
2 Regulation of Iron Absorption and Distribution

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Summary

- Iron absorption and distribution are determined by the activities of iron transport and storage proteins.
- Absorption of iron by the epithelial cells of the proximal gastrointestinal tract is a highly regulated physiological process that maintains body iron homeostasis.
- Iron efflux from cells occurs through the transporter ferroportin.
- Hepcidin is a plasma peptide that regulates plasma iron levels by controlling the iron export function of ferroportin and thereby also regulates iron absorption and distribution.
- Other mechanisms exist within cells to modulate the activity of iron transport and storage systems in response to cellular iron levels.
- Disordered iron distribution in the body occurs in chronic inflammatory conditions.

Key Words: DMT1; iron overload; iron turnover; ferroportin; transferrin receptor 1; transferrin receptor 2; Dcytb; HCP1; hephaestin; ceruloplasmin; transferrin; ferritin; IRP1; IRP2; erythropoiesis; hepcidin

1. INTRODUCTION AND SCOPE OF REVIEW

Iron absorption and distribution are determined by the activities of iron transport and storage proteins. Early work identified transferrin (Tf), transferrin receptor (TFR1), ferritin and haemosiderin as the major transport and storage proteins, while the past decade has seen the discovery of an additional series of proteins (DMT1, ferroportin, hephaestin, Dcytb, HCP1 and STEAP3) that provide a framework for explaining the molecular basis for iron absorption and cell iron uptake and release (Dunn, Rahmanto, & Richardson, 2007; Latunde-Dada, Simpson, & Mckie, 2006).

The regulation of iron metabolism was initially understood at a cellular level by the discovery of the effects of iron regulatory proteins IRP1 and IRP2 and their action in controlling the levels of transferrin receptor and ferritin (Rouault, 2006). Our understanding of whole body iron metabolism has been revolutionised by the discovery of hepcidin, a plasma peptide that regulates plasma iron levels by controlling the iron export function of ferroportin (Ganz & Nemeth, 2006). These discoveries have been extensively reviewed by others (see references below), and this chapter brings together key features of these findings and suggests some areas where more work is needed.

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2. IRON UPTAKE AND RELEASE BY CELLS

Cellular iron movements in mammals are explained by membrane transport steps which are coupled to oxidation or reduction of iron ions. The latter is necessary as proteins that hold iron tightly (transferrin in plasma or ferritin inside cells) bind the more stable oxidised iron ion, Fe(III) (ferric or Fe^{3+}), while iron seems to move around within cells and across membranes as the more soluble but unstable, reduced Fe(II) (ferrous or Fe^{2+}) ion. Iron enters cells via DMT1 [for divalent metal transporter 1, also called Slc11a2, DCT1 or nramp2 (Andrews, Fleming, & Gunshin, 1999)] and a single electron reduction step is associated with this process [catalysed by STEAP3 in reticulocytes (Ohgami et al., 2005) and Dcytb (also called Cybrd1) (Mckie et al., 2001) in duodenal epithelial cells (see later)]. The influx of iron into cells is driven by a proton gradient as DMT1 is a coupled co-transporter of H^+ and Fe^{2+} (Courville, Chaloupka, & Cellier, 2006; Gunshin et al., 1997). Iron leaves cells via ferroportin (IREG1, MTP1), a process that is coupled to oxidation to Fe^{3+} catalysed by hephaestin (in the gut) or ceruloplasmin in plasma.

Iron outside cells is normally tightly bound to the plasma iron transport protein, transferrin. This protein is bound at the cell surface by transferrin receptor, then internalised via clathrin-coated pits to endosomes where acidification of the endosomal lumen leads to dissociation of iron from transferrin (VanEijk & deJong, 1992), allowing reduction by STEAP3 and then transport by DMT1. Iron can also enter cells via fluid-phase internalisation (pinocytosis; Richardson, Chua, & Baker, 1999) of transferrin or by uptake of non-transferrin-bound plasma iron. The latter is important in iron-overload conditions. The finding that DMT1 knockout mice can still take up iron into certain tissues (Gunshin et al., 2005) suggests other transporters for iron exist. There is some evidence that iron can be transported into cells via transporters for other metals, e.g. Zn or Ca transporters [(Liuzzi, Aydemir, Nam, Knutson, & Cousins, 2006; Oudit, Trivieri, Khaper, Liu, & Backx, 2006), although the latter is controversial (Ludwiczek et al., 2007; Savigni, Wege, Cliff, Meesters, & Morgan, 2003)], or by other transporters (Mwanjewe & Grover, 2004) and these may be important in iron-overload conditions. A second transferrin receptor has been found (TFR2; Kawabata et al., 1999); however, this protein seems to be mainly involved in the regulation of iron metabolism as its loss leads to iron overload rather than iron deficiency (Wallace, Summerville, Lusby, & Subramaniam, 2005) (see below). Phagocytic cells can take up iron via internalisation of particles, bacteria or cells, e.g. senescent red cells, and phagocytic cells have both DMT1 and an additional iron transporter, nramp1 (SLC11a1; Blackwell, Searle, Mohamed, & White, 2003; Nevo & Nelson, 2006). This latter transporter is thought to reduce the viability and growth of phagocytosed microorganisms by transporting metals across the phagosome membrane (Courville, Chaloupka, & Cellier, 2006; Nevo & Nelson, 2006). The exact direction of metal transport by nramp1 (into or out of the phagosome) is disputed (Nevo & Nelson, 2006); thus there is uncertainty about the precise function of nramp1. There are, however, many reports of association between genetic variants of nramp1 and susceptibility to infections and autoimmune disease (Blackwell, Searle, Mohamed, & White, 2003); thus this transporter is important in immune function.

