

- 40 Andreassen H, Rungby J, Dahlerup JF *et al.* (1997) Inflammatory bowel disease and osteoporosis. *Scand J Gastroenterol* 32, 1247–1255.
- 41 Vogel A, Strassburg CP, Manns MP (2002) Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. *Hepatology* 35, 126–131.
- 42 Fan L, Tu X, Zhu Y *et al.* (2005) Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. *J Gastroenterol Hepatol* 20, 249–255.

2.3.12 Normal iron metabolism

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Introduction

Iron is an essential nutrient, but one with considerable potential for toxicity. It is therefore understandable that the uptake and disposition of iron are controlled by elaborate physiological mechanisms. Although the highly regulated nature of iron metabolism has been recognized for decades, the mechanisms governing its regulation have only recently been elucidated. This has been made possible by the discovery of a variety of proteins involved in iron transport, as well as the iron-regulatory hormone, hepcidin. The aim of this chapter is to provide an overview of iron metabolism with an emphasis on these new discoveries, particularly as they relate to the liver.

Overview of iron metabolism

Before describing these discoveries, a brief review of iron metabolism is necessary. Iron metabolism is a highly conservative process characterized by recycling. The body of the average adult male contains approximately 5 g of iron, of which the single largest component is the haemoglobin contained in the erythrocytes. At the end of their relatively short lifespan, these cells are destroyed, their haemoglobin catabolized and the resulting iron made available for reuse in the synthesis of haemoglobin, myoglobin or any of a number of iron-requiring enzymes, including the cytochrome P450 system, ribonucleotide reductase and the prolyl hydroxylases. In an iron-replete individual, some iron is stored, primarily in the liver, spleen and bone marrow. Hence, once absorbed, iron is conserved. This observation is underscored by the fact that there is no regulated pathway for the excretion of iron, and daily iron loss is negligible, resulting mostly from desquamation of cells.

Given the lack of a regulated means of excreting iron, the control of iron uptake is clearly of paramount importance. The duodenum and proximal jejunum are the main sites of absorption of dietary iron. Haem iron is absorbed more efficiently than non-haem iron, apparently by endocytosis of the intact iron–protoporphyrin complex at the enterocyte brush border.

Iron is then liberated from the haem moiety by the action of haem oxygenase and enters the intracellular iron pool from which it can be transferred across the basolateral membrane, bind to transferrin and enter the circulation. In contrast, the absorption of non-haem iron is more limited, in part as a result of its more complex uptake. As detailed below, absorption of non-haem iron requires reduction of ferric iron at the brush border membrane, followed by internalization by a proton-coupled transporter. Presumably, once iron derived from non-haem sources enters the intracellular iron pool within the enterocyte, its fate is similar to that of haem-derived iron. It is worth noting that, although a great deal has been learned about the mechanisms controlling iron absorption, iron stores exert a major influence on this process under physiological conditions but the means by which iron stores are sensed remains unclear.

Iron transport and uptake mechanisms

Because of the ability of iron to catalyse the production of reactive intermediates, its uptake and transit through the body require mechanisms to diminish its reactivity and thus prevent free radical generation. One of the means by which this is accomplished is by the binding of iron to proteins for transportation and storage. Thus, iron is transported in the blood bound to transferrin. Each molecule of transferrin can bind two atoms of ferric iron. Transferrin-bound iron is taken up at the cell membrane by the interaction of transferrin with the extracellular ligand-binding domain of the transferrin receptor 1 (TfR1). Upon binding of transferrin to TfR1, the entire complex is internalized by receptor-mediated endocytosis. Iron dissociates from transferrin in the acidic milieu of the endosome and then enters the intracellular iron pool, from which it is incorporated into iron-containing proteins, while apo-transferrin and TfR1 are recycled to the cell membrane.

