

Iron- Physiology & Pharmacology

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PHYSIOLOGY OF IRON

The importance of iron

Iron is the fourth most abundant element of the earth's crust and the second most abundant metal. It is also an essential nutrient required by every human cell.

Its atomic structure gives rise to a number of biochemically useful properties, including the unusual capacity to both donate and accept electrons, and to reversibly bind to ligands such as oxygen and nitrogen. It can exist in various oxidation states (from **-2** to **+6**), the principal being **Fe²⁺** and **Fe³⁺**. The body exploits the unique properties of iron by incorporating it into hundreds of different enzymatic and non-enzymatic proteins that are crucial to a wide range of physiological functions:

FUNCTION	PROTEIN
Oxygen transport and storage	Haemoglobin in red blood cells transports oxygen in the blood, and myoglobin stores oxygen in muscles
Oxygen homeostasis	An iron-dependent prolyl hydroxylase plays a critical role in the physiologic response to hypoxia
Electron transport and energy production	Cytochromes and dehydrogenases are crucial components of mitochondrial electron transport for ATP synthesis
Metabolism and detoxification	Cytochromes are also involved in the metabolism of biological molecules, drugs and pollutants
Antioxidant activity	Catalase and peroxidases metabolise hydrogen peroxide to reduce the risk of oxidative cellular damage
Beneficial pro-oxidant activity	Myeloperoxidase synthesizes reactive oxygen species with neutrophils to aid bacterial cell killing
DNA synthesis	Ribonucleotide reductase converts ribonucleotides to deoxyribonucleotides.
Neurological function	Cofactor in the synthesis of neurotransmitters and myelin

The biochemical reactivity of iron – namely its ability to both donate and accept electrons – means that it can be harmful when present in high concentrations. Unbound iron can catalyse the formation of reactive oxygen species that cause intra- and extra-cellular damage. Thus, when not bound to functional proteins (functional iron), free iron is sequestered by the iron transport protein transferrin (transport iron) or stored by ferritin or as haemosiderin (storage iron). Iron levels must be tightly regulated to prevent damage caused by either too much or too little iron.

Distribution of iron

Body iron content is approximately 4-5 g in well-nourished populations, which corresponds to a concentration of about 50 mg iron/kg body weight in men and about 40 mg iron/kg BWT in women. Approximately 60% is present in the form of haemoglobin in red blood cells

Most of the rest is contained in ferritin complexes that are found in all cells, but most commonly in the bone marrow, liver, and spleen. The ferritin molecule is composed of an apoferritin protein shell, composed of 24 polypeptide subunits (of 2 types, named L & H) with an overall molecular weight of approximately 500 kDa, enclosing a crystalline ferrihydrite core of up to 4,500 ferric iron atoms. The liver's stores of ferritin are the primary physiologic source of reserve iron in the body. Haemosiderin is an aggregated, iron-rich ferritin degradation product with very low apoferritin content, found in some phagocytic cells (such as macrophages). Its iron content is not readily available.

TYPE OF IRON	CONCENTRATION (MG IRON/KG BODY WEIGHT)	
	Men (% of total)	Women (% of total)
Functional Iron		
Haemoglobin	31 [62]	28 [70]
Myoglobin	5 [10]	4 [10]
Haem enzymes	1 [2]	1 [2.5]
Non-haem enzymes	1 [2]	1 [2.5]
Transport iron		
Transferrin	<1 (0.2 [0.4])	<1 (0.2 [0.5])
Storage iron		
Ferritin	8 [16]	4 [10]
Haemosiderin	4 [8]	2 [5]
Total	~50	~40

Systemic and cellular regulation of iron

Human iron homeostasis is regulated at two different levels, the **systemic** level and the **cellular** level, by two very distinct control systems.

Systemic iron levels are controlled by the hepcidin/ferroportin system- the regulated absorption of dietary iron by enterocytes, the cells that line the interior of the intestines, is balanced against the uncontrolled loss of iron from epithelial sloughing, sweat, injuries and blood loss. In addition, systemic iron is continuously recycled.

Control of **cellular iron levels** is accomplished using mechanisms involving the expression of particular iron regulatory and transport proteins, described as the IRE/IRP system. It seems very likely that there is higher level coordination between the systemic and cellular systems, and future work will define the detail.

Plasma iron

The key to systemic iron supply and homeostasis lies in the regulation of adequate plasma iron levels. Iron circulates in plasma bound to the 80-kDa glycoprotein **transferrin**. The liver is the main site of transferrin synthesis but other tissues and organs, including the brain, also produce transferrin. The main role of transferrin is to deliver iron from macrophages and duodenal absorption to all tissues. Transferrin has two high-affinity binding sites for Fe³⁺.

Transferrin binding (1) maintains iron in a soluble form, (2) serves as a major vehicle for iron delivery into cells (via the transferrin receptor, TfR1), and (3) limits the generation of toxic radicals.