Iron efflux from cells occurs through the transporter ferroportin (Abboud & Haile, 2000; Donovan et al., 2000; Mckie et al., 2000). This is thought to be a passive transport (i.e. down the concentration gradient) (McGregor, 2006); however, the strong binding of iron by extracellular transferrin means that iron will tend to leave cells if ferroportin is active. Ferroportin expression is confined to cells with a specific iron efflux function, i.e. placenta, duodenum, macrophages, hepatocytes and brain endothelial cells (Rouault & Cooperman, 2006). Loss of ferroportin leads to severe anaemia and death in unborn mice or zebrafish (Donovan et al., 2005), emphasising the

importance of this protein in placental iron transfer. Tissue-specific knockout of ferroportin confirmed that this protein is essential for intestinal iron absorption (Donovan et al., 2005). Efflux is linked to oxidation as ferroportin seems to transport Fe^{2+} ions (McGregor, 2006) while extracellular iron is Fe^{3+} bound to transferrin. Hephaestin and ceruloplasmin are both important for this oxidation as shown by the effects of mutation or loss of these genes in mice (Harris, Durley, Man, & Gitlin, 1999; Wallace, Summerville, Lusby, & Subramaniam, 2005) and aceruloplasminemia in humans (Harris et al., 1995; Yoshida et al., 1995). Loss of hephaestin leads to anaemia due to reduced export of iron from the gut (Vulpe et al., 1999) while loss of ceruloplasmin leads to build up of iron in macrophages due to inefficient efflux of iron from these cells (Harris, Durley, Man, & Gitlin, 1999). Thus there is some tissue specificity in the utilisation of these two proteins for the oxidation step.

Excess iron within cells is stored as Fe^{3+} ions within the storage protein ferritin. Cellular iron uptake from, and release as, ferritin has been reported (Gelvan, Fibach, MeyronHoltz, & Konijn, 1996; Sibille, Kondo, & Aisen, 1988); however, this is not likely to be a major pathway for cellular iron transport in the normal situation (see Konijn, Gelvan, MeyronHoltz, & Fibach, 1997; Ponka & Richardson, 1997).

3. MECHANISMS OF IRON ABSORPTION AND LOSS BY MAMMALS

Absorption of iron by the epithelial cells of the proximal gastrointestinal tract is a highly regulated physiological process that maintains body iron homeostasis (Miret, Simpson, & Mckie, 2003). The intestine therefore attunes enterocyte apical iron influx, storage, transcellular transit and/or basolateral efflux in response to systemic requirements. Absorption of iron, therefore, correlates with the body's iron status or requirements under normal physiological conditions (Cox & Peters, 1980; Finch, 1994). Thus, while iron absorption is elevated during depletion (Finch, 1994), hypoxia (Simpson, 1996), development (Anderson, Walsh, Powell, & Halliday, 1991) and pregnancy (Gambling et al., 2001; Millard, Frazer, Wilkins, & Anderson, 2004), it is depressed in secondary iron-overload conditions (Gavin, McCarthy, & Garry, 1994). However, aberrations such as anaemia of inflammation and mutations of iron metabolism genes exert diverse consequences on the magnitude of iron absorption (Fleming, et al., 1997, 1998; Pietrangelo, 2006; Vulpe et al., 1999). The molecular mechanisms underlying these processes and the expression and regulation of the genes and proteins involved have been deciphered to a considerable extent in the past decade.

Dietary iron is broadly classified into haem and non-haem, and each has a separate and distinct mode of uptake by the enterocytes (Carpenter & Mahoney, 1992). Haem is organically bound to the porphyrin moiety of haemoglobin and myoglobin in meat and is believed to be absorbed intact as a metalloporphyrin complex (Wyllie & Kaufman, 1982) after proteolytic cleavage of globin. Haem is highly bioavailable, it is less influenced by dietary constituents, and meals with high haem content (i.e. high meat content) confer an enhancing influence on non-haem iron sources in a composite meal (Carpenter & Mahoney, 1992). Advances in recent years have revealed the molecular mechanism of non-haem iron absorption while that for haem is still emerging.

Earlier evidence showed the presence of a haem receptor in the intestine of pig (Grasbeck, Majuri, Kouvonen, & Tenhunen, 1982) and humans (Worthington, Cohn, Miller, Luo, & Berg, 2001). Furthermore, electron microscopy revealed apical uptake of haem and its subsequent endocytosis in pits at the base of the microvilli, before transit into lysosomes (Parmley, Barton, Conrad, Austin, & Holland, 1981). Temperature-dependent and saturable-specific uptake of

haem or haem analogues has been shown in cultured cells such as Caco-2, K562 and HepG2 (Uc, Stokes, & Britigan, 2004; Worthington, Cohn, Miller, Luo, & Berg, 2001). Absorbed haem is catabolised by haem oxygenase 1 (HO-1) to release inorganic iron that enters the cytosolic pool. Haem carrier protein 1 (HCP1) was cloned and characterised (Shayeghi et al., 2005) as a putative haem transport protein (Fig. 1), but recent evidence shows that it is more active as a transporter of folate (Qiu et al., 2006). Breast cancer resistance protein (BCRP/ABCG2) and Feline leukemic virus protein C (FLVCR) recently reported (Krishnamurthy et al., 2004; Quigley et al., 2004) as haem efflux proteins are also localised in the intestine. These haem transport proteins exhibit a broad substrate spectrum and their specific functions in the gut are not fully characterised; however, they function to export excess haem or other dietary porphyrins or structurally similar compounds (Fig. 1). Non-haem iron, on the other hand, consists of mostly ferric ion and its absorption is subject to a synergism of enhancers and inhibitory components of diets and gut secretions (Cox, Mazurier, Spik, Montreuil, & Peters, 1979; Fairweather-Tait, 1989; Miret, Simpson, & Mckie, 2003).

