

Pathophysiology of iron overload

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Excess iron can cause serious cellular and tissue damage through its ability to promote formation of highly reactive hydroxyl radicals by electron transfer. Such free radicals can damage proteins, lipids, and DNA, leading to destruction of organelles, cell death, and fibrosis. Excess iron from blood transfusions and from elevated gastrointestinal absorption of dietary iron accumulates in the tissues and contributes to increased pools of unsequestered labile iron. The clinical effects of excess tissue iron include heart disease, hepatic dysfunction, and derangement of the endocrine system. Iron chelation therapy can detoxify excess labile iron, reduce absolute levels of tissue iron, and reverse iron-mediated disease.

How is iron toxic?

In healthy subjects, when iron metabolism is in balance, iron is absorbed from the diet at a rate equivalent to ~1 to 2 mg each day, and is lost at a similar rate through sloughed epithelial cells and blood loss [1]. After absorption from the duodenum, iron enters the plasma where it is complexed with transferrin, a

77 kDa single-chain polypeptide with high affinity for Fe^{3+} . Transferrin-bound iron in the plasma is the main pool supplying iron to the erythron, which cycles 20 to 30 mg of iron each day, as well as to hepatocytes and other parenchyma, which cycle approximately one tenth of this amount (Fig. 1). Within cells, iron is stored complexed with ferritin, a large multimeric protein able to accommodate ~4500 Fe^{3+} ions. A small proportion of ferritin is found in the blood (serum ferritin).

Patients receiving repeated red-blood-cell transfusions for anemia not related to blood loss have a considerably increased iron intake. For example, a splenectomized patient with thalassemia major maintaining a mean hemoglobin level of 12 g/dL requires ~300 mL of blood per kg body weight per year, which equates to around 24 mg of iron per day; a >10-fold increase on normal iron absorption rates [2]. Excess iron from blood transfusions is initially processed by macrophages, which digest senescent erythrocytes and return iron to the plasma transferrin pool. This extrinsic transfusional iron load may saturate the available uncomplexed transferrin leading to the for-

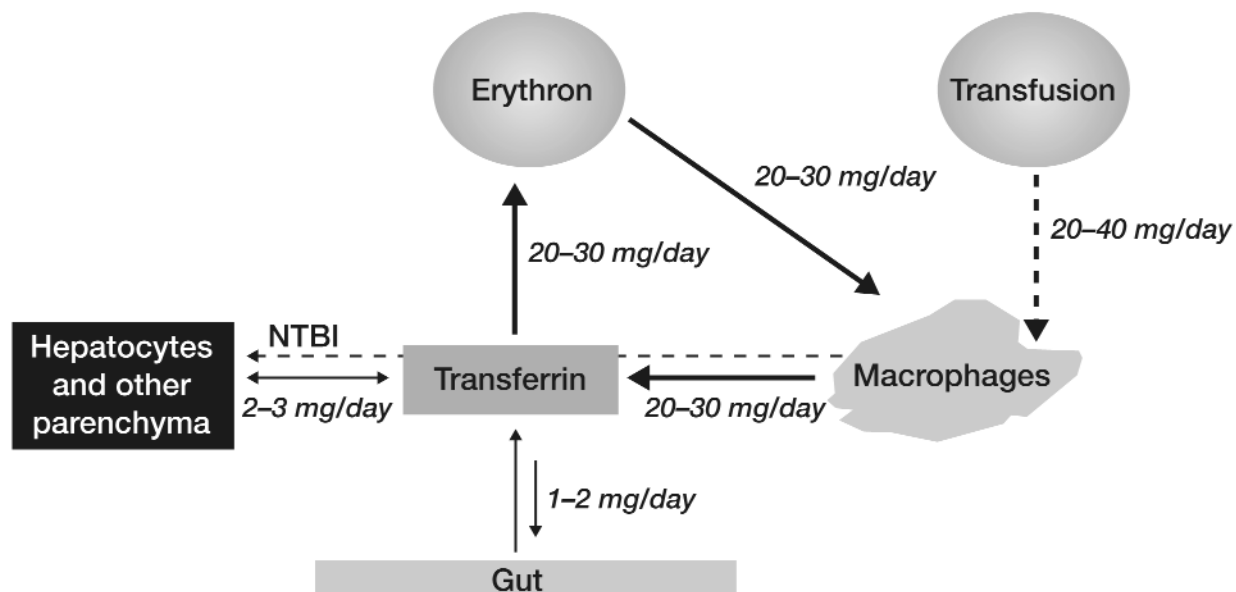
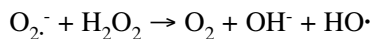