Plasma transferrin is normally about 30% saturated with iron. A transferrin saturation <16% indicates iron deficiency, whereas >45% saturation is a sign of iron overload. When the saturation exceeds 60%, *non-transferrin-bound iron* begins to accumulate in the circulation and to damage parenchymal cells.

The homeostatic system thus has to maintain transferrin saturation at physiological levels, responding to signals from pathways that consume iron (such as erythropoiesis) and sending signals to the cells that supply iron to the blood stream.

Iron is released into the circulation from:

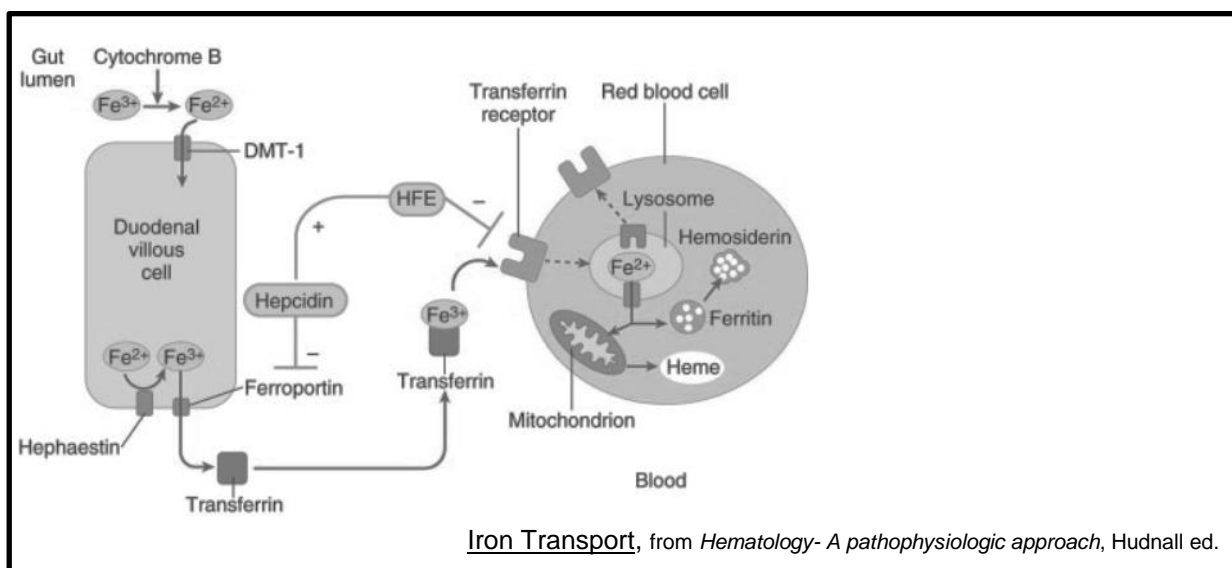
(1) *macrophages of the reticuloendothelial system*, which internally recycle 20–25 mg of iron from senescent erythrocytes per day, and

(2) *duodenal enterocytes*, which absorb 1–2 mg of dietary iron per day.

Hepatocytes play a dual role in systemic iron metabolism: they are the major site of iron storage and they secrete the regulatory hormone **hepcidin**. Hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter **ferroportin** on the surface of iron-releasing cells, triggering its degradation and hence reducing iron transfer to transferrin. Inherited and acquired disorders that alter hepcidin production therefore cause iron deficiency (high hepcidin levels) or iron overload (hepcidin deficiency).

Assessing the concentration of serum ferritin is a clinically useful measure of iron storage. Iron is stored inside cells as ferritin. Small amounts of ferritin are secreted into the plasma, proportional to the

amount within the cells. Low serum ferritin levels indicate depleted stores, whereas increased levels may indicate iron overload. Inflammatory conditions (or infections, cancer, and liver disorders) can also increase serum ferritin. The physiological function(s) of serum ferritin and its source (that is, whether it is derived from damaged cells or actively secreted by a regulated mechanism) still remain to be defined. Serum ferritin is predominantly composed of L chain subunits, partially glycosylated, and iron-poor.



Iron Absorption

Most of the iron in the diet is in the ferric (Fe^{3+}) form, whereas it is the ferrous (Fe^{2+}) form that is absorbed. Gastric secretions dissolve the iron and permit it to form soluble complexes with ascorbic acid and other substances that aid its reduction to the Fe^{2+} form. The importance of this function in humans is indicated by the fact that iron deficiency anaemia often complicates partial gastrectomy. Inorganic dietary iron is absorbed at the brush border of duodenal enterocytes via the **divalent metal transporter 1 (DMT1)**. A membrane-associated ferrireductase, **duodenal cytochrome B**, must first reduce any iron in its oxidized state before this absorption can occur. Haem iron is absorbed by a **haem importer protein** of uncertain identity, and is released intracellularly from its porphyrin by haemoxygenase, mainly by the inducible haemoxygenase 1 (HOX1). Within the enterocyte, some of the Fe^{2+} is converted to Fe^{3+} and bound to ferritin. The rest of the cytosolic iron binds to the basolateral Fe^{2+} transporter, **ferroportin**, and is exported to the interstitial fluid. Enterocytic iron export through ferroportin requires **hephaestin**, a membrane-bound multicopper oxidase homologous to serum caeruloplasmin, which oxidases Fe^{2+} to Fe^{3+} for loading onto transferrin. In the presence of hepcidin, ferroportin is internalized and degraded. Thus, iron exportation is blocked. Inversely, in the absence of hepcidin, ferroportin is maintained on the cell membrane, and iron transportation is facilitated.

