contrast, in iron deficiency, transport of iron into plasma transferrin is enhanced.<sup>7</sup>

Uptake, sequestration, and export of iron are achieved by regulatory proteins that sense intracellular iron levels. There are 2 cytosolic iron regulatory proteins 1 and 2 that are present in most tissues. These proteins regulate intracellular iron levels and expression of iron metabolism protein by binding to RNA stem loops within transcripts of iron metabolism proteins.<sup>8</sup> Expression of the transferrin receptors, ferritin, and the ferrous iron exporter can be regulated by hepatocytes to prevent the accumulation of excess iron.<sup>8</sup>

Iron is transported in plasma by binding to transferrin, an 80-kd glycoprotein that is synthesized in the liver.<sup>8</sup> Each molecule of transferrin binds to 2 atoms of ferrous iron, which then will be oxidized to the ferric state. To remove the iron from transferrin, ferric iron is reduced to ferrous iron by reducing substances in plasma. Under normal conditions, one third of the transferrin is saturated with iron, which provides a significant binding capacity to bind free iron in plasma. Thus, under normal circumstances, no free iron exists in plasma. The major function of plasma transferrin is to maintain extracellular iron in soluble form and deliver it to the cells, including erythroid precursors for hemoglobin synthesis.<sup>8</sup>

Iron is transported to brain via the blood-brain barrier that is composed of endothelial cells in small vessels throughout the brain that contain tight junctions, which regulate the brain iron levels.<sup>9,10</sup> In addition, fenestrated blood vessels in the choroid plexus that produce cerebrospinal fluid (CSF) also regulate brain iron.<sup>9,11</sup>

There are several mechanisms for the transport of iron to the brain. One mechanism is that transferrin binds to the transferrin receptors on the plasma membrane of neurons and glial cells and the transferrin-receptor complex enters the cell as an endosome.<sup>12</sup> In the endosomal compartment, iron is released from transferrin. After releasing iron, the transferrin and transferrin-receptor complex are recycled to the plasma via the proton-driven divalent metal transport protein 1,<sup>9</sup> which forms a transmembrane channel that permits water-soluble metals, such as iron, cobalt, and cadmium, to cross the lipid-rich environment of the cell membrane.<sup>13</sup> Another suggested mechanism for brain cell iron uptake is that iron detaches from transferrin within barrier cells (eg, neurons and glial cells), and then apotransferrin (iron-free transferrin) recycles to the blood and iron is released as non-transferrin-bound iron into the brain interstitium.<sup>14</sup> A 97-kd protein (known as melanotransferrin and belonging to the transferrin family) has been suggested as another route for transportation of iron to the brain.<sup>15</sup>

It has been shown that transferrin uptake and the ratio of iron to transferrin uptake by the brain decrease with age, and the transferrin recycling time increases with age.<sup>16,17</sup> It also has been reported that in the developing brain, when iron requirements are high, transferrin receptors are present in larger numbers and function more efficiently than in later life.<sup>16,17</sup>

Ferritin is a storage iron protein that exists in all tissues, including liver, spleen, bone marrow, skeletal muscle, and brain. It consists of 2 light and heavy subunits (light, 19 kd; heavy, 21 kd) and can store up to 4,500 iron atoms in each ferritin molecule.<sup>18</sup> The ratio of heavy to light chains varies in different tissues. For example, ferritins with more light chains are found in liver, which can store more iron, and ferritins with heavy chains that contain less iron exist in heart and brain.<sup>18</sup> Very small amounts of ferritin normally circulate in the plasma, and the ferritin comes from the storage pool of body iron. Therefore, the plasma ferritin level is a good marker for assessing body iron stores.<sup>3</sup> In the liver, most of the ferritin is stored in the parenchymal cells. In other tissues, such as the spleen and bone marrow, iron is stored in the mononuclear phagocytic cells.<sup>19</sup> In the brain, ferritin is found in microglia and oligodendroglia.<sup>20</sup>

When the protein shells of the ferritin are degraded, iron is aggregated into hemosiderin granules, which are insoluble. When body iron storage is normal, only small amounts of hemosiderin are found in the reticuloendothelial cells in the bone marrow, spleen, and liver. In iron-overload states, most of the iron is stored in the form of hemosiderin. Distribution of nonheme iron stored in the brain has been studied using magnetic resonance imaging (MRI), owing to iron's magnetic property. The concentrations of free iron ions in the brain are negligible (at physiologic pH), so the only nonheme iron-containing compounds with sufficient concentration to provide tissue magnetic resonance contrast are ferritin and hemosiderin.<sup>21</sup>