Fig. 1. Simplified iron turnover in humans. Schematic diagram showing the major physiological routes of iron transfer in humans (solid arrows), together with the route of transfusional iron in transfusional iron overload (dotted arrows).

mation of nontransferrin-bound iron (NTBI) in the plasma. This form of iron is rapidly taken into hepatic and other parenchymal cells through uptake mechanisms that are independent of transferrin-mediated uptake [3]. NTBI is also formed by excessive ineffective erythropoiesis. In patients with disorders of hemopoiesis, such transfusional iron loading may be exacerbated by increased iron absorption from the gut due to erythropoietin-induced erythroid expansion [4]. Excess iron in the plasma is ultimately taken into cells and deposited in the form of ferritin and ferritin-based precipitates called hemosiderins that are visible histologically. The majority of iron deposited in this way accumulates in the liver.

Iron is able to cycle between ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms through the donation or acceptance of an electron. While this electron transfer capacity is necessary for the function of many iron-coordinating biomolecules such as cytochromes and hemoglobin, it also allows iron to catalyze the formation of highly reactive hydroxyl radicals via the Haber Weiss reaction. In this reaction, comparatively poorly reactive superoxide and hydrogen peroxide yield molecular oxygen, hydroxide ions, and highly reactive hydroxyl radicals:



Owing to their high reactivity, hydroxyl radicals can cause oxidative damage affecting lipid, protein, and DNA molecules. Effects on lipids are likely to play a major role in iron-mediated oxidative damage. Initial abstraction of a hydrogen atom ($\text{H}\cdot$) from a lipid by an hydroxyl radical (to yield a water molecule) can result in molecular rearrangement, lipid peroxidation, and the formation of a peroxy radical that is able to propagate further lipid peroxidation in a chain reaction. The end result is decomposition of lipid molecules with concomitant effects on

the integrity of organelles. One consequence of such damage is enzyme leakage from lysosomes and related failure of cellular compartmentalization, which can lead to cell death. Another effect is an increased production of transforming growth factor $\beta 1$, which leads to increased collagen synthesis and fibrosis. Overall, therefore, iron-induced oxidative damage can lead to cell death and/or fibrosis (Fig. 2) [5].

Which forms of iron are most toxic?

Toxicity of iron may be considered from two different perspectives: the presence and effects of pools of labile iron such as NTBI, and the presence and effects of iron deposits in the tissues. Looking at iron from each perspective can yield valuable insights into mechanisms of iron toxicity. Pools of labile iron consist of iron not bound to dedicated proteins, which may correspond with physiological turnover pools of iron transiently 'free' before being recomplexed. Within cells, this pool of iron is termed the labile iron pool (LIP). Such pools account for a small proportion of the absolute tissue levels of iron, most of which is in the form of ferritin and hemosiderins.

