Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism

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Key Messages

- Iron is a paradoxical nutrient with potential for great benefit but also for causing harm if it is not appropriately chaperoned and regulated.
- In the past 20 years advances in molecular biology have elucidated many of the mechanisms by which humans tread the delicate balancing act to derive optimal benefit; key amongst these is the discovery of hepcidin which acts as the master regulator of iron.
- The new insights derived from these basic-science discoveries show that humans exert considerable physiological effort into excluding iron in the face of inflammation and infection, and hence that eliminating these will be at least as important in combating global iron deficiency as efforts directed towards improving diets.

Keywords

Iron · Hepcidin · Ferroportin · Iron deficiency · Anemia

Abstract

The fact that humans must balance their need for iron against its potential for causing harm has been known for several centuries, but the molecular mechanisms by which we achieve this feat have only been revealed in the last 2 decades. Chief amongst these is the discovery of the master-regulatory liver-derived hormone hepcidin. By switching off ferroportin in enterocytes and macrophages, hepcidin exerts fine control over both iron absorption and its distribution among tissues. Hepcidin expression is downregulated by low iron status and active erythropoiesis and upregulated by iron overload and infection and/or inflammation. The latter mechanism explains the etiology of the anemia of chronic infection. Pharmaceutical companies are actively developing hepcidin agonists and antagonists to combat iron overload and anemia, respectively. In a global health context the discovery of hepcidin shines a new light on the world’s most prevalent micronutrient problem; iron deficiency and its consequent anemia. It is now apparent that humans are not poorly designed to absorb dietary iron, but rather are exerting a tonic downregulation of iron absorption to protect themselves against infection. These new insights suggest that interventions to reduce infections and inflammation will be at least as effective as dietary interventions and that the latter will not succeed without the former.

Introduction

Iron is an intriguing nutrient with many paradoxical characteristics. It is the most abundant element on our planet and yet is hard for living organisms to access due to its very low solubility. Its ability to switch easily be-
tween the ferric (Fe$^{3+}$) and ferrous (Fe$^{2+}$) state is key to its usefulness in many biological reactions, but managing the electron state also poses a challenge. Iron is an essential and usually highly beneficial nutrient, but at the same time can wreak havoc if it is not appropriately chaperoned to prevent it from causing oxidant damage. In humans these chaperone processes are also critical in withholding iron from bacterial pathogens and maintaining relatively hypoferremic conditions such that microorganisms will not be able to multiply rapidly and can be dealt with by other arms of the innate and adaptive immune systems. This much has been known for many decades with the chemical details of the chaperone mechanisms starting to emerge after Schade’s first descriptions in Science of the iron withholding and bacteriostatic qualities of ovotransferrin and transferrin [1, 2].

Within the past 2 decades advances in molecular biology methods have revealed the finer details of how iron is absorbed, transported, and utilized. Of special relevance has been the discovery of hepcidin: the master regulator of iron. These insights have transformed our understanding of iron metabolism and have immediate clinical relevance as summarized here.

**Twentieth-Century Concepts of Iron Absorption and Regulation**

By the 1930s the legendary scientific duo of McCance and Widdowson [3] had concluded that iron homeostasis in humans is controlled by regulating intestinal absorption. Initial attempts to assess the efficiency of intestinal iron absorption from different foods and diets started with fastidious and very time-consuming balance studies [4, 5]. These were superseded by stable isotopic tracer methods which assessed iron utilization (the aggregate outcome of absorption and ultimate utilization for heme production) in the single isotope mode [6], or true absorption and utilization in the double isotope mode in which one isotope is administered orally and the other intravenously [7].