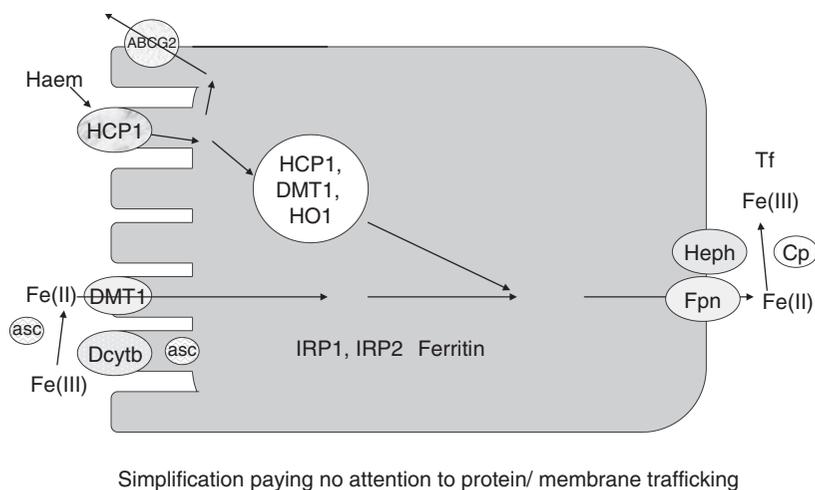


Fig. 1. Enterocyte iron transport mechanisms. HO1, haem oxygenase 1; HCP1, haem carrier protein 1; Cp, ceruloplasmin; Heph, hephaestin; Fpn, ferroportin; DMT1, divalent metal transporter 1; asc, ascorbate.

Luminal factors (particularly ascorbic acid) from the diet or secretions, along with DcytB or Cybrd1, reduce ferric ions to ferrous for uptake at the apical surface of the enterocytes (Fairweather-Tait, 1989; Mckie et al., 2001) (Fig. 1). Ferric reductase redundancy reported (Gunshin et al., 2005) in DcytB knockout mice might not represent the situation in scorbutic species such as guinea pigs and humans or in conditions of high iron demands. STEAP metal ion reductases have also been localised in the intestine (Ohgami et al., 2005). Ferrous ion is driven by proton-coupled electrogenic transport into the cytosol of enterocytes by DMT1 (Fig. 1). In addition to Fe^{2+} transport, DMT1 has been functionally implicated in the mucosal proton-dependent uptake of other divalent metals including Co^{2+} , Mn^{2+} , Cd^{2+} , Cu^{2+} , Ni^{2+} and Pb^{2+} (Gunshin et al., 1997). DMT1 has four mRNA splice variants, differing in the presence or absence of the 3' iron-response element (IRE), regulatory exon at the 5' end, tissue and functional specificities (Mackenzie, Takanaga, Hubert, Rolfs, & Hediger, 2007). Mutations in the DMT1 gene (G185R) cause microcytic anaemia (Fleming et al., 1997, 1998) respectively in mouse (mk)

and rat (Belgrade), and the latter is also reported to show liver iron loading (Thompson, Molina, Brain, & Wessling-Resnick, 2006). In man, mutations in DMT1 leading to loss of active protein from the plasma membrane cause microcytic anaemia with liver iron loading (Beaumont et al., 2006; Iolascon et al., 2005; Lam-Yuk-Tseung, Touret, Grinstein, & Gros, 2005). While the global knockout of DMT1 in mice is lethal due to its specific roles in the intestine and erythropoiesis, selective ablation revealed it is non-essential in the hepatocytes and placenta (Gunshin et al., 2005). Selective knockout of intestinal DMT1 confirms that this transporter is essential for intestinal iron absorption in adult mice (Gunshin et al., 2005).

Absorbed iron enters an uncharacterised pool in the cytosol and its fate is thence subject to systemic regulation. When the body is iron-replete, significant amounts of iron are retained and deposited as ferritin in the cytosol and this is sloughed off into the gut lumen during exfoliation of mature enterocytes. However, in situations of high systemic iron demands or depletion, absorbed iron is trafficked into the circulation through the coordinating actions of the efflux protein ferroportin (Ireg 1, MTP1) and the ferroxidase, hephaestin, a homologue of ceruloplasmin (Vulpe et al., 1999) (Fig. 1). The transit of exogenous iron into circulation (basolateral iron transfer) represents a regulatory set-point involving ferroportin–hepcidin interaction and this is expounded further below. Several mutations in the iron efflux protein ferroportin have been described (Pietrangelo, 2006) and the phenotype generally is tissue iron loading often coupled with hypoferremia (DeDomenico et al., 2005). Similarly, hephaestin-mutant mice (sla) exhibit duodenal iron loading with defective transfer of iron to the body, which results in anaemia (Vulpe et al., 1999). It has been reported that this defect in iron transfer can be overcome by feeding iron-deficient diets (Edwards, Ursillo, & Hoke, 1975), showing that an alternative oxidation mechanism exists in duodenum and this could be explained by ceruloplasmin which was found to contribute to intestinal iron transfer in circumstances of increased iron demand (Cherukuri et al., 2005). Plasma ceruloplasmin can be increased by iron deficiency (Mukhopadhyay, Mazumder, & Fox, 2000).