Iron in the LIP and NTBI is available to become involved in electron transfer reactions and thus to catalyze production of hydroxyl radicals. There is good in vitro evidence linking LIP iron to lipid peroxidation and organelle damage [6]. Moreover, studies show that NTBI can promote lipid peroxidation in vitro [7]. However, in the clinical setting, evidence for the toxicity of labile iron pools is sparse. No direct link has been found between NTBI or LIP concentration and clinically manifest organ damage. Nevertheless, there is some indirect evidence for the clinical significance of labile iron pools. One example is the depletion of antioxidant levels observed in patients with iron overload. A recent analysis of 48 transfusion-dependent β -thalassemia patients found severe depletion of

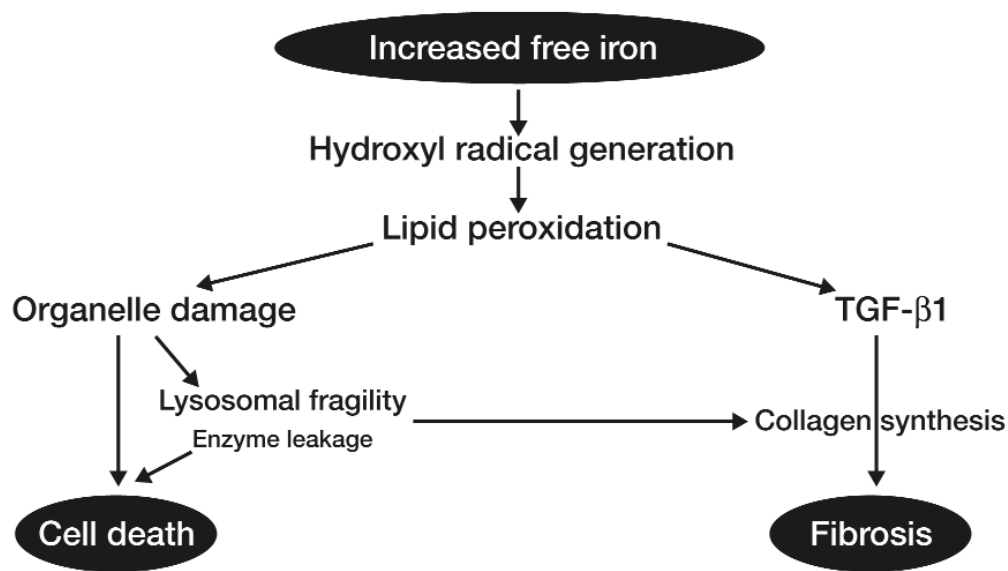


Fig. 2. Consequences of iron-mediated toxicity. Schematic diagram showing the mechanisms through which 'free' iron may cause damage.

(Adapted from Cohen AR and Porter JB. Transfusion and iron chelation therapy in thalassemia and sickle cell anemia. In: *Disorders of Hemoglobin: genetics, pathophysiology and clinical management*. Cambridge University Press. Steinberg MH et al [Eds];2001:979–1027.)

plasma levels of lycopene, ubiquinol, ubiquinone, vitamin A, vitamin E, β -carotene and vitamin C [8]. There was also a significant correlation between plasma NTBI concentration and antioxidant depletion. Further indirect evidence comes from the observed effects of short-term iron chelation therapy to remove excess iron. In patients with iron-mediated cardiac damage, such short-term therapy improves cardiac function within a few days or weeks, a timescale which allows only minimal reduction in total body storage iron [9]. This suggests that reduction of LIP accounts for the beneficial effect rather than the removal of storage iron in the form of ferritin or hemosiderins.

There is good evidence for the harmful clinical effects of increased absolute tissue levels of iron. Serum ferritin concentration mirrors total body iron load over the long term and is used as an indicator of overall iron burden. At our own center and others, it has been shown that patients with lower serum ferritin levels have better long-term survival than those with higher levels [10]. Moreover, there appears to be an association between liver iron concentration (LIC) and survival [11]. However, absolute tissue levels of iron alone do not provide a complete picture. For example, the concentration of iron in different tissues at *post mortem* does not correlate with damage to those organs. In particular, no direct link has been established between the concentration of storage iron in the heart and cardiac damage or death.

Where is iron toxic?