The abundance of TfR1 is regulated by cellular iron status, while the identical mechanism controls expression of the iron-storage protein ferritin in an inverse manner. Cellular iron content determines the composition of a cytosolic protein termed the iron regulatory protein 1 (IRP1). Under iron-replete conditions, IRP1 contains a 4Fe–4S cluster that is unable to bind to iron-responsive elements (IRE) in the mRNAs of TfR1 and ferritin. When cellular iron content is low, the iron–sulphur cluster is disassembled, liberating an apo-IRP that binds to specific stem–loop structures in the 3′ or 5′ untranslated regions (UTRs) of the mRNAs encoding these proteins. In the case of TfR1, the IREs are located in the 3′ UTR, and binding of IRP1 increases the stability of the message and enhances the synthesis of TfR1. Conversely, binding of IRP1 to the IREs in the 5′ UTR of ferritin mRNA mediates translation repression. Thus, under iron-replete conditions, there is more rapid turnover of TfR1 mRNA, leading to diminished translation and cell-surface expression of TfR1, reduced uptake of transferrin-bound iron and an expanded capacity for iron storage through increased synthesis

of ferritin. Cellular iron deficiency, on the other hand, reverses these phenomena by promoting TfR1 expression and uptake of transferrin-bound iron while diminishing synthesis of ferritin.

In contrast to TfR1, the recently described transferrin receptor 2 (TfR2) has a rather restricted tissue distribution, with the liver the predominant site of expression, and lower levels reported in erythroid precursors and enterocytes [1]. Interestingly, both the 3' and the 5' UTRs of TfR2 mRNA lack IREs, indicating that its regulation differs from that of TfR1. Consistent with this observation, hepatic TfR2 expression is not downregulated by iron overload [2]. Given that the liver is a major site for iron storage, the high level of expression of TfR2 and its lack of responsiveness to iron status might be viewed as a protective mechanism, selectively diverting iron to hepatocytes under conditions in which circulating levels of transferrin-bound iron are high and peripheral iron stores are replete. However, the notion that TfR2 merely serves as a reserve is contradicted by the finding that subjects with mutations affecting TfR2 develop a form of non-*HFE* haemochromatosis (type 3) [3]. These observations indicate that TfR2 must serve additional functions, perhaps playing a role in modulating intestinal iron uptake and/or sensing of body iron stores.

In normal individuals, nearly all cellular acquisition of iron from blood occurs via transferrin receptor-mediated uptake, as virtually all the iron in the circulation is bound to transferrin. In circumstances in which the binding capacity of transferrin becomes saturated, as for example in iron loading disorders, iron forms low-molecular-weight complexes, the most abundant of which is iron citrate. It has been known for years that hepatic clearance of this non-transferrin-bound iron (NTBI) is rapid and highly efficient. Furthermore, studies in isolated perfused rat livers and cultured hepatocytes indicated that hepatic uptake of NTBI involves a membrane carrier protein whose iron transport function is subject to competition by other divalent metal ions. Based on these characteristics, it appears that the recently discovered divalent metal transporter 1 (DMT1; also known as DCT1 and Nramp-2) is the major transporter accounting for hepatic uptake of NTBI.

Using a cDNA library prepared from iron-deficient rat intestine, the DMT1 transcript was identified by its ability to increase iron uptake in *Xenopus* oocytes [4]. DMT1 has subsequently been shown to transport various divalent metal ions in a manner that is coupled to the transport of protons. Although DMT1 mRNA is broadly expressed in mammalian tissues including liver, its highest level of expression is found in the proximal intestine, consistent with its role in the absorption of dietary non-haem iron. Two isoforms of DMT1 have been described. The form of DMT1 that predominates in the intestine has an IRE in its 3' UTR, indicating that the stability of this transcript is regulated by cellular iron status in a manner similar to that of TfR1. Reciprocal changes in duodenal DMT1 expression *vis-à-vis* iron status have been demonstrated in iron-deficient rats and in humans with iron deficiency and iron overload [5]. Collectively, these data provide evidence for a negative feedback

loop in which iron status regulates intestinal DMT1 expression, which in turn controls iron uptake.