Because iron cannot be excreted from the organism in a regulated way, iron absorption represents the critically controlled process. Normally, only 1–2 mg of iron per day are absorbed to compensate for iron losses, for example by sloughing of intestinal epithelial cells, desquamation of skin and urinary cells, blood loss, or sweat. Iron absorption can be enhanced when the needs are higher (for example, because of increased erythropoiesis or pregnancy) and suppressed in iron overload. The lack of an active mechanism for iron excretion explains the development of iron overload when the regulation of iron absorption is defective or bypassed (e.g. blood transfusions & IV iron therapy).

Iron Recycling

Less than 10% of the daily iron needs are met by intestinal absorption, and the rest is covered by macrophages that recycle iron internally to maintain plasma iron levels. The bulk of recycled iron (about 25 mg/day) is used by erythroblasts for haemoglobin synthesis. The amount of plasma iron is just over 10% of the amount used daily, which means that plasma iron is turned over many times each day.

Macrophages phagocytose aged or damaged erythrocytes and catabolize haem using haemoxygenase. NRAMP1 (natural resistance-associated macrophages protein 1), a divalent metal transporter homologous to DMT1, is expressed within phagolysosomal membranes and participates in iron export from phagocytic vesicles. Export of ferrous iron from macrophages occurs via ferroportin.

Because it has such a central role in systemic iron homeostasis, ferroportin expression in macrophages is closely regulated:

- 1) ferroportin transcription is induced by erythrophagocytosis and haem iron,
- 2) translation of ferritin is regulated by the IRE/IRP system
- 3) protein stability of ferritin is regulated by hepcidin

Ferroportin-mediated iron export is coupled to the function of the multicopper oxidase caeruloplasmin, a protein synthesized and secreted by the liver. Caeruloplasmin-deficient humans show hepatocyte and macrophage iron accumulation.

Systemic Iron Homeostasis: More about the Iron Hormone Hepcidin

Control of iron balance in the whole body requires communication between sites of uptake, utilization and storage. Hepcidin (encoded by the HAMP gene) is the central regulatory molecule of systemic iron homeostasis. It is a defensin family member with strong links to innate immunity. The bioactive, mature 25 amino acid peptide is generated from an 84 amino acid prepropeptide by furin cleavage in the liver. Hepcidin is secreted from hepatocytes and circulates in plasma bound to α 2-macroglobulin. Hepcidin clearance occurs via the kidney or by codegradation with ferroportin.

Hepcidin binds to ferroportin, triggers its internalization, ubiquitination, and subsequent lysosomal degradation. In the duodenal enterocyte, hepcidin-dependent regulation of ferroportin reduces dietary iron absorption, i.e. it is a negative regulator of iron absorption. In the macrophage (and possibly the hepatocyte), hepcidin activity attenuates cellular iron release by reducing ferroportin. Hepcidin expression in hepatocytes is regulated by multiple, in part opposing signals, which include:

- 1) systemic iron availability (i.e. levels of diferric transferrin, Tf-Fe₂): \uparrow Tf-Fe₂ \rightarrow \uparrow hepcidin
- 2) hepatic iron stores: if high \rightarrow \uparrow hepcidin
- 3) erythropoietic activity: anaemia \rightarrow \downarrow hepcidin
- 4) hypoxia \rightarrow \downarrow hepcidin
- 5) inflammatory/infectious states: IL1 & IL6 \rightarrow \uparrow hepcidin
- 6) non-genetic iron overload \rightarrow \uparrow hepcidin

These different regulatory inputs are integrated transcriptionally.

Cellular Iron Homeostasis: The IRE/IRP System

Coordination of iron uptake, utilization, and storage to assure the availability of appropriate supplies and to prevent toxicity is as crucial on the cellular level as it is on the systemic level. In contrast to systemic iron metabolism, though, cellular iron traffic also involves regulated iron **excretion**.

Cellular Iron Uptake

Cells take up iron-loaded (diferric) transferrin, **Tf-Fe₂**, via the high-affinity transferrin receptor, **TfR1**, as their major source of iron. The Tf-Fe₂/TfR1 complex is internalized by clathrin-dependent endocytosis. Acidification in the endosomes, likely through the pump action of a Na⁺-H⁺-ATPase, triggers release of iron by conformational changes in both transferrin and its receptor. Freed iron is reduced from Fe³⁺ to Fe²⁺ by metalloreductases for transport into the cytosol via the endosomal protein DMT1 (also found on the apical membrane of enterocytes where it mediates systemic iron absorption). The transferrin cycle is completed when the endosome returns to and fuses with the plasma membrane, returning apotransferrin to the circulation and TfR1 to the plasma membrane and allowing both molecules to start the cycle again.