Most of our experience of iron toxicity is based on transfusion-dependent patients with β -thalassemia. The predominant cause of death in these patients is heart disease, which can occur as early as the first decade of life, followed by infection, liver disease, and malignancy [12]. Paradoxically, cardiac iron load is typically a fraction of that found in the liver, even in patients dying of heart failure. The reasons for the heart's susceptibility to iron overload are not clear. The elevated risk of infection in iron overload may be due to the depletion of apo-transferrin and its iron-scavenging capacity that normally deprives bacteria of iron [13]. In the liver, fibrosis and cirrhosis are common manifestations of iron overload and are likely to be consequences of oxidative stress due to the high iron load in this organ. In addition to these effects, a major source of long-term morbidity in iron overload is derangement of the endocrine system leading to hypogonadism with concomitant effects on development, as well as hypothyroidism and diabetes.

In the early stages of iron overload, the distribution of iron in transfusional overload differs from that seen with absorptional overload [1,14]. Excess iron from blood transfusions is initially deposited in the reticulo-endothelial system. By contrast, in hereditary hemochromatosis initial iron deposition is predominantly found in hepatocytes. As more iron accumulates, the patterns of distribution converge. There is a characteristic distribution of iron in the liver and endocrine glands, with very little iron found in the brain or skeletal muscle. This distribution probably reflects the pattern of uptake of NTBI by the respective tissues. During iron chelation therapy, the picture

may change further due to differential rates of iron removal from the different tissues [15]. While tissue levels of iron may vary between patients and may not always correlate directly with toxicity in a particular organ, there is nevertheless a clear link between overall iron burden and global toxicity. The LIC correlates well with total body iron load in the setting of thalassemia [16], and is used by convention as the key indicator of iron burden. LIC can be measured either by liver biopsy or noninvasively using MR imaging or other magnetic techniques.

Even though cardiac disease is the major cause of death in patients with iron overload, no direct association has been found between cardiac iron concentration and cardiac dysfunction or death. This lack of association may partly reflect the difficulty of obtaining data on cardiac iron load, a problem that is increasingly being addressed by the use of noninvasive measurement techniques such as MR imaging and magnetic susceptibility. Emerging evidence from these techniques suggests that iron loading in the heart does not necessarily correlate with total body iron load or LIC. Fig. 3 illustrates examples of discordance between liver and heart iron deposition revealed by MR imaging [17].



Fig. 3. Discordance of liver and heart iron deposition. The upper panel shows a patient with severe cardiac iron deposition but minimal liver iron deposition (heart darker than liver). The lower panel shows a patient with normal myocardial iron but severe liver iron overload (liver darker than heart). (From Anderson LJ et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;22:2171-9; with permission.)

How can chelators decrease toxicity?

Iron chelators are able to complex with iron to facilitate its removal in the urine or bile. The aims of chelation therapy are to reduce the levels of toxic iron, ie, LIP within cells and NTBI outside cells, and to reduce the overall burden of iron in the form of ferritin and hemosiderin deposits. A balance must be struck between minimizing excess iron in 'free iron' turnover pools, and sequestering so much iron that physiological iron turnover is impeded. In the latter case, over-chelation can inhibit metalloenzymes and lead to neurotoxicity, bone marrow toxicity, and growth failure.

The likely direct targets for iron chelation and removal are turnover iron pools within liver cells and other organ parenchyma (LIP), and in the plasma (NTBI). Within liver cells, chelation aims to limit the size of the LIP and thus minimize production of new ferritin, as well as to capture iron as ferritin molecules are turned over periodically. Outside cells, chelation should intercept the iron that is released from macrophages, which would otherwise be destined for binding to transferrin. Extracellular chelation should ideally also sequester NTBI, which is likely to be toxic and is also able to contribute to intracellular LIP and iron loading of parenchymal cells by entering cells via a calcium-dependent uptake mechanism or via dedicated metal transporters. As plasma NTBI is highly labile, levels decline rapidly in the presence of a chelator. However, they also rapidly return to their original levels after the chelator is removed. Fig. 4 shows the effect of intravenous infusion of the chelator deferoxamine (Desferal®) on plasma NTBI levels. Plasma NTBI decreases rapidly soon after an infusion begins, but due to the short plasma half-life of deferoxamine, it returns to its previous levels almost as soon as the infusion is stopped [18].