Less is known about the regulation and function of DMT1 in the liver. Data concerning the regulation of DMT1 expression by iron status in the liver are inconsistent, with some studies reporting that iron deficiency increases DMT1 mRNA in a manner similar to that of the intestine, while others find no change with altered iron status. Rather surprisingly, livers of rats fed an iron-deficient diet are reported to lack DMT1 immunoreactivity, while those from animals fed an iron-enriched diet demonstrate a pattern of DMT1 reactivity similar to that in the livers of animals fed a control diet, only much more intense [6]. This finding may reflect induction of the non-IRE-regulated form of DMT1, which contains metal responsive elements in its 5' regulatory region. Nonetheless, it is unclear why the IRE-regulated form of DMT1 (which is also present in liver) is repressed in liver under conditions of iron deficiency. It is evident that the regulation of DMT1 expression in the liver requires further study.

Questions also remain regarding the cellular localization of DMT1 in the liver. While *in situ* hybridization of DMT1 mRNA demonstrated diffuse, low-level expression confined to hepatocytes, results of quantitative polymerase chain reaction (PCR) studies on carefully isolated liver cell populations indicate that DMT1 transcripts are present in hepatocytes, sinusoidal endothelial cells, Kupffer cells and hepatic stellate cells, with the highest levels of expression observed in the last two cell types [7]. These results are particularly interesting in view of the finding that DMT1 immunoreactivity in normal rat liver is observed along the sinusoids, consistent with expression of the transporter on the microvilli of hepatocytes, where it can take up NTBI (or other divalent metals) from the subendothelial space. However, based on the PCR results and the immunostaining, one or more types of sinusoidal lining cells may also acquire iron from sinusoidal blood via DMT1. Further studies are needed to determine the sources and significance of non-parenchymal cell DMT1 expression.

Another issue relevant to DMT1 in liver is the mechanism by which iron is reduced prior to uptake by the transporter. Under physiological conditions, iron exists predominantly in the ferric (+3) state. Uptake by cellular transport systems requires that iron undergoes reduction to the ferrous (+2) state. Recent studies have identified a ferric reductase that is highly expressed in the proximal intestine, termed duodenal cytochrome *b* (Dcytb) [8]. A haem protein, Dcytb, is upregulated by conditions that stimulate iron absorption, including iron deficiency, chronic anaemia and hypoxia. The mechanism by which its expression is upregulated in these conditions is unclear, as there are no obvious IREs in the mRNA of Dcytb. Nevertheless, the localization of Dcytb on the brush border of duodenal enterocytes closely mirrors that of DMT1, supporting the concept that Dcytb supplies ferrous iron to DMT1. Presumably, hepatocellular uptake of NTBI via DMT1 has a similar requirement for ferrous iron. However, whether the reductase that serves this

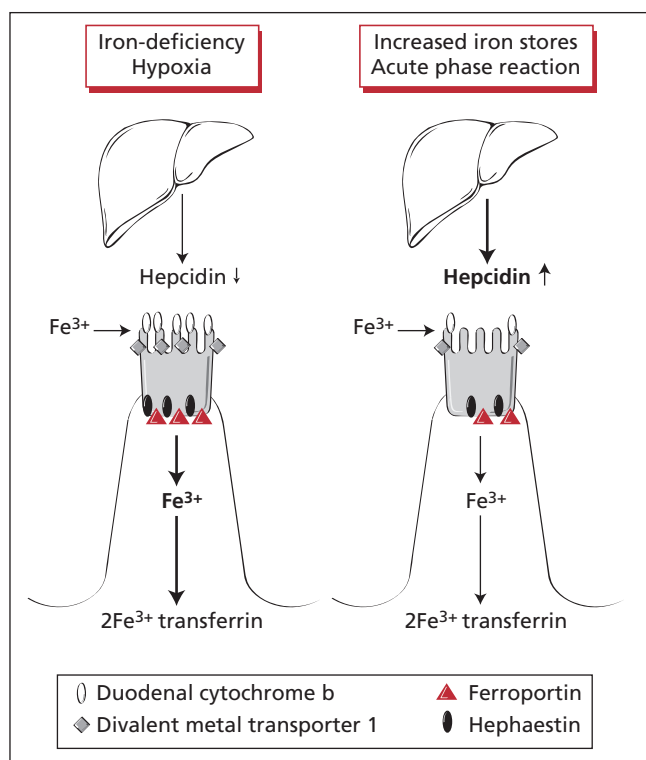


Fig. 1 Schematic representation of effects of various stimuli on hepcidin and its effects on iron transporters in the enterocyte. Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; Fpn, ferroportin; Heph, hephaestin.

function on hepatocytes (and other liver cells that express DMT1) is identical to Dcytb is currently unknown.