There also seem to be transferrin-independent routes of iron uptake, and receptor-mediated endocytosis of other forms of protein-bound iron represents an additional means for specific cell types to take up iron. Serum ferritin can enter cells via at least 2 different ferritin receptors. Finally, specialized cells, e.g. enterocytes, are able to acquire iron in the form of haem. Cells also acquire haem indirectly. Macrophages obtain haem by phagocytosis and processing of senescent red blood cells. In plasma, haemoglobin and free haem arising from intravascular haemolysis are cleared by specific scavenger systems: haemoglobin forms a complex with haptoglobin that is delivered to reticuloendothelial cells via CD163-mediated endocytosis. Free plasma haem binds to hemopexin and the complex is endocytosed via the CD91 receptor present on the surface of macrophages, hepatocytes, and other cell types.

Cellular Iron Export

Iron export occurs from many cells but it is particularly important in cells that maintain plasma iron levels, i.e. macrophages and duodenal enterocytes. As mentioned before, ferroportin transports Fe₂⁺ and acts in concert with either of the ferroxidases hephaestin (enterocytes) or caeruloplasmin (other cell types) that facilitate iron extraction from the ferroportin channel and subsequent loading onto plasma transferrin. Caeruloplasmin and hephaestin are both copper dependent & this explains the importance of the copper status for iron metabolism.

Erythroblasts: In order to avoid dangerous iron and/or haem excess (excess haem triggers apoptosis in proerythroblasts), erythroblasts store excess iron as ferritin or export it via **ferroportin**.

Erythroblasts express a *ferroportin* messenger RNA (mRNA) isoform (1b) that lacks the 5' IRE and thus evades potential translational repression by IRPs. This isoform is susceptible to hepcidin degradation and may provide erythroid precursors with a mechanism to respond to systemic iron availability. Additionally, erythroblasts have the capacity to export excess haem (for example, when globin synthesis is limiting).

Cellular Iron Storage

Iron from the cytoplasmic "labile iron pool" (LIP) that is not used by the cell or exported is stored within the nanocavity of ferritin heteropolymers made of 24 subunits of heavy and light chains. Ferritin provides cells with a means to lock up excess iron in a redox inactive form to prevent iron-mediated cell and tissue damage. It also serves as an iron store which can be mobilized by both proteasomal and lysosomal ferritin degradation. A few cell types (heart, testis, pancreas, kidneys) have homopolymeric (FtMt subunit) H-type ferritin present in their mitochondria which protects these organelles against iron-mediated toxicity. Unlike cytosolic ferritin, mitochondrial ferritin subunit expression is not (directly) controlled by the IRPs (see below).

Regulation of Cellular Iron Metabolism

While key aspects of systemic iron metabolism are regulated transcriptionally (hepcidin expression) and posttranslationally (ferroportin function by hepcidin), cellular iron homeostasis is regulated posttranscriptionally by iron regulatory protein 1 (IRP1) and IRP2. These two RNA-binding proteins interact with hairpin structures known as iron-regulating elements (IREs), which are present in the untranslated regions of target mRNAs for proteins involved in iron storage, utilization and export. IRP-binding to IREs responds to cellular iron levels.

Intracellular Iron Trafficking and Utilisation

How iron moves within cells remains poorly understood. In the cytoplasm, Fe²⁺ iron is directly bound to proteins such as ribonucleotide reductase, but most iron is transferred to mitochondria, where it is incorporated into **bioactive haem** and **Fe/S cluster prosthetic groups**. Iron is imported into mitochondria by the inner membrane protein **mitoferrin 1**; this process is facilitated by the ABCB10 protein, which is thought to stabilize mitoferrin 1. Potentially, iron may also be directly transported from endosomes into mitochondria by a "kiss-and-run mechanism" through a direct contact between both organelles, effectively bypassing the cytosol.

In the mitochondria, iron is delivered to the Fe/S cluster biosynthetic machinery, or inserted into protoporphyrin IX by ferrochelatase to form haem. To coordinate the synthesis of the haem precursor protoporphyrin IX with iron availability, δ -aminolaevulinic acid synthase 2, the erythroid-specific first enzyme of protoporphyrin IX synthesis, is post-transcriptionally regulated by iron via the iron-responsive element/iron-regulatory protein (IRE/IRP) system. Haem is exported from the mitochondria via a yet undefined mechanism for incorporation into proteins throughout the cell.

By making haem and Fe/S clusters, mitochondria represent the major subcellular site of iron utilisation and as such play a central role in the control of cellular iron metabolism. A current theory of how mitochondria influence cellular iron metabolism states that cells sense mitochondrial iron insufficiency via an Fe/S cluster-dependent factor and respond by increasing mitochondrial iron levels; a haem intermediate could also be involved. Diversion of iron to mitochondria depletes the cytosol, thereby stimulating IRP binding to IREs. This in turn increases cellular iron uptake (TfR1, DMT1) and diminishes iron storage (ferritin) and export (ferroportin), so that more iron becomes available. In erythroid cells, IRP activation furthermore inhibits haem synthesis to avoid the accumulation of toxic metabolic intermediates until mitochondrial iron sufficiency is restored.