Intestinal absorption of both dietary forms (haem and non-haem) of iron occurs principally in the duodenum (Forth & Rummel, 1973), and absorption declines progressively down the gastrointestinal tract in parallel with the expression of key proteins involved in the transport machinery, especially Dcytb and Fpn (Gunshin et al., 1997; LatundeDada et al., 2002; Mckie et al., 2000). Moreover, the expression of these proteins is remarkably adaptive and responsive to local and systemic regulation. Early reports have shown biphasic kinetics of iron uptake into mucosal cells and transfer to the body (Forth & Rummel, 1973). An initial rapid phase of iron uptake and basolateral transfer precedes a slower serosal transfer period lasting about 6–24 h following ingestion of iron (Forth & Rummel, 1973). Recent molecular studies have reinforced this idea as the repression of the rapid phase is due in part to the local downregulation of the genes involved in the uptake process (Frazer et al., 2003), while the lag phase corresponds in duration with the time taken to respond to systemic signal. Efflux of iron from the intestine was suggested (Marx, 1979) as the rate-determining step in the absorption process and this has now been ascribed to the regulation of ferroportin in the basolateral enterocyte (Chen et al., 2003).

4. IRON TRANSPORT ROUND THE BODY AND TISSUE UPTAKE

Absorbed iron binds to transferrin (Fig. 2) and this, as well as non-transferrin-bound iron (NTBI), which is seen in pathological conditions of iron overload such as haemochromatosis, transfusional siderosis and in hypotransferrinemic (Hpx) mice, enters the circulation where excess is deposited in the liver. Diferric-Tf forms a complex with transferrin receptors, which can be

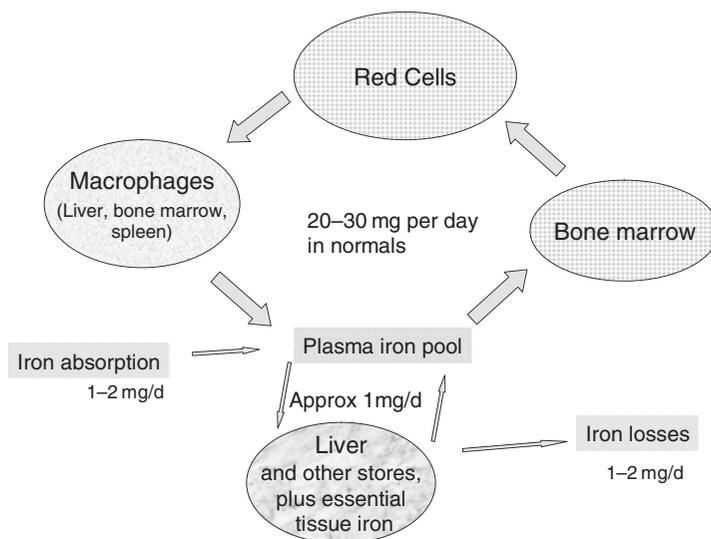


Fig. 2. Body iron compartments and fluxes.

endocytosed into hepatocytes where iron is released through DMT1 in acidified endosomes and the receptor is recycled back to the cell surface. This pathway is, however, insignificant as targeted mutation of the DMT1 gene did not obliterate iron uptake in the hepatocyte (Gunshin et al., 2005). Liver also takes up plasma (including iron) by fluid-phase pinocytosis and this can contribute to overall rates of liver iron uptake. NTBI is taken into the hepatocyte by unknown mechanisms although calcium channels, zinc transporters, e.g. ZIP 14, and transient receptor canonical protein TRPC have been proposed as candidates (Liuzzi, Aydemir, Nam, Knutson, & Cousins, 2006; Mwanjewe & Grover, 2004; Oudit et al., 2003). These channels contribute to massive iron loading of the liver in some pathological conditions (Chua, Olynyk, Leedman, & Trinder, 2004; van der et al., 2006) and also cardiomyopathy and endocrinopathy of iron overload. Excess haem in the circulation is similarly endocytosed in the liver by the CD91 haemopexin receptor on hepatocytes and macrophages (Hvidberg et al., 2005). This, as well as haemoglobin-CD163 (haptoglobin receptor on macrophages) endocytosis, represents an acute-phase response to offset elevated levels of free haem in the circulation under pathological conditions such as haemorrhage, haemolysis and rhabdomyolysis.