Iron mobilization and export (see Fig. 1)

Given that the liver is a site of iron storage under physiological conditions, it is apparent that there must be mechanisms by which iron stored in the liver can be mobilized and exported to extrahepatic tissues. Under normal physiological circumstances, Kupffer cells play a prominent role in interorgan iron trafficking. One of the primary sites of erythrocyte turnover, Kupffer cells, along with the reticuloendothelial cells of the spleen and bone marrow, ingest senescent or damaged red blood cells, catabolize the haemoglobin and release the iron. Collectively, the quantity of iron that is recycled from erythrocytes through the macrophage compartment on a daily basis is severalfold greater than that taken up through the intestine. Hence, the contribution of Kupffer cells to total body iron economy is both qualitatively and quantitatively important. It is therefore not surprising that Kupffer cells are the major type of liver cell that express a recently described iron exporter, ferroportin (Fpn; also known as Ireg1 and MTP1) [9–11].

In the intestine, Fpn expression is upregulated by iron deficiency and anaemia. Although this makes sense from a

physiological perspective, it is somewhat surprising in view of the presence of IREs in the 5' UTR of Fpn mRNA, rather than in the 3' UTR as would be expected, given the similarity of its regulation to that of DMT1 and TfR1. As illustrated by the example of ferritin, binding of IRPs to IREs in the 5' region generally results in translational repression. It has been proposed that the IRE in the 5' UTR may be non-functional in the intestine. If so, this suggests that Fpn expression may be regulated by different mechanisms in different tissues and/or cell types, as the intensity of Fpn staining of Kupffer cells in murine livers is reported to increase with iron loading [11].

Consistent with its role in iron absorption, Fpn is expressed at high levels along the basolateral membrane in mature enterocytes of the duodenal villi. In addition, Fpn transcripts are also detected in liver, spleen, kidney and placenta. In murine liver, hepatocytes as well as Kupffer cells show immunoreactivity for Fpn, albeit less intense. The quantitative PCR study on isolated cells from rat livers discussed above reported similar levels of Fpn transcripts in hepatocytes, Kupffer cells and stellate cells, and lower levels in sinusoidal endothelial cells [7]; however, Fpn protein has not been demonstrated in the last two cell types. Interestingly, the subcellular localization of Fpn appears to differ between hepatocytes and Kupffer cells, being localized to the plasma membrane along the sinusoidal border in the former and cytoplasmic in the latter [11]. It has been proposed that the intracellular localization of Fpn in Kupffer cells (which is also observed in RAW267.4 cells, a murine macrophage cell line) indicates that Fpn does not directly export iron across the plasma membrane in these cells but, rather, that it may participate in intracellular trafficking of iron, perhaps through the secretory pathway. Further studies are needed to determine whether Fpn is involved in multiple pathways of iron export.

Like cellular uptake of iron, efflux of iron from cells requires ferroxidase activity. It has been known for some time that ceruloplasmin, a copper-containing plasma ferroxidase synthesized by hepatocytes, plays an important role in iron homeostasis. Aceruloplasminaemia results in a form of iron overload that is recapitulated in mice with a targeted disruption of the ceruloplasmin gene [12]. Interestingly, although the ceruloplasmin knockout mice accumulate iron in both hepatocytes and Kupffer cells, intestinal iron absorption is unaffected by ceruloplasmin deficiency.

The recent discovery of a homologue of ceruloplasmin that is expressed at high levels in intestinal villi, termed hephaestin, probably accounts for this observation [13]. Despite their similarities, the function of hephaestin is distinct from that of ceruloplasmin, as mutations in hephaestin lead to iron deficiency rather than iron overload. This is illustrated by the sex-linked anaemia (*sla*) mouse, in which a mutation in hephaestin impairs the transfer of iron out of the enterocyte, resulting in microcytic, hypochromic anaemia. The divergent phenotypes of the ceruloplasmin knockout and the *sla* mouse appear to indicate that one ferroxidase has little if any ability to substitute functionally for the other. As hephaestin is membrane bound, this may indicate a