IRON STUDIES

Measured indices include:

- **Serum iron**- considerable daily variation; not very useful
- **Serum transferrin (or total iron binding capacity, TIBC)** - TIBC is a direct measure of the levels of transferrin; transferrin levels are reduced in inflammatory processes.

- **Transferrin saturation** - Level of transferrin saturation is particularly helpful in assessment of early stages of iron overload, with levels > 55% for males and > 50% for females indicative of iron overload (should be fasting level for more accurate assessment)
- **Serum ferritin** - Small amount of circulating serum ferritin reflects body iron stores; normal range 15 – 300 mcg/L (reference ranges vary depending on the method used). Levels < 15 mcg/L reflect absent / reduced iron stores. Elevated levels may reflect iron overload but will be increased in liver disease, inflammation or malignant disease. In the presence of inflammation, a level of > 100 mcg/l generally excludes iron deficiency
- **Soluble transferrin receptor** - Transferrin receptors are present on cell surfaces and are responsible for the internalization of transferrin resulting in intracellular release of iron. In the absence of adequate iron stores, expression of transferrin receptors increases. The amount of soluble transferrin receptor closely reflects iron stores and is not affected by inflammatory processes. Increased levels of soluble transferrin receptor are also found in conditions of increased red cell turnover (e.g. haemolysis)

Expected findings: “most helpful test” given in bold

	IRON-DEFICIENCY ANAEMIA *	ANAEMIA OF CHRONIC DISEASE	IRON-DEFICIENCY & INFLAMMATION	ACUTE-PHASE RESPONSE	IRON OVERLOAD
Serum iron	decreased	decreased	decreased	decreased	increased
Serum transferrin, TIBC	increased	decreased	decreased (low normal)	decreased	decreased or normal
Transferrin saturation	decreased	decreased	normal or decreased	decreased	increased
Serum ferritin	decreased	normal (>100 mcg/L)	“normal”	increased	increased
Soluble transferrin receptor	increased	normal	increased	normal	decreased

***Iron-deficiency anaemia:** Unless there is major bleeding, overt iron-deficiency anaemia develops progressively. The first sign of iron supply-demand mismatch is a reduction in serum ferritin, indicating that iron stores are being drawn on as iron is mobilised from the liver and reticuloendothelial system. However, serum iron, total iron-binding capacity (TIBC), and red cell morphology remain normal until iron stores are exhausted. Afterwards, serum iron levels decrease, while TIBC increases in an attempt to raise iron absorption. Dysfunctional erythropoiesis and microcytosis only occur once the transferrin saturation drops below 15%. Only then do anaemia and low haemoglobin levels develop.

PHARMACOLOGY OF IRON THERAPY

Oral iron supplements:

Indications: treatment of iron-deficiency anaemia; prevention of iron-deficiency anaemia in pregnancy; supplementation in regular blood donors to prevent subclinical iron deficiency.

Of course, the primary cause of the iron deficiency must be actively sought and corrected. The goal of iron supplementation is two-fold: to reverse anaemia and to replenish iron stores. The expected response to a course of oral iron is a reticulocytosis in 3-5 days, peaking after one week, followed within three weeks by a rise in haemoglobin. An increase in haemoglobin by 1g/dL after one month qualifies as an adequate response. The British Society of Gastroenterology suggests that oral iron therapy should be continued for three months after normalization of the haemoglobin levels to ensure that the iron stores are replenished.

Preparations:

The ferrous salts (including ferrous sulphate, ferrous gluconate, and ferrous fumarate) are the most commonly prescribed preparations. Ferrous (Fe²⁺) forms are more soluble than the dietary ferric (Fe³⁺) form, with twice the absorbability. The estimated absorption rate of the ferrous salts is 10-15%, with no difference found in absorbability between the different formulations. Note, though, that the different ferrous salts contain differing quantities of elemental iron per tablet. The equivalent of 60 mg of elemental iron is 300 mg ferrous sulphate heptahydrate, 180 mg ferrous fumarate or 500 mg of

ferrous gluconate. Oral solutions of ferrous iron salts are available for use in children, but can cause black staining of teeth.

For the treatment of iron-deficiency anaemia in adults, the recommended daily dose of elemental iron is in the range of 150 to 200 mg/day; for children the dose is 3-6 mg iron/kg body weight/day. Because absorption is so poor, thrice-daily dosing has traditionally been recommended to reverse iron-deficiency. For example, a single ferrous sulphate 300mg tablet contains 60mg of elemental iron, so thrice-daily dosing provides 180mg of elemental iron per day, well within the recommended daily range of 150-200mg for iron-deficient patients. If the absorption rate is 10%, then after a month of therapy about 500mg of bioavailable iron should have accumulated, which can produce 500mL of packed red blood cells and a haemoglobin increase of 2g/dL.