Deferoxamine is the current reference standard iron chelator, and more than 35 years of clinical use has demonstrated ability to reduce iron burden and maintain a negative iron balance in frequently-transfused patients. However, the clinical utility of deferoxamine is compromised by its poor bioavailability and short plasma half-life. These properties generally necessitate a regimen of subcutaneous or intravenous infusion over many hours, on most days every week, the aim being to maximize the duration of effective chelation and minimize the persistence of excessive 'free' iron pools.

An oral iron chelator, deferiprone (Ferriprox®), became available in Europe in 1999. However, deferiprone is currently not approved for use in the USA or Canada due to concerns about its efficacy and safety. The issues are controversial, and mainly relate to the ability of deferiprone to remove iron from the liver in a subset of patients, and the possible risk of severe neutropenia, which necessitates regular monitoring of neutrophil count. More recently, a new once-daily oral iron chelator ICL670 (deferasirox, Exjade®), has been developed and is currently in clinical trials. Available results suggest that ICL670 at doses of 20 and 30 mg/kg/day may be as effective as deferoxamine with a safety and tolerability profile acceptable for long-term use [19].

In contrast to the rapid effects of chelation on labile iron pools, the reduction of ferritin and hemosiderin iron deposits by chelation is a slow process. This is because the pool of 'uncoordinated' iron available for chelation at any one time is small compared with the overall iron burden. Even with an iron removal rate of 0.4 to 0.5 mg/kg/day, it may take years for a patient with severe iron overload to achieve acceptable levels of iron. To put the issue into perspective, in the era before iron chelation therapy was available, transfusion-dependent

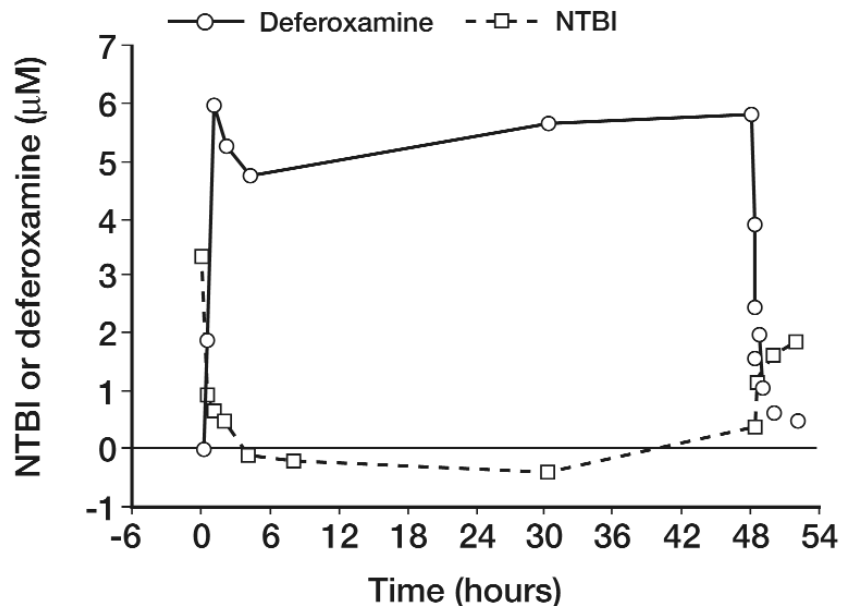


Fig. 4. Effect of 48-hour deferoxamine intravenous infusion on NTBI. The effect of deferoxamine intravenous infusion at 50 mg/kg/24 hours on NTBI is shown in a single patient with thalassemia major on starting the deferoxamine infusion and on stopping the infusion at 48 hours. (From Porter JB et al. Kinetics of removal and reappearance of nontransferrin-bound plasma iron with deferoxamine therapy. *Blood* 1996;88(2):705-13; with permission.)