The distribution of iron and transferrin in the brain is not equal, and the distribution patterns are not similar. MRI studies have shown that the distribution of iron in the brain is uneven; the highest concentrations of iron are observed in the globus pallidus, dentate gyrus, thalamus, putamen, substantia nigra, and red nucleus (associated with Parkinson disease [PD], Alzheimer disease [AD], Huntington chorea, amyotrophic lateral sclerosis [ALS]).<sup>22</sup> The highest concentrations of transferrin are found in the hippocampus and the cortical region.<sup>23</sup> Quantitation of nonheme brain iron by MRI is helpful in the diagnosis and monitoring of different neurologic diseases.<sup>24</sup>

## Iron Deficiency in the Brain

Iron deficiency has been proposed to have adverse effects on brain function, causing cognitive and learning

impairment in infants and young children.<sup>25</sup> The severity and duration of iron deficiency are important indicators in brain disorders associated with iron deficiency. It has been suggested that iron and transferrin deficiency might be responsible for degeneration of the C1 and C2 areas of the hippocampus.<sup>26</sup> Unfortunately, supplementation with iron has not proven effective, especially if the iron deficiency occurred during a critical state of brain development and neural differentiation (when changes are irreversible). The alteration of neurotransmitters such as noradrenaline, serotonin, and dopamine during a state of iron deficiency might explain some of the behavioral and developmental changes observed in human infants.<sup>27,28</sup>

### Restless Legs Syndrome

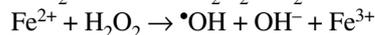
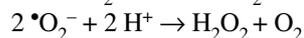
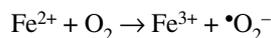
Motor deterioration is suggested to be associated with iron deficiency in the brain.<sup>28</sup> There is some evidence indicating that iron deficiency alters motor activity<sup>29</sup> and circadian patterns of motor activity.<sup>28,30</sup> The association of restless legs syndrome (RLS) with iron deficiency anemia that can be improved with iron consumption has been known for decades.<sup>31</sup> Because RLS is seen more frequently in pregnant women (12%-20%),<sup>32</sup> it has been suggested that RLS also might be associated with iron deficiency.<sup>33</sup> Although one study claimed that serum ferritin and transferrin levels were not different between patients with RLS and control subjects, the patients with RLS had significantly lower CSF ferritin levels and higher CSF transferrin levels than did the control subjects.<sup>34</sup> Thus, it was concluded that impaired transportation of iron across the blood-brain barrier might be related to RLS, and changes in iron homeostasis play the crucial role in pathophysiology of RLS.<sup>33,35,36</sup>

### Excess Brain Iron

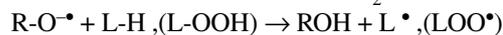
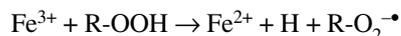
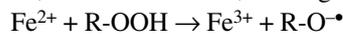
Iron accumulates in the normal aging brain and in greater amounts in several neurologic disorders.<sup>37</sup> In the presence of excess extracellular iron, such as in hemochromatosis, the iron-carrying capacity of serum transferrin increases. The increased iron-carrying capacity of transferrin and increased rate of iron transportation across the blood-brain barrier result in accumulation of iron in the brain cells.<sup>38</sup>

### Hazards of Free Iron

Iron is known to catalyze the formation of reactive oxygen species (ROS), such as hydroxyl radical, and initiation or enhancement of lipid peroxidation, by reacting with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) via the Fenton reaction.<sup>39</sup> ROS are highly reactive oxygen-containing radicals that can easily react with other molecules, such as protein, DNA, lipids, and antioxidants.<sup>40,41</sup>



Iron also can react with lipid peroxides in a way similar to its reaction with H<sub>2</sub>O<sub>2</sub> and produce alkoxy (R-O•) and peroxy radicals (R-O<sub>2</sub>•). The resulting peroxy radical leads to propagation of lipid peroxidation.<sup>42</sup> Lipid (L) peroxidation can proceed until the lipid radicals interact with one another and/or a "chain breaker," such as vitamin E, forming a nonradical species.