The liver is the central organ that controls iron storage and release by ferroportin into circulation through the regulation of hepcidin expression (discussed below). Iron release encompasses quantitatively large amounts of iron (about 20–25 mg in man) entering the circulation via reticuloendothelial erythrophagocytosis of senescent red blood cells (Fig. 2). Iron released from macrophages binds to transferrin and supplies the high demand for iron by developing erythroid progenitor cells via the TFR1/STEAP3/DMT1 mechanism. An adequate amount of new erythrocytes is therefore produced to replenish depletion by senescence and prevent anaemia. A minor uptake of Tf-bound iron probably occurs in the polarised epithelial cells of the kidney through megalin-dependent, cubilin-mediated endocytosis (Kozyraki et al., 2001). The dynamic and high turnover rate of Tf-bound iron emphasizes the importance of the regulation of tissue iron distribution and kinetics in maintaining homeostatic equilibrium (Gehrke et al., 2003; Uchida et al., 1983). Systemic iron circulation therefore precisely supplies metabolic and physiological requirements while averting excessive tissue deposition. Consequently, plasma iron

concentration is kept within a narrow range in spite of variable fluxes within body compartments (Fig. 2). The maintenance of plasma iron levels through efflux from macrophages of the RES and absorption from the intestine is regulated by ferroportin through its interaction with hepcidin (Nemeth, Tuttle, et al., 2004) (see below). A summary of the proteins involved in iron absorption and regulation is shown in Table 1.

Table 1
Summary of Putative Functions of Proteins Involved in Intestinal Iron Absorption and Regulation

<i>Proteins</i>	<i>Localisation</i>	<i>Function</i>
<i>Mucosa Iron Uptake</i>		
Duodenal cytochrome b (Dcytb)	Brush border apical membrane	Ferrireductase, supplies Fe ²⁺ to DMT1
Divalent metal transporter (DMT1)	Brush border apical membrane, particularly during enhanced uptake	Uptake of iron and other divalent metals into enterocytes
Iron-responsive protein (IRP1, IRP2)	Cytosol	Regulate mRNA expression of DMT1, TFR1 and ferritin
HCP1	Brush border apical membrane	Putative haem transporter/folate transporter
ABCG2	Brush border apical membrane	Possibly apical haem efflux protein
<i>Intracellular Processing</i>		
Ferritin	Cytosol	Enterocyte storage of excess iron
Haem oxygenase 1 (HO1)	Microsomes (lysosomes)	Haem degradation and iron release and recycling of Hb iron
<i>Basolateral Transfer</i>		
Ferroportin	Basolateral membrane	Iron export from enterocytes and cells
Hephaestin	Basolateral/supranuclear region	Basolateral membrane-bound ferroxidase
Ceruloplasmin	Serosal side	Cu-binding-plasma ferroxidase
<i>Regulation of Iron Absorption</i>		
HFE (Hfe)	Liver/crypt enterocyte	Senses iron status, interacts with TfR1 and TfR2
Transferrin receptor 1 (TfR1)	Liver/crypt enterocyte	Membrane receptor for Tf. May be involved with HFE in the regulation of hepcidin expression
Transferrin receptor 2 (TfR2)	Liver/crypt enterocyte (?)	Membrane receptor for Tf. May be involved with HFE in the regulation of hepcidin expression
Haemojuvelin	Liver, heart, muscle, haematopoietic cells	Membrane and soluble forms associate with bone morphogenic protein in regulating hepcidin expression
Hepcidin	Liver, heart?	Antimicrobial peptide that inhibit iron absorption from the enterocytes and efflux in macrophages and placenta

Disordered iron distribution in the body occurs in chronic inflammatory conditions as iron is sequestered in macrophages and the enterocyte to induce hypoferrremia (Nemeth, Rivera, et al., 2004). This situation is believed to be part of the host defence strategy against microbial infection. This acute-phase response elicits expression of inflammatory cytokines to induce hepcidin production (inhibition of iron release through hepcidin–ferroportin interaction is discussed below). In iron overload, specific tissues and even specific cells within tissues are affected. In the most common types of genetic haemochromatosis, some epithelial cells (pancreatic acinar cells and hepatocytes) become very highly iron-loaded while some muscles (especially smooth muscle including heart) show a lower loading, but this can be clinically significant. Other cells are relatively spared from iron loading for reasons that are not well understood. The differential distribution of transferrin receptors or DMT1 between cells is likely to be important as is the presence of ferroportin in some cells. The presence of iron uptake via non-DMT1 pathways may also be significant.

5. REGULATION AT THE CELLULAR LEVEL

Iron uptake and release by cells are controlled locally by post-transcriptional regulation of the levels of TFR1, DMT1, ferritin and ferroportin. The mechanism of this regulation is by iron-dependent activity of IRP1 and IRP2. IRP1 binds messenger RNAs (mRNAs) for these proteins only when cellular iron levels are low. When iron is abundant, IRP1 has a complete iron–sulphur centre bound to it and functions as cytoplasmic aconitase. IRP2 also binds mRNAs when iron levels are low; however, when iron is abundant, IRP2 is targeted for degradation by an iron-dependent mechanism (Rouault, 2006). Thus both IRPs bind mRNAs for key cellular iron proteins when iron levels are low. The effect of this binding is to block translation of mRNAs for proteins that remove iron from the cytosol, i.e. the storage protein ferritin, the efflux protein ferroportin, or in erythroid cells the rate-limiting enzyme for haem biosynthesis, delta amino laevulinic acid synthase (ALAS) which indirectly promotes iron removal from the cytosol for incorporation into haem. The effect of IRP binding to mRNAs is different for uptake proteins which deliver iron to the cytosol, i.e. TFR1 or DMT1. Whereas the ferritin and ferroportin mRNAs have IRP-binding sites (called iron-responsive elements or IREs) in their 5' end near the initiation codons for translation, the TFR1 and DMT1 IREs are in the 3' untranslated region of the mRNA, where the effect of IRP binding is to prevent degradation of the mRNA, thus elevating the levels available for translation and leading to increased levels of DMT1 and TFR1.