requirement for close physical proximity of the ferroxidase to Fpn for efficient iron export, at least in the enterocyte. In this context, it is interesting to contrast hepatocytes, which have low levels of Fpn protein and lack detectable hephaestin transcripts, with Kupffer cells, which have more robust levels of Fpn and express hephaestin transcripts, albeit at levels that are considerably lower than the intestine [7]. Taken together, these observations suggest either that the ferroxidase activity of ceruloplasmin can indeed substitute for hephaestin in Fpn-expressing cells in the liver (but not in the intestine), or that hepatocytes and possibly Kupffer cells as well may employ additional means to promote iron export, such as upregulation of hephaestin in response to iron loading and/or the expression of alternative exporters or ferroxidases.

Another gene involved in iron metabolism that is highly expressed in the liver as well as the intestine is HFE (see also Chapter 16.2, Haemochromatosis). Originally identified on the basis of a high frequency of HFE mutations in patients with genetic haemochromatosis, wild-type HFE protein forms a complex at the plasma membrane with TfR1 and β 2-microglobulin. Studies in transfected cells indicate that the stoichiometry of these components influences the rate of recycling of TfR1, thus modulating iron uptake [14]. Nonetheless, the precise mechanism whereby HFE mutations lead to iron loading remains speculative. While immunohistochemistry for HFE demonstrates a distinctive pattern of intracellular perinuclear staining in the epithelial cells of the small intestine [15], immunoreactivity for HFE in liver has been variously ascribed to bile ducts, sinusoidal lining cells, Kupffer cells and endothelial cells. Furthermore, these studies are at variance with results of PCR and Western blot analysis of isolated liver cells demonstrating that hepatocytes are the major source of HFE in rat liver, with a minor contribution from Kupffer cells [7]. Additional studies are needed to resolve this discrepancy and provide further insight into the function of HFE.

In contrast to the genes encoding HFE and proteins involved in iron transport, haemojuvelin mRNA is not abundantly expressed in the intestine. Rather, the highest levels of expression are seen in skeletal muscle, with lower levels in cardiac muscle and liver, where haemojuvelin transcripts are localized to periportal hepatocytes. Haemojuvelin was discovered by positional cloning of the locus associated with juvenile haemochromatosis [16]. Subsequently, two groups have reported that targeted deletion of haemojuvelin in mice results in iron overload [17,18]. While the function of haemojuvelin is unknown, it has been proposed that haemojuvelin is 'upstream' of hepcidin in the pathways controlling iron metabolism, as both patients with iron overload resulting from haemojuvelin mutations and haemojuvelin knockout mice fail to respond to their iron burden with an appropriate increase in hepcidin. A direct interaction between these two proteins seems unlikely, however, given that hepatic expression of haemojuvelin is not altered in mice treated with parenteral iron, while the same livers show a robust increase in hepcidin mRNA. Thus, the available

data demonstrate lack of responsiveness of haemojuvelin to iron as well as divergent regulation of haemojuvelin and hepcidin in normal animals treated with iron.

A major advance in the understanding of iron metabolism was the discovery of the iron regulatory hormone hepcidin. Hepcidin was originally identified as an antimicrobial peptide isolated from human urine [19]. The liver is the predominant source of hepcidin, where the 84-amino-acid prepropeptide is synthesized and cleaved to yield 20- and 25-amino-acid peptides that are released into the circulation and filtered by the kidney. Consistent with release into the blood from hepatocytes, hepcidin immunoreactivity is observed along the sinusoidal borders of hepatocyte membranes, with accentuated staining of periportal (zone 1) hepatocytes [20].

The initial finding linking hepcidin to iron metabolism came about through the use of subtractive hybridization to identify genes upregulated by iron overload in murine livers [21]. Later studies demonstrated that hepcidin knockout mice develop a form of iron overload reminiscent of hereditary haemochromatosis [22], while mice with overexpression of hepcidin have severe iron-deficiency anaemia [23]. These data led to the conclusion that hepcidin is a negative regulator of intestinal iron absorption, an inference that has since been confirmed by administration of synthetic hepcidin to rodents.