Iron compounds like free hemoglobin also can catalyze peroxidation of purified arachidonic acid and other polyunsaturated fatty acids within normal cell membranes in the presence of H<sub>2</sub>O<sub>2</sub> and •O<sub>2</sub><sup>-</sup>.<sup>43</sup> Furthermore, the addition of purified hemoglobin (even in the absence of H<sub>2</sub>O<sub>2</sub> and •O<sub>2</sub><sup>-</sup>) can result in brisk peroxidation of lipids in murine brain homogenate.<sup>44</sup> The hemoglobin and/or iron-mediated reactions in the brain are catalyzed paradoxically by the (usually) antioxidant compound, ascorbic acid, and are blocked by an iron chelator such as desferrioxamine.<sup>45</sup>

### Hemochromatosis

Hemochromatosis is the most prominent iron-related disorder in which pathologic symptoms arise from an excessive uptake of dietary iron and its deposition in many organs, including the brain. The gene involved in hemochromatosis is known as *HFE*. The function of the HFE protein is to complex with the transferrin receptor on the cell membrane and to lower the affinity of the receptor for transferrin. Although defects in the *HFE* gene are not the only cause of iron-storage disease, homozygous mutation of the Cys282Tyr *HFE* is responsible for the severe type of iron-storage disorder.<sup>46,47</sup> The presence of the HFE protein on blood vessels, choroid plexus, and ependymal cells can influence brain iron uptake.<sup>46,47</sup> Iron deposition in hemochromatotic patients is observed in the choroid plexus, pituitary, and circumventricular organs.<sup>48,49</sup> It is suggested that mutations in the hemochromatosis gene *HFE* that can result in increased iron accumulation in the brain might be a risk factor for AD and parkinsonism associated with hemochromatosis.<sup>50,51</sup>

### Alzheimer Disease

AD is a progressive degenerative disease with a gradual deterioration in memory, cognition, behavior, and the ability to perform activities of daily living. Evidence of increased brain metal levels such as iron and copper has been associated with AD.<sup>52</sup>

The amyloid-β (Aβ) plaques in the brain are the hallmark pathologic features of AD, which are derived from the cleavage of amyloid precursor protein (APP). Deposition of fibrillar

aggregates of A $\beta$  in the brain parenchyma, which is caused by A $\beta$  overproduction, impaired clearance, or both, has been hypothesized to explain the cause of AD.<sup>53</sup> APP has binding sites in its amino-terminal domain and in the A $\beta$  domain for copper and iron. High levels of iron may interact with the A $\beta$  peptide,<sup>52</sup> leading to the reduction of molecular oxygen to superoxide and eventually to H<sub>2</sub>O<sub>2</sub> by reducing iron. It also has been demonstrated that overexpression of the carboxyl-terminal fragment of APP (A $\beta$ ) significantly reduces the level of copper and iron in the transgenic mouse brain. This suggests a role for APP and A $\beta$  in physiologic metal regulation in AD.<sup>54</sup>

In addition, overexpression of melanotransferrin has been reported in AD. Because purified melanotransferrin can bind iron, it has been proposed as another protein that also might be involved in iron transportation.<sup>55</sup>

The role of iron in the pathogenesis of AD is thought to be related to enhanced oxidative stress mediated by free iron. Transferrin, ferritin, and iron regulatory protein 2 also have been associated with neurodegeneration in AD. The latter might be responsible for the disturbance in brain iron homeostasis and the overall decompartmentalization of iron and the resulting oxidative processes suggestive of AD.<sup>54</sup>

### Friedreich Ataxia

Friedreich ataxia is an autosomal recessive neurodegenerative disease characterized by degenerative atrophy of the posterior columns of the spinal cord followed by the spinocerebellar tracts and corticospinal motor tracts, leading to progressive ataxia, sensory loss, and muscle weakness. It also is associated with scoliosis, foot deformity, and heart disease and has ophthalmic manifestations.