This mechanism allows cells some degree of direct control over their own iron levels and allows them to make ferritin to store away excess iron in a relatively non-toxic form. In erythroid cells the control of ALAS by iron levels helps to ensure coupling of iron supply to haem biosynthesis (Ponka, 2002).

IRPs can also be affected by reactive oxygen and nitrogen species (ROS, RNS) and their activity can be altered by kinase/phosphatase-mediated phosphorylation–dephosphorylation reactions (Wallander, Leibold, & Eisenstein, 2006); thus indirect responses to iron levels and an integration of cellular defence mechanisms are possible (see also below). Note that DMT1 exists in alternately spliced forms, only some of which have IREs. These forms have specific tissue distributions (Hubert & Hentze, 2002); thus iron-dependent control of DMT1 activity varies between tissues. The best understood tissue is the gut and this is described in detail below.

Loss of IRP1 by gene knockout leads to changes only in kidney and brown fat, and it seems that IRP2 is more generally important in regulating iron metabolism *in vivo* (Meyron-Holtz et al., 2004). Loss of IRP2 leads to more widespread alterations in iron metabolism, with a mild deficit

in erythron iron uptake and altered iron distribution between storage compartments in the liver and spleen (Galy et al., 2005) and a late-onset neurodegeneration (LaVaute et al., 2001) implying an important role for IRP2 in the brain. Loss of IRP2 combined with reduction in IRP1 results in anaemia with a more severe neurodegeneration (Smith et al., 2004). Overall total body iron appears to be approximately normal in IRP2 KO mice [R Simpson estimate from data in (Galy et al., 2005)] and radio-iron absorption was found to be normal, despite local increased iron storage in the absorptive enterocytes (Galy et al., 2005). Mutations in IREs within single mRNAs highlight the relatively subtle and tissue-specific roles of the IRE/IRP system; thus hereditary cataracts result from mutations in the L-ferritin IRE in humans, while in mice loss of the ferroportin IRE results in a complex series of changes in tissue iron accumulation, which are not yet fully understood (Mok, Mlodnicka, Hentze, Muckenthaler, & Schumacher, 2006).

There must be other mechanisms for control of protein levels and activity by iron; however, these are not as well understood as the IRP/IRE system. One system that has been well studied is the hypoxia gene response system which is controlled by Hifs (hypoxia-inducible factors) (Metzen & Ratcliffe, 2004). Hifs are transcription factors that activate transcription of genes with appropriate promoter-response elements. The levels of Hifs are controlled by a complex signalling system that responds to cellular levels of oxygen, iron and ascorbate (Jones, Trowbridge, & Harris, 2006; Metzen & Ratcliffe, 2004; Pan et al., 2007). On the other hand, genes with hypoxia-response elements in their promoters (including TFR1 and hepcidin) can potentially respond to oxygen levels; thus there is a regulatory mechanism operating at the cellular level that can integrate control of oxygen and iron-responsive genes. Calreticulin is reported to be an iron-binding protein (Conrad, Umbreit, & Moore, 1993) whose levels are altered by iron (Nunez, Osorio, Tapia, Vergara, & Mura, 2001). Calreticulin has also been reported to alter synthesis of the transcription factor C/EBPalpha (Timchenko, Iakova, Welm, Cai, & Timchenko, 2002) which controls transcription of a variety of genes including hepcidin (Courselaud et al., 2002). There is also evidence that the redox-responsive transcription factor, NfkappaB, can respond to cellular iron levels (Templeton & Liu, 2003); thus NfkappaB-responsive genes may also respond to iron levels. Thus iron levels can potentially control transcription of a wide range of genes, while several signalling pathways may also affect iron metabolism.

Specifically, iron-sensitive transcription factors remain elusive in mammals, and until they are identified, transcriptional control of genes by iron remains poorly understood and seems to be somewhat indirect, operating via transcription factors that have other duties and potentially integrate several diverse signalling pathways.

A third level at which control exists is the cellular localisation of proteins, such as DMT1, being controlled by iron. This has been shown to occur in intestinal enterocytes, with the amounts of DMT1 at the brush border membrane decreasing in response to oral iron dosing (K. Y. Yeh, M. Yeh, Watkins, RodriguezParis, & Glass, 2000). Different DMT1 isoforms also recycle between the cell surface and the intracellular sites with distinctive kinetics, thus allowing differential expression of DMT1 at the cell surface depending on tissue-specific alternate splicing of the mRNA (Thomson, Rogers, & Leedman, 1999).

6. REGULATION OF WHOLE BODY IRON STATUS

Total body iron levels are maintained primarily by controlling duodenal iron absorption from the diet. Iron is lost from the body via uncontrolled blood losses (mainly gastrointestinal in men, with menstrual losses also significant in women of child-bearing age), epithelial cell losses (mainly

gastrointestinal tract, skin and kidney epithelial cells) and via secretions (tears, sweat and gastrointestinal secretions). Figure 3 shows the relative importance of these losses. Body iron losses vary somewhat in proportion to body iron levels, e.g. blood iron losses will presumably be less in anaemia and epithelial cell iron losses may be greater in iron overload; however, there is no close linkage of body iron losses to the levels of iron stores or tissue iron requirements. Absorption of dietary iron is, on the other hand, closely linked to iron stores and iron requirement and is therefore the main way the body controls its iron content (Hallberg, Hulthen, & Gramatkovski, 1997). Iron losses are seen as obligatory, uncontrolled losses and these are balanced by the regulation of iron absorption by duodenum. The mechanisms by which this regulation is brought about are now being understood at the molecular level (see hepcidin section below). There is some evidence that re-absorption of intestinal iron losses in the colon can be important; however, this has been little studied (Takeuchi et al., 2005).