An understanding of how hepcidin regulates systemic iron absorption caused some researchers to rethink oral iron dosing. In 2015, Moretti et al. published their findings: A large oral dose of iron taken in the morning causes an increase in the plasma iron level. This stimulates an increase in hepcidin, which then interferes with the absorption of an iron dose taken later in the day. The suppression of iron absorption could last as long as 48 hours. They concluded that providing lower dosages and avoiding twice-daily dosing actually maximizes fractional iron absorption, and their results support supplementation with 40-80 mg of iron taken every other day.

Side effects of oral iron therapy, namely constipation, diarrhoea, black stools, heartburn, nausea, and epigastric pain, affect 35-59% of patients and limit compliance. The upper GI side effects, such as nausea and epigastric pain, are more dose-dependent and can be managed with lower or less frequent dosing initially, while lower GI effects such as altered bowel habits, are less related to dosing. There is no difference in GI side-effects between equivalent dosages of the different ferrous salt preparations. Taking iron with meals to minimize GI upset reduces absorption by almost 50% (phytates in cereal, tannins in tea, and foods rich in calcium hinder absorption).

Enteric-coated formulations of ferrous salts have been developed to decrease the prevalence of GI upset and reduce the dosing schedule. The problem with these is that the iron may not be absorbed in the duodenum. The estimated bioavailability of the enteric-coated preparations is 30% of the regular oral preparations.

A polysaccharide-iron complex formulation has been designed to minimize GI upset via delayed iron release in the intestines. The combination of ferric iron and low molecular weight polysaccharide contains 150mg elemental iron. Intestinal absorption is significantly less than that of equivalent dosages of ferrous salts, though GI side-effects are much less severe.

Carbonyl iron is used as a substitute for ferrous sulphate. It has a slower release of iron as it relies on gastric acid for its solubilisation and is more expensive than ferrous sulphate. The slower release affords the agent greater safety if ingested by children. On a milligram-for-milligram basis, it is 70% as efficacious as ferrous sulphate. It is claimed to cause fewer GI effects.

Co-administration with vitamin C: Ascorbic acid theoretically improves absorption by reducing iron to a ferrous Fe²⁺ state to optimize its solubility. Increasing doses of vitamin C exhibit a dose-dependent response in iron absorption during concomitant administration in healthy volunteers. Co-administration of 500mg vitamin C results in a 48% increase in the absorption of 30mg of elemental iron.

Intravenous iron therapy:

Treatment with IV iron presents several advantages over oral iron such as a faster and higher increase in Hb levels and replenishment of body iron stores. There are extended **indications** for intravenous iron compared with oral iron:

MAIN CLINICAL INDICATIONS FOR INTRAVENOUS IRON TREATMENT	
1. Intolerance of oral iron or non-compliance with an oral iron regimen	
2. In acquired or hereditary decreased intestinal iron absorption	Intestinal malabsorption syndromes, inflammatory bowel disease, after gastrectomy/bariatric surgery, iron-resistant iron-deficiency anaemia
3. In cases with severe iron-deficiency anaemia because of continuous or uncontrolled blood loss and/or because of increased iron needs	Hereditary haemorrhagic telangiectasias, angiodysplasia due to other causes, pregnancy (after 14 weeks), postpartum anaemia, patients scheduled for surgery
4. In cases of functional iron deficiency* particularly when an erythropoiesis-stimulating agent is being used	Anaemia of chronic kidney disease, inflammatory diseases, anaemia of cancer
5. Other circumstances	Autologous blood donation for elective surgery, Jehovah's Witnesses

* Functional iron deficiency is characterized by the presence of adequate iron stores as defined by conventional criteria but an inability to sufficiently mobilize this iron, particularly when erythropoiesis is stimulated by an erythropoiesis-stimulating agent. These patients, who often have inflammatory disease, respond faster & more completely to high doses of IV iron for treatment of anaemia. But, what is the underlying mechanism? In these patients, a small proportion of the infused iron is delivered in the ferric form into the plasma and taken up by transferrin. Most of the administered iron dose is taken up by the macrophages. The iron overload of the macrophages in the RES may cause a 'bypass' of the hepcidin-induced block, allowing over-expression of ferroportin and allowing a flow to the bone marrow, transported by transferrin, to sustain erythropoiesis. In addition, in autoimmune diseases, macrophage iron loading may inhibit pro-inflammatory immune effector pathways, thus reducing disease activity (anti-inflammatory effect).

As free iron may lead to the production of hydroxyl radicals with potential toxicity to tissues, iron deficiency should be confirmed by ferritin levels before use of parenteral preparations.

Contraindications include a history of anaphylaxis or reactions to parenteral iron therapy, first trimester of pregnancy, active acute or chronic infection and chronic liver disease

Intravenous iron classification

Most IV iron formulations are colloids, comprising an iron-oxyhydroxide core (Fe³⁺) stabilized by a carbohydrate outer shell that maintains iron in a colloid form and controls its release. Formulations differ in iron core size and in the type and density of the surrounding carbohydrate shell. The stronger the iron complex, the slower the rate of iron release. IV iron complexes can generally be classified as labile or robust (kinetic variability) and as weak or strong (thermodynamic variability) with all possible intermediates. Each iron-carbohydrate complex enters the RES macrophages of the liver, spleen, and bone marrow where the shell is broken down and the iron is released from the complex. The released iron is either transported into storage pools or is transported via plasma transferrin for its incorporation into haemoglobin.