patients with thalassemia major might accumulate so much iron by the age of 20 years that LIC exceeded 40 mg Fe/g dry weight. This can be compared with the LIC values widely considered as normal (<1.6), associated with increased risk of complications (>7) and at high risk of cardiac disease and early death (>15 mg Fe/g dry weight) [20]. In a recent study, continuous intravenous infusion of deferoxamine was able to reduce LIC by approximately 7 mg Fe/g dry weight over 12 months (Table) [21]. However, this sustained degree of chelation represents an ideal. Many patients using deferoxamine with subcutaneous infusion pumps are unable to achieve this rate of iron removal because of difficulties with compliance.

Iron chelation therapy is also effective in reducing the concentration of iron in the heart. Measurement of cardiac iron concentration is not straightforward, and until recently necessitated cardiac biopsy, which might yield inaccurate results due to the heterogeneous distribution of iron in the myocardium. However, the advent of MR imaging has led to the development of a noninvasive method for the measurement of cardiac iron called MR imaging T2-star (T2*). MR imaging T2* measures local magnetic field differences that are increased with iron deposition [16]. Low MR imaging T2* values indicate heavy iron loading. The Table shows the effects of continuous intravenous infusion of deferoxamine on cardiac and liver MR imaging T2*, LIC, and resting left ventricular ejection fraction in iron-overloaded patients with heart failure [20]. By 3 months, mean ejection fraction increased to within the normal range while cardiac iron load as assessed by MR imaging T2* was improved to a much smaller degree. Cardiac iron load continued to improve and by 12 months the cardiac MR imaging T2* had increased significantly. These data confirm earlier results showing significant improvements in resting left ventricular ejection fraction and reversal of serious arrhythmias after continuous intravenous deferoxamine infusion [9].

Conclusions

Iron can be toxic as a result of its ability to catalyze the formation of free radicals that cause oxidative damage to lipids,

proteins, and DNA. In iron overload, excess iron accumulates in the tissues in the form of ferritin and hemosiderin. These increased deposits of storage iron contribute to increased pools of labile turnover iron that have been implicated in oxidative damage. Although most clinical experience of iron overload comes from thalassemia, evidence is also emerging in the context of myelodysplastic syndrome and is reviewed in this supplement by Professor Norbert Gattermann. The liver and endocrine glands are among the tissues that accumulate the highest iron concentrations, although concentration does not necessarily correlate with toxicity. In fact, iron-mediated heart disease is the leading cause of death in transfusion-dependent patients with β -thalassemia, although liver disease and endocrine dysfunction also cause considerable morbidity and mortality. Overall iron burden correlates well with clinical outcome, and changes in iron burden are reflected by long-term trends in serum ferritin levels. Iron chelation therapy aims to remove excess iron from labile iron pools without removing so much iron that physiological processes are disturbed. Iron chelators can reverse iron-mediated disease in the short term, and can reduce overall iron burden over the long term.

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Table

Effect of continuous intravenous deferoxamine infusion on cardiac and liver iron burden and cardiac function

Mean values	Myocardial T2* (ms)	Liver T2* (ms)	LIC (mg Fe/g dw)	LVEF (%)
Normal range	>20	>19	<1.6	61–81
Baseline	5.1 ± 1.9	1.8 ± 1.0	9.6 ± 4.3	52 ± 7.1
3 months	6.9 ± 2.1	3.4 ± 1.8	6.0 ± 5.6	61 ± 8.1
6 months	7.5 ± 2.5	6.9 ± 5.3	2.9 ± 1.9	62 ± 7.9
12 months	8.1 ± 2.8	10.3 ± 9.2	2.1 ± 1.5	63 ± 6.4
<i>p</i> ^a	0.003	0.01	0.001	0.03

Abbreviations: LVEF, resting left ventricular ejection fraction; dw, dry weight.

^aInitial versus final measurement.

(Data from Anderson LJ et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol* 2004;127:348-55; with permission.)

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