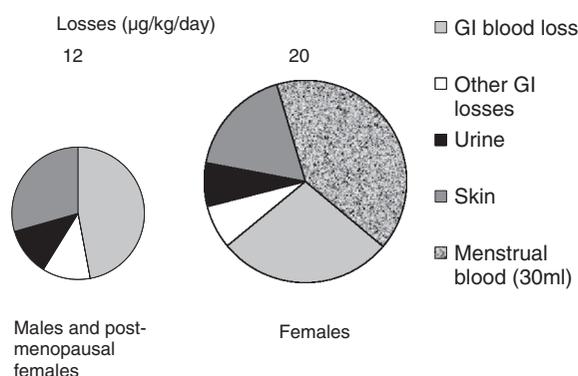


Fig. 3. Body iron losses. The charts indicate the relative size of iron losses from the body; the numbers show the total losses in µg/kg body weight/day.

Regulation of body iron levels therefore requires a sensing mechanism that can regulate iron absorption in response to decreased iron levels in critical compartments, thereby maintaining homeostasis. The key compartments appear to be the erythroid compartment, plasma transferrin and tissue iron stores. Production of hepcidin is thought to be regulated in response to decreased iron stores or plasma diferric transferrin (Steele, Frazer, & Anderson, 2005), thereby providing a homeostatic loop that regulates plasma iron and iron stores by its blocking effect on iron efflux from enterocytes, hepatocytes and macrophages. Deranged body iron homeostasis occurs in several diseases, all of which seem to affect hepcidin production, and this is discussed in detail below. Mutations in ferroportin can lead to loss of sensitivity to hepcidin (DeDomenico et al., 2005; Schimanski et al., 2005) or loss of ferroportin activity (McGregor et al., 2005; Sham et al., 2005), leading to inappropriate iron accumulation in iron stores. Mutations that directly affect hepcidin levels are described below.

Inappropriate body iron losses occur mainly through increased bleeding as losses through sweat and urine are relatively small (Beard & Tobin, 2000). These can result from gastrointestinal diseases (e.g. cancer, ulcers, Annibale et al., 2001), parasites (Crompton & Nesheim, 2002), post operatively (Wallis, Wells, Whitehead, & Brewster, 2005), NSAID treatment (Ferrara, Coppola, Coppola, & Capozzi, 2006) or from increased menstrual losses (Ferrara, Coppola, Coppola, & Capozzi, 2006; Hallberg, Hulthen, Bengtsson, Lapidus, & Lindstedt, 1995). These can rapidly

lead to iron deficiency when the body's normal homeostatic mechanism of increased duodenal iron absorption fails to compensate, either because of lack of sufficient dietary iron in a suitably available form (Ferrara, Coppola, Coppola, & Capozzi, 2006) or because of a failure to absorb sufficient iron due to gastrointestinal disease (e.g. gastritis & or IBD (Annibale et al., 2001; Gasche & Kulnigg, 2006)) or chronic inflammation (Ganz & Nemeth, 2006). In these conditions the underlying cause of iron loss has to be addressed. Iron deficiency can arise over a long period where there is a chronic condition that reduces iron absorption, e.g. a chronic inflammatory condition as noted above or poor dietary iron supply. Rapid growth rates or pregnancy combined with poor supply of dietary iron can lead to more rapid onset of iron deficiency (Milman, 2006).

7. HEPCIDIN IN THE REGULATION OF IRON ABSORPTION AND DISTRIBUTION

Hepcidin (hepatic bactericidal protein) is a liver-expressed antimicrobial peptide (LEAP1) found in the blood (Krause et al., 2000) and in urine (Park, Valore, Waring, & Ganz, 2001). Hepcidin gene is called HAMP (hepcidin antimicrobial peptide (OMIM)) and is expressed predominantly in the liver (Nicolas et al., 2002; Park, Valore, Waring, & Ganz, 2001; Pigeon et al., 2001). It is also expressed in the heart, pancreas, lungs and haematopoietic cells (Peyssonnaud et al., 2006). The bioactive 25 amino acid hepcidin peptide is rich in β -sheet which is stabilized by conserved disulfide bonds in a hairpin configuration (Kemna, Tjalsma, Podust, & Swinkels, 2007). Hepcidin's role in iron metabolism was serendipitously discovered in USF knockout mice that displayed a massive iron overload phenotype, characteristic of haemochromatosis (Nicolas et al., 2001). In contrast, however, transgenic mice over-expressing hepcidin developed a severe iron-deficient phenotype (Nicolas, Bennoun, et al., 2002). Furthermore, hepcidin expression is increased after the administration of large oral doses of iron (Frazer et al., 2003) when iron stores are elevated (Pigeon et al., 2001) or in inflammation (Nemeth, Rivera, et al., 2004; Nicolas, Chauvet, et al., 2002) and decreased under conditions of anaemia (Nicolas, Chauvet, et al., 2002), iron deficiency (Frazer et al., 2002) and hypotransferrinemia (Weinstein et al., 2002). Compelling direct evidence revealed that the injection of hepcidin into mice resulted in decreased absorption of iron (Laftah et al., 2004), while a hepcidin-secreting tumour was found to cause anaemia (Weinstein et al., 2002).