In addition to iron status, hepcidin expression is modulated by hypoxia and inflammation [24]. In the latter case, hepcidin is an acute-phase reactant, and its induction in response to inflammatory mediators accounts for several phenomena associated with anaemia of chronic disease [25,26]. Although the responsiveness of hepcidin to interleukin-1, interleukin-6 and tumour necrosis factor- α is established, the mechanism by which these mediators influence hepcidin expression is not known. Evidence to date indicates that hepcidin expression is regulated primarily at the transcriptional level. CCAAT enhancer binding protein α , a transcription factor involved in the control of many hepatocyte-specific genes, is a major positive regulator of hepcidin gene expression in the basal state [27]. However, the mechanisms involved in the induction or suppression of hepcidin expression in response to various stimuli have not been delineated, including the means by which iron status modulates hepcidin. There are no apparent IREs in the hepcidin transcript. Furthermore, isolated hepatocytes do not respond to exogenous iron in cell culture [21], suggesting that, *in vivo*, some other cell type senses iron levels and communicates that information to hepatocytes. Although Kupffer cells are likely candidates for this function, recent studies in rodents have shown an appropriate hepcidin response to iron following elimination of Kupffer cells [28,29]. Hence, this aspect of hepcidin biology remains poorly understood.

Subsequent studies have provided insight into the mechanisms by which hepcidin modulates iron absorption. Within a week of being placed on a low-iron diet, rats show a twofold increase in intestinal iron absorption that is temporally associated with a significant drop in hepatic hepcidin expression, and

increases in duodenal mRNAs for Dcytb, DMT1 and Fpn [30] (see Fig. 1). Although the increase in Fpn mRNA under these circumstances is of relatively small magnitude, the increase in Fpn protein is more substantial. A similar pattern is seen in the intestine of hepcidin knockout mice, providing additional evidence that hepcidin suppresses the expression of these iron transporters. While the role of hepcidin in the regulation of Dcytb and DMT1 has not been characterized, several reports have established that Fpn is a major target of hepcidin's action. As suggested by the observations discussed above, hepcidin appears to regulate Fpn expression by two distinct mechanisms. The first is at the level of Fpn transcripts, which are decreased following stimulation of endogenous hepcidin production or administration of recombinant hepcidin [31]. The second involves binding of hepcidin to Fpn at the cell membrane, causing internalization and degradation of Fpn, thus diminishing iron transfer [32]. These mechanisms are clearly not mutually exclusive and, while either or both probably contribute to the decrease in intestinal iron absorption in response to hepcidin, it is unclear at present whether Fpn expression in liver cells is regulated in the same manner. In mice treated with iron, intestinal Fpn expression is low, consistent with the known effects of hepcidin. In the liver, however, Fpn is increased, particularly in Kupffer cells [11]. This may result from enhanced translation due to the presence of the IRE in the 5' UTR of Fpn mRNA. If so, this effect must predominate over the hepcidin-induced increase in Fpn turnover. Alternatively, the distinctive intracellular pattern of Fpn in Kupffer cells implies that Fpn may not physically interact with hepcidin in macrophages, again raising the possibility of differential regulation of Fpn in liver vs. intestine. Further characterization of the effect of hepcidin on the regulation of iron transporters in the liver and its physiological consequences will help to clarify these issues.

The development of iron overload in hepcidin knockout mice [22] and humans with mutations in the hepcidin gene [33] is clearly explicable by the effects of hepcidin on intestinal iron absorption. Since the discovery of hepcidin, several authors have reported that hepcidin expression fails to increase in response to increased iron stores in other disease states characterized by iron loading. For example, hepcidin expression is inappropriately low in iron-loaded subjects with hereditary haemochromatosis [34] and haemojuvelin mutations [16]. Similar findings are reported in a variety of iron-loading anaemias [35]. These observations have led to the concept that 'upstream' iron-related proteins such as HFE and haemojuvelin must in some way control hepcidin expression such that, when these proteins are mutated, dysregulation of hepcidin results. It is clear that the response of hepcidin is defective in all these disorders, as 'appropriate' levels of hepcidin would prevent the progressive accumulation of iron. Nonetheless, it is unclear whether there are direct interactions between hepcidin and other 'upstream' iron regulatory proteins, or whether endogenous iron loading (secondary to HFE or haemojuvelin mutations, for example, as opposed to administration of dietary or parenteral iron) leads to aberrant sensing of

iron stores, thus accounting for the dysregulation of hepcidin expression, which then becomes the final common pathway of iron overload. Efforts to elucidate this question are limited by the current lack of knowledge regarding the mechanism by which iron status regulates hepcidin expression.