Friedreich ataxia has been established as a trinucleotide repeat disorder, and the encoded protein was named frataxin.<sup>56</sup> Frataxin, a mitochondrial protein, is suggested to have a role in mitochondrial iron transport or in iron-sulfur assembly and transport. High levels of iron in the mitochondria can react with superoxide ( $\bullet\text{O}_2^-$ ) and H<sub>2</sub>O<sub>2</sub> to produce the hydroxyl radical ( $\bullet\text{OH}$ ), which can oxidize cellular components, damage respiratory chain complexes, and result in cellular injury and, eventually, cell death. Brain and heart cells depend highly on aerobic metabolism and are more susceptible to free radical generation in mitochondria.<sup>56,57</sup> The effects of treatment with the antioxidants coenzyme Q and vitamin E in patients with Friedreich ataxia are being studied, and preliminary results seem to be promising.<sup>58</sup>

### Parkinson Disease

PD is a progressive disorder that manifests as tremor at rest, bradykinesia, gait abnormalities, rigidity, postural dysfunction, and loss of balance. For many years, scientists have tried to find a connection between iron in the substantia nigra and the pathophysiology of PD.

Iron has been suggested to be responsible for nigrostriatal dopamine neuron degeneration in PD owing to its ability to produce toxic ROS and cause lipid peroxidation.<sup>59</sup> Iron deposits in degenerating neurons of the substantia nigra and in Lewy bodies<sup>60</sup> can have deleterious effects on the extrapyramidal system and on psychomotor function.<sup>61</sup> The presence of the pigment, neuromelanin, in the substantia nigra in PD also might result in iron accumulation, because neuromelanin can function like ferritin and store iron.<sup>62</sup> Overexpression of lactoferrin (a protein that reversibly binds iron) receptors on neurons and microvessels in regions of neuronal degeneration in PD-affected brain tissue suggests a possible link to iron overload in affected brain regions.<sup>60</sup> All these mechanisms suggest that disturbances in iron homeostasis and metabolism in PD occur at several levels, such as iron uptake, storage, intracellular metabolism, release, and posttranscriptional control.<sup>63</sup> As indicated in the preceding text, a disturbance in iron homeostasis can provide a favorable condition in which free iron, via generation of ROS, causes permanent tissue damage.

### Type I Neurodegeneration With Brain Iron Accumulation

Type I neurodegeneration with brain iron accumulation (NBIA-1) formerly was known as Hallervorden-Spatz syndrome or pantothenate kinase-associated neurodegeneration. NBIA-1 is a rare, genetically determined neurodegenerative disorder characterized by extrapyramidal dysfunction and mental deterioration. Iron accumulates mainly in the globus pallidus and the pars reticularis of the substantia nigra, which is shown as brown-pigmented iron deposits.<sup>64</sup> MRI contrast shows a centrally located high signal in the globus pallidus named the "eye-of-the-tiger" that is characteristic of NBIA-1.<sup>65,66</sup> The mutation in a novel pantothenate kinase gene, *PANK2*, is predicted to cause the accumulation of cysteine, which binds iron and causes oxidative stress in the iron-rich globus pallidus.<sup>67</sup>

### Multiple Sclerosis

Multiple sclerosis is a neuroinflammatory condition in which the oligodendrocyte and its product myelin are the targets of attack by mononuclear cells. By using immunocytochemical and histochemical staining methods, high concentrations of ferritin<sup>68</sup> and iron are observed in oligodendrocytes.<sup>69</sup> During stresses such as hypoxia, oligodendrocytes can increase their synthesis of ferritin.<sup>70</sup> Ferritin, by releasing iron, is fully capable of providing elements (such as free iron) that through oxidative processes can cause cellular injury.<sup>71</sup> In 1 study, 5 autopsy specimens from patients with multiple sclerosis showed positive iron reactions in sections surrounding demyelinated plaques.<sup>72</sup> It was speculated that positive reactions found within blood vessels of gray matter near the demyelination area also might be due to extravasated blood.<sup>72</sup> In contrast with the latter observation,<sup>72</sup> microscopic

examinations of autopsy materials from patients with multiple sclerosis (32 paraffin-embedded blocks containing demyelinated plaques) failed to detect iron within or around areas of demyelination.<sup>73</sup> Obviously, more studies are needed to ascertain whether iron accumulation in or around multiple sclerosis plaques is the primary cause of the tissue damage or a consequence of damage caused by other insults (which can cause decompartmentalization of iron).