Substantive evidence has accrued to implicate hepcidin as a peptide that inhibits the release of iron into circulation from the intestine, hepatic and splenic macrophages and the placenta (Rivera et al., 2005). Hepcidin was found to interact with ferroportin at the apical surface of HEK 293 cells causing its internalization and degradation (Nemeth, Tuttle, et al., 2004) in endolysosomes, thus providing a mechanism whereby cellular iron efflux by ferroportin is inhibited by hepcidin. This direct interaction of hepcidin with ferroportin mediates *in vivo* iron absorption in the duodenum as well as iron efflux from hepatocytes and macrophages (DeDomenico et al., 2005; Njajou et al., 2001). Hepcidin is therefore a negative regulator of iron efflux in these tissues. In the case of enterocytes, decreased ferroportin could lead to iron build up in enterocytes, which in turn downregulates iron uptake from the intestinal lumen; however, a more direct effect of hepcidin on enterocyte iron uptake is also possible (Yamaji, Sharp, Ramesh, & Srai, 2004).

The mechanism by which hepcidin production is regulated in hepatocytes in response to the iron requirements of the whole organism is the subject of intense ongoing investigation. Delineation of the regulatory mechanisms of hepcidin expression has been achieved from studies of iron-loading phenotypes in humans and mice (see above). However, compelling evidence indicates that

the BMP/Smad, STAT3 and ERK/p38 MAP kinase signal transduction pathways are involved in the transcriptional regulation of hepcidin expression (Babitt et al., 2006; Calzolari et al., 2006; Verga Falzacappa et al., 2007; Wrighting & Andrews, 2006).

Advances in understanding the regulation of hepcidin induction seem to be progressing faster than knowledge of signals that repress its expression as seen in iron depletion, hypoxia or increased erythropoiesis (Gardenghi et al., 2007; Latunde-Dada, Vulpe, Anderson, Simpson, & Mckie, 2004; Nicolas, Chauvet, et al., 2002). Anaemia (decreased circulating haemoglobin) leads to increased erythropoiesis, which also seems to regulate hepcidin production to increase iron absorption. The mechanism of this link between the bone marrow (site of erythropoiesis) and the liver (site of hepcidin production) is unclear but may involve plasma diferric transferrin levels or some other factor (e.g. 5-amino laevulinic acid (Laftah, Raja, Simpson, & Peters, 2003; Laftah et al., 2003, 2004) or erythropoietic factors such as GDF15 (Tanno et al., 2007)). While the mechanism of the erythropoietic signal is still unknown, it overrides the iron-sensing regulation in certain iron-loading conditions such as thalassemia intermedia. It seems apparent therefore that a signal which modulates iron absorption originates in the bone marrow as a result of ineffective erythropoiesis and causes the suppression of hepcidin expression. This is perhaps the reason why anaemia that involves peripheral destruction of mature red blood cells, e.g. sickle cell, hereditary spherocytosis or autoimmune haemolytic anaemia, does not enhance iron absorption.

Oxygen delivery to liver also seems to regulate hepcidin production (Nicolas, Chauvet, et al., 2002), thereby providing a mechanism whereby hypoxia increases iron absorption (Laftah et al., 2005). Moreover, a recent study in cultured HepG2 cells showed that reactive oxygen species (ROS) repress hepcidin gene expression by inhibiting C/EBP α and STAT3 binding to hepcidin promoter during hypoxia (Choi, Cho, Kim, & Park, 2007), while a study of the Hif (hypoxia-inducible factor) pathway suggested this may also regulate hepcidin synthesis (Peyssonnaud et al., 2007). Other factors that can regulate iron absorption include gender-related factors (e.g. sex hormones, Hershko & Eilon, 1974), pregnancy and inflammation. These all affect hepcidin levels (Courselaud et al., 2004; Millard, Frazer, Wilkins, & Anderson, 2004; Nicolas, Chauvet, et al., 2002), which in turn controls iron absorption.

8. CONCLUDING OVERVIEW AND UNKNOWNNS

The model for iron regulation of iron absorption and distribution described above remains qualitative but seems to contain mechanisms that can explain most of the observed features of these processes. The main gaps are the identity of the 'erythroid factor' that can stimulate iron absorption even in the face of iron loading and possible gender-specific factors and minor genetic factors which could combine with known components to modulate body iron levels and distribution. Details of the signalling pathways that regulate hepcidin are still emerging. The reasons why only specific cells load up with iron in iron-overload diseases and the reasons why some cells seem to be more susceptible to damage by iron are poorly understood. Other challenges are quantitative – there is no widely available quantitative assay for hepcidin, and when one becomes available, measurements of this key regulator need to be combined with measurements of other parameters of iron metabolism (many of which have ceased to be widely measured, e.g. plasma iron turnover, plasma/serum diferric transferrin levels, iron absorption rates). The resulting data will then allow the plasma iron-hepcidin-iron absorption regulation model to be tested thoroughly, in particular to explain the high variability in reported hepcidin levels (even within

normal groups) and the poor correlation between plasma iron levels or transferrin saturation and iron absorption (e.g. Walters, Jacobs, Worwood, Trevett, & Thomson, 1975). Only then will the adequacy of our knowledge be apparent.

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