References

- 1 Kawabata H, Yang R, Hirama T *et al.* (1999) Molecular cloning of transferrin receptor 2. *J Biol Chem* 274, 20826–20832.
- 2 Fleming RE, Migas MC, Holden CC *et al.* (2000) Transferrin receptor 2: continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis. *Proc Natl Acad Sci USA* 97, 2214–2219.
- 3 Roetto A, Totaro A, Piperno A *et al.* (2001) New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. *Blood* 97, 2555–2560.
- 4 Gunshin H, Mackenzie B, Berger UV *et al.* (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388, 482–488.
- 5 Zoller H, Koch RO, Theurl I *et al.* (2001) Expression of duodenal iron transporters divalent-metal iron transporter 1 and ferroportin 1 in iron deficiency and iron overload. *Gastroenterology* 120, 1412–1419.
- 6 Trinder D, Oates PS, Thomas C *et al.* (2000) Localisation of divalent metal transporter 1 (DMT1) to the microvillus membrane of rat duodenal enterocytes in iron deficiency, but to hepatocytes in iron overload. *Gut* 46, 270–276.
- 7 Zhang A-S, Xiong S, Tsukamoto H *et al.* (2004) Localization of iron metabolism-related mRNAs in rat liver indicate that HFE is expressed predominantly in hepatocytes. *Blood* 103, 1509–1514.
- 8 McKie AT, Barrow D, Latunde-Dada GO *et al.* (2001) An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 291, 1755–1758.
- 9 Donovan A, Brownlie A, Zhou Y *et al.* (2000) Positional cloning of zebrafish *ferroportin1* identifies a conserved vertebrate iron exporter. *Nature* 403, 776–781.
- 10 McKie AT, Marciani P, Rolfs A *et al.* (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 5, 299–309.
- 11 Abboud S, Haile DJ (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. *J Biol Chem* 275, 19906–19912.
- 12 Harris ZL, Durley AP, Man TK *et al.* (1999) Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc Natl Acad Sci USA* 96, 10812–10817.
- 13 Vulpe CD, Kuo Y-M, Murphy TL *et al.* (1999) Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the *sla* mouse. *Nature Genet* 21, 195–199.
- 14 Waheed A, Grubb JH, Zhou XY *et al.* (2002) Regulation of transferrin-mediated iron uptake by HFE, the protein defective in hereditary hemochromatosis. *Proc Natl Acad Sci USA* 99, 3117–3122.
- 15 Parkkila S, Waheed A, Britton RS *et al.* (1997) Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. *Proc Natl Acad Sci USA* 94, 2534–2539.
- 16 Papanikolaou G, Samuels ME, Ludwig EH *et al.* (2004) Mutations in *HFE2* cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nature Genet* 36, 77–82.