### Cerebrovascular Disorders

Intracerebral hemorrhage is a common type of stroke that often is fatal. The hematoma within brain parenchyma leads to a series of events, which can result in secondary insults. Severe neurologic deficits can occur if the patient survives the ictus. Iron overload and up-regulation of transferrin, transferrin receptors, and ferritin in the brain have been reported in these subjects, suggesting a role for iron in the outcome of intracerebral hemorrhage.<sup>74</sup> Iron can exacerbate the damage caused by ischemic stroke by generating ROS and causing lipid peroxidation. Iron overload has been shown to have some effects on the infarct size after middle cerebral artery occlusion. This adds to the oxidative stress that occurs during an ischemic episode facilitated by iron overload disrupting the blood-brain barrier.<sup>75</sup>

### Aceruloplasminemia

Aceruloplasminemia is a genetically determined disorder with symptoms of blepharospasm, retinal degeneration, diabetes mellitus, and neurodegeneration.<sup>76</sup> There is an absence of circulating plasma ceruloplasmin owing to mutation in the *Cp* gene.<sup>76</sup> Ceruloplasmin is a 132-kd  $\alpha_2$ -globulin that binds to copper (6-8 copper atoms per ceruloplasmin molecule) and contains about 95% of the total plasma copper.<sup>76</sup> Ceruloplasmin is involved mostly in redox reactions in plasma. It can act as an antioxidant and a prooxidant. Also, ceruloplasmin has ferroxidase activity and, therefore, can oxidize ferrous iron to ferric iron and facilitate the uptake of free iron by transferrin. In addition, in aceruloplasminemia, the serum ferritin level is elevated, mild anemia is present, the serum iron level is low, and a profound hepatic iron overload is present. Interestingly, iron deposition was detected more in the astrocytes than in the neurons.<sup>77</sup> Dementia and cerebellar ataxia due to iron accumulation in the basal ganglia of the brain are seen in aceruloplasminemia.<sup>76</sup> Again, the possible consequence of increased iron in the brain of patients with aceruloplasminemia is the generation of ROS and lipid peroxidation that can result in tissue injury and eventual neuronal cell death in the brain.<sup>78</sup>

### Other Brain Disorders

In addition to the conditions described in previous sections, there are several more conditions in which iron

deposition in the brain has been found. For instance, in a case of a special type of hepatocerebral encephalopathy, iron deposition was found in the central gyrus, superior temporal gyrus, medial and lateral occipitotemporal gyrus, and middle temporal gyrus of the occipital lobe.<sup>79</sup> Whether iron accumulation in the latter case is the primary cause of tissue injury or the consequence of other insults to the tissue is not understood at this time. ALS is another disorder in which increased iron-mediated oxidative changes are suggested to be associated with the tissue damage seen in this disorder. ALS is one of the most common neurodegenerative disorders, with degeneration of cortical and spinal motor neurons. As mentioned, iron-mediated oxidative stress is suggested to be one of the main factors involved in the pathogenesis of ALS.<sup>80</sup>

Other conditions including neuroferrinopathy, Huntington disease, and progressive supranuclear palsy also have been shown to have elevated iron levels in the brain.<sup>10,81,82</sup> Because iron might have a central role in the pathogenesis of the aforementioned disorders, it seems reasonable to think of iron itself a potential marker for these disorders. Indeed, one interesting study suggests that serum free (non-protein bound) iron may be used as a marker to identify neonates in whom neurodisability will develop.<sup>83</sup> It is a reasonable thought and a relatively easy approach to start looking for better markers for laboratory diagnosis of these devastating disorders.

## Conclusion

We summarized findings from many studies on different neurodegenerative disorders. Although these diseases have their own distinctive features, they all had one thing in common: accumulating iron in the brain. Increased iron in the brain, which is rich in oxygen and fatty acids, provides an ideal environment for oxidative stress and possible irreparable tissue damage. If, indeed, iron and/or oxidative processes are involved in the pathogenesis of neurodegenerative disorders, approaches such as iron chelation therapy and antioxidant supplements might help slow the degenerative processes or ameliorate brain tissue injury. Indeed, a recent study introduces a new potent iron chelator, VK-28 (5-[4-(2-hydroxyethyl) piperazine-1-ylmethyl]-quinoline-8-ol), that can cross the blood-brain barrier and inhibit iron-induced mitochondrial membrane lipid peroxidation and can protect neurons.<sup>84</sup> Obviously, more work is needed to better understand the role of iron in the pathogenesis of neurologic disorders and to design therapeutic agents for more effective management and amelioration of these devastating diseases.

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