Older formulations

- 1) *Ferric hydroxide*: first iron compound for parenteral use; introduced early in the 20th century; lacked a carbohydrate shell → immediate iron release and severe toxic reactions; recommended only in extraordinary circumstances.
- 2) *Imferon*®: first high-molecular-weight iron dextran [HMW-ID] for intramuscular and IV use; introduced in 1954. HMW-ID consists of an iron oxyhydroxide core, which is surrounded by a carbohydrate shell made of polymers of dextran. This carbohydrate shell controls the release of free iron from the complex and also limits the total dose that can be given at any one administration. The bioavailability of iron occurs via uptake of iron dextran particles into the reticuloendothelial system (RES) with subsequent breakdown. High incidence of serious adverse events, especially the well-known dextran-induced anaphylactic reactions, led to its recommendation only when extreme clinical conditions were present and other options unavailable; removed from the market in 1991.

In 1989, recombinant human erythropoietin was introduced for clinical use, and it was recognized that absolute or functional iron deficiency was the commonest cause for erythropoietin failure in chronic kidney disease patients. IV iron therapy was shown to play an essential role in achieving and maintaining target Hb levels in CKD patients. This provided the impetus to develop better IV iron formulations.

- 3) *INFeD*®: low-molecular-weight iron dextran (LMW-ID); approved by FDA for clinical use in 1992; can be administered as an IV bolus or total dose infusion (TDI) with doses up to 1000 mg. Much lower risk of adverse events compared with the now-withdrawn HMW-ID compounds.
- 4) *Dexferrum*®: a HMW-ID, approved by FDA in 1996; administered as an IV bolus or total dose infusion (TDI) with doses up to 1000 mg; required a test dose and had a black box warning; no longer available in Europe.
- 5) *Ferrlecit*®: sodium ferric gluconate in sucrose (FG), after having been available in Europe for many years, was introduced into the American market in 1999 as a safer alternative to iron dextran with a lower risk of severe hypersensitivity reactions

- 6) *Iron sucrose* (IS) (Venofer®) was approved in the US in November 2000, and there is more published literature about this drug than any other IV iron preparation. IS can be safely administered as a 15-30 minute infusion in doses of 200-300 mg; the maximum weekly dose should not exceed 600 mg. If higher-than-recommended doses are not infused, AEs are rarely observed. The main disadvantage of IS is the need for multiple infusions as the maximum weekly dose should not exceed 600 mg (200 mg IV, 1-3 times/week).

The incidence of serious life-threatening anaphylaxis with IS is 0.002% vs 0.6-2.3% and 0.04% with HMW-ID and FG, respectively. Black box warnings do not appear in the directions for use of either FG or IS and a test dose is not required.

Newer formulations

Three new IV iron compounds have been released for clinical use in patients with iron-deficiency anaemia in the last 10 years. All three of these new compounds have better safety profiles than the more traditional IV preparations, particularly because these products may be given more rapidly and in larger doses than their predecessors.

- 1) *Ferric carboxymaltose* (FCM): dextran-free parenteral iron product; first new agent approved for rapid and high-dose replenishment of depleted iron stores; iron complex that consists of a ferric hydroxide core stabilized by a carbohydrate shell- the design of the macromolecular ferric hydroxide carbohydrate complex allows controlled delivery of iron to the cells of the RES and subsequent delivery to the iron-binding proteins, ferritin and transferrin, with minimal risk of releasing large amounts of ionic iron into the serum. Stable, with very low immunogenic potential. Large doses (15 mg/kg; maximum of 1000 mg/infusion) may be administered in a single and rapid (15-minute) infusion without the need for a test dose.
- 2) *Ferumoxytol* (FeraHeme®): Approved by the FDA in 2009 for iron replenishment in adult CKD patients with iron-deficiency anaemia. It consists of a superparamagnetic iron oxide that is coated with a carbohydrate shell, which helps to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the RES macrophages. It can be administered as a relatively large dose (max 510 mg) in a rapid (< 20 seconds) session without test dose requirement. The main adverse effects are serious hypersensitivity reactions (0.2%) and transient interference with the diagnostic ability of magnetic resonance imaging for up to 3 months.
- 3) *Iron isomaltoside 1000* (Monofer®): introduced into Europe in 2010; a non-branched, non-anaphylactic carbohydrate, structurally different from the branched polysaccharides used in iron dextran. Very low immunogenic potential and a very low content of free iron and can therefore be administered as a rapid high dose infusion of up to 2000 mg without a test dose, which offers the possibility of providing one-dose iron repletion. Monofer® does not have a colloid structure; rather, the linear oligosaccharide isomaltoside 1000 allows the formation of a matrix with interchanging iron and carbohydrate.