- 17 Huang FW, Pinkus JL, Pinkus GS *et al.* (2005) A mouse model of juvenile hemochromatosis. *J Clin Invest* 115, 2187–2191.
- 18 Niederkofler V, Salie R, Arber S (2005) Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. *J Clin Invest* 115, 2180–2186.
- 19 Park CH, Valore EV, Waring AJ *et al.* (2001) Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 276, 7806–7810.
- 20 Kulaksiz H, Gehrke SG, Janetzko A *et al.* (2004) Pro-hepcidin: expression and cell specific localisation in the liver and its regulation in hereditary haemochromatosis, chronic renal insufficiency, and renal anaemia. *Gut* 53, 735–743.
- 21 Pigeon C, Ilyin G, Courselaud B *et al.* (2001) A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol Chem* 276, 7811–7819.
- 22 Nicolas G, Bennoun M, Devaux I *et al.* (2001) Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci USA* 98, 8780–8785.
- 23 Nicolas G, Bennoun M, Porteu A *et al.* (2002) Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci USA* 99, 4596–4601.
- 24 Nicolas G, Chauvet C, Viatte L *et al.* (2002) The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 110, 1037–1044.
- 25 Nemeth E, Rivera S, Gabayan V *et al.* (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 113, 1271–1276.
- 26 Lee P, Peng H, Gelbart T *et al.* (2005) Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proc Natl Acad Sci USA* 102, 1906–1910.
- 27 Courselaud B, Pigeon C, Inoue Y *et al.* (2002) C/EBP α regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. *J Biol Chem* 277, 41163–41170.
- 28 Montosi G, Corradini E, Garuti C *et al.* (2005) Kupffer cells and macrophages are not required for hepatic hepcidin activation during iron overload. *Hepatology* 41, 545–552.
- 29 Lou D-Q, Lesbordes J-C, Nicolas G *et al.* (2005) Iron- and inflammation-induced hepcidin gene expression in mice is not mediated by Kupffer cells *in vivo*. *Hepatology* 41, 1056–1064.
- 30 Frazer DM, Wilkins SJ, Becker EM *et al.* (2002) Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. *Gastroenterology* 123, 835–844.
- 31 Yeh K-Y, Yeh M, Glass J (2004) Hepcidin regulation of ferroportin 1 expression in the liver and intestine of the rat. *Am J Physiol* 286, G385–G394.
- 32 Nemeth E, Tuttle MS, Powelson J *et al.* (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 306, 2090–2093.
- 33 Roetto A, Papanikolaou G, Politou M *et al.* (2003) Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nature Genet* 33, 21–22.
- 34 Bridle KR, Frazer DM, Wilkins SJ *et al.* (2003) Disrupted hepcidin regulation in *HFE*-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet* 361, 669–673.
- 35 Papanikolaou G, Tzilianos M, Christakis JI *et al.* (2005) Hepcidin in iron overload disorders. *Blood* 105, 4103–4105.

2.3.13 Normal copper metabolism and lowering copper to subnormal levels for therapeutic purposes

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Introduction

In this chapter, we will first provide a review of current knowledge about copper metabolism. Copper is an essential trace element, and the normal diet contains an average of about 1.0 mg. This is about 25% more than is required, and most of the excess is normally excreted by the liver into the bile for loss in the stool. Hence, the liver is important in regulating copper balance and other aspects of metabolism. Excellent progress has been made in understanding copper metabolism in the body, thanks in part to discoveries of the genes that cause two copper-related diseases, ATP7A for Menkes' disease and ATP7B for Wilson's disease (see Chapter 16.1). Progress has also been helped by the elucidation of the roles of copper chaperones, evolutionarily conserved genes whose protein products facilitate transfer of copper to target proteins or vesicles. In the second part of the chapter, a new area involving copper will be reviewed, that of the therapeutic use of lowering copper to subnormal levels to treat cancer and diseases of inflammation and fibrosis.

Copper metabolism and its role in health and disease

Introduction

The essentiality of copper in human health has been recognized for more than 70 years. Severe copper deficiency, whether genetic or acquired, can produce devastating disease and death. The toxic properties of copper were brought to the forefront of scientific attention when a disease described by Wilson in the early 1900s called 'hepatolenticular degeneration' was later discovered to be due to copper accumulation and toxicity [1]. The liver plays a prominent role in copper distribution to organs and regulates overall system homeostasis. Bile, not urine, for eventual loss in the stool, is the major excretory route for copper. Normal urine copper loss is 20–50 $\mu\text{g}/\text{day}$, whereas stool copper loss is in the order of 1.0 mg/day. Transport through the blood to the absorption surfaces of cells has yet to be clarified with certainty. Transport through the membrane and into cytosolic proteins, however, is becoming better understood [2]. The discovery of two structurally related membrane-bound Cu-ATPases, ATP7A and ATP7B, defective in Menkes' and Wilson's diseases, respectively, has provided insight into intracellular copper movement and control of its excretion from the cell. These discoveries have also provided unprecedented biochemical insights into diseases of copper metabolism and have formed the basis upon which much of the current theories of cellular copper movement and homeostasis rest.