In Australia and New Zealand, *iron polymaltose* (Ferrum H® & Ferrosig®) is registered for IM and IV use. It has an iron content of 50 mg/mL, and can be administered as a total-dose infusion, with a maximum dose of 2500 mg in patients >35 kg.

A patient's total body iron deficit can be calculated using the *Ganzoni formula*:

**total body iron deficit, or cumulative iron dose (mg) =
body weight* (kg) x (target Hb – actual Hb in g/L) x 0.24** + iron stores (mg)*****

*Use ideal body weight in overweight patients. If underweight, use actual body weight

**The factor 0.24= 0.0034 x 0.07 x 1,000: For this calculation the iron content of haemoglobin = 0.34%, blood volume = 7% of the bodyweight, and 1,000 is the conversion from g to mg.

***Iron stores:

<35 kg body weight: iron depot = 15 mg/kg body weight

≥35 kg body weight: iron depot = 500 mg

For example a 70 kg female with Hb 8 g/dL has an iron deficit of:

$70 \times (150 - 80) \times 0.24 + 500 = 1676 \text{ mg}$ i.e. approx. 1700 mg

	High-molecular-weight iron dextran	Low-molecular-weight iron dextran	Iron gluconate	Iron sucrose	Ferric carboxymaltose	Ferumoxytol	Iron isomaltoside 1000
Brand name	Dexferrum ®	INFeD ®	Ferrlecit ®	Venofer ®	Ferinject ® Injectafer ®	FeraHeme ®	Monofer ®
Carbohydrate shell	Dextran (branched polysaccharide)	Dextran (branched polysaccharide)	Gluconate (monosaccharide)	Sucrose (disaccharide)	Carboxymaltose (branched polysaccharide)	Polyglucose sorbitol carboxymethylether	Isomaltoside (linear oligosaccharide)
Complex type	Type I Robust & strong	Type I Robust & strong	Type III Labile & weak	Type II Semi-robust & moderately strong	Type I Robust & strong	Type I Robust & strong	Type I Robust & strong
Molecular weight (kDa)	265	165	289-440	30-60	150	750	150
Initial V _D (L)	3.5	3.5	6	3.4	3.5	3.16	3.4
Plasma half-life (h)	60	20	1	6	16	15	20
Labile iron release	-	-	+++	+-	-	-	-
Direct iron donation to transferrin (% injected dose)	1-2	1-2	5-6	4-5	1-2	<1	<1
Iron content (mg/mL)	50	50	12.5	20	50	30	100
Maximum single dose (mg)	20mg/kg	20mg/kg	125	200-300	15mg/kg (max 1000mg in one infusion)	510	20mg/kg
Total-dose infusion possible	Yes	Yes	No	No	No	No	Yes
Premedication	TDI only	No	No	No	No	No	No
Test dose required	Yes	Yes	No	No	No	No	No
Black box warning	Yes	Yes	No	No	N/A	No	N/A
Preservative	None	None	Benzyl alcohol	None	None	None	None
Life-threatening ADEs (x 10 ⁶ doses)	113	3.3	0.9	0.6	?		?

Potential negative effects of IV iron, of concern but not all fully clarified:

- 1) *acute hypersensitivity reactions*: much rarer with decreasing use of HMW-ID, but can be life-threatening. The two likeliest mechanisms are immunological IgE-mediated responses, for example, to the dextran component of IV iron preparations containing this molecule, and complement activation-related pseudo-allergy (CARPA).
- 2) *vasoactive reactions*, which are due to the appearance of nontransferrin-bound or free (labile) iron in the circulation, include a drop in blood pressure, acute edema of extremities, and acute onset of diarrhoea when large IV iron doses are administered rapidly
- 3) If iron release exceeds binding capacity or if transferrin is oversaturated, toxic unbound iron results in ↑ *oxidative stress & free radical formation*—believed to lead to coronary artery inflammation, with atherosclerosis development and *long-term CV risk*. Iron may also cause LDL oxidation → coronary artery damage. MI occurring with the use of IV iron (possibly caused by ↑ serum ferritin levels) has been reported
- 4) *promotion of tumour growth* in patients with cancer
- 5) *endothelial dysfunction*
- 6) *renal tubular damage*- iron sucrose
- 7) theoretical risk of *infection*: elemental iron is an essential growth factor for bacteria, with many species expressing iron transport proteins that compete with transferrin; available evidence suggests no increased risk of sepsis, but some practitioners withhold IV iron if acute infection
- 8) *iron overload*: ↑ risk for liver disease (cirrhosis, cancer), MI or CCF, DM, osteoarthritis, osteoporosis, metabolic syndrome, hypothyroidism, hypogonadism and in some cases premature death. Iron overload can accelerate neurodegenerative diseases e.g. Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy and multiple sclerosis.

References: Moretti D et al. *Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women.* Blood 2015; 126(17):1981-1989

Further reading: Anderson G & McLaren G ed. *Iron Physiology & Pathophysiology in Humans.* Humana Press, 2012.