Arising from these and related studies came the “textbook” knowledge of iron absorption [8] which, simply put, was: (a) that iron absorption is a dynamic process with auto-regulatory feedback (hence, for instance, pregnant women with greater iron needs upregulate their absorption); (b) that heme iron is better absorbed and utilized than non-heme iron; (c) that iron absorption is inhibited by various binding agents in foods (such as phytates and phenolic compounds); (d) that iron absorption is enhanced by co-ingestion of vitamin C; (e) that there is competition for absorption between iron and zinc; and (f) that there is no active process to excrete iron from the body. These were the basic tenets around which most iron interventions were designed and most recommendations were set. Of particular impact was an underlying assumption that the human gut was poorly designed to absorb iron. This prompted extreme practices in which, for instance, young children were (still are) deemed to require 2 mg/kg iron (when their actual daily need is a very small fraction of this), were given highly absorbable iron, often with vitamin C, and dosing was advised to occur between meals to avoid interference by phytates or related “antinutrients.” These recommendations for highly nonphysiological dosing regimens have probably caused considerable iatrogenic harm judging from the adverse outcomes of many randomized trials of iron supplementation to children [9]. With the modern insights summarized below, and viewed from a more nuanced understanding of the origins and “intentions” of the finely evolved systems for iron handling, these failures in public health practice are unsurprising. We now know that, far from being ill-designed to absorb iron, children in unhygienic environments are working hard to exclude iron from their systems; and the processes mediating this iron blockade are now well understood.

**Molecular Mechanisms of Iron Absorption at the Intestinal Lumen**

It seems remarkable that the mammalian divalent cation transporter 1 (DCT1; now termed DMT1) was only discovered and cloned in the rat 20 years ago by Gunshin et al. [10] at Harvard. In the original description in the rat the investigators described the 12 transmembrane loops, its tissue distribution (very high in enterocytes lining the villi of the duodenum but with some expression in most tissues studied), and the fact that mRNA levels were highly upregulated by dietary iron deficiency [10]. They also correctly predicted that the hereditary hemochromatosis gene (HFE) was likely to be part of the iron-sensing regulation of DCT1.

These first discoveries led the way to our current understanding of the molecular mechanism of iron absorption [11]. The absorption of non-heme iron requires that it be reduced to Fe$^{2+}$ by the action of duodenal cytochrome B (Dcytb) which co-localizes with DMT1 in the brush border membrane [12]. Once inside the enterocyte, iron can either be stored in ferritin (and later lost when the cells are sloughed) or can traverse the cell and enter the circulation via ferroportin. Ferroportin

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**Hepcidin-Mediated Regulation of Iron Absorption and Metabolism**

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Fig. 1. Iron absorption, recycling, and organ distribution. Values for an adult human male. Reproduced with permission from Hentze et al. [16].

Fig. 2. Hepcidin is the dominant regulator of iron absorption and incorporation into erythrocytes. Data from young Gambian children with postmalarial anemia and control anemic subjects without recent malaria. Reproduced with permission from Doherty and colleagues [22].

Fig. 3. Current knowledge of the key pathways in the regulation of hepcidin expression. Three main molecular pathways have been identified that modulate hepcidin transcription: JAK/STAT3, BMP/SMAD, and HFE/TfR2 signaling pathway. IL6-induced inflammatory stimuli activate the JAK/STAT3 pathway which in turn triggers hepcidin production. Increased hepatic cellular iron induces BMP6 expression which then interacts with BMPR and HJV, forming a complex, thereby activating the SMAD pathway. The SMAD pathway involves phosphorylation of SMAD1, 5, and 8 (pSMADs), formation of the pSMADs/SMAD4 complex, and subsequent translocation of this complex to the nucleus to activate hepcidin gene expression. Extracellular Tf-Fe\textsuperscript{2+} mediates a second iron signal. When transferrin saturation increases, Tf-Fe\textsuperscript{2+} displaces HFE from TfR1. HFE then interacts with TfR2 to form the HFE/TfR2 complex. Consequently, this complex activates hepcidin transcription via the HJV/BMP/SMAD and/or ERK/MAPK signaling pathways. Furthermore, TMPRSS6 and furin cleave HJV to form a soluble HJV, which can selectively inhibit BMP-induced hepcidin expression. Furin and TMPRSS6 can be regulated by hypoxia and via HIF1. Erythropoietic drive may also control hepcidin expression by EPO production. EPO subsequently stimulates erythroferrone expression, which acts together with TWSG1 to inhibit hepatic hepcidin expression by suppressing the BMP/SMAD pathway. JAK, Janus-associated kinase; STAT, signal transducer and activator of transcription; HFE, human hemochromatosis protein; TfR, transferrin receptor; SMAD, sons of mothers against decapentaplegic; BMP, bone morphogenetic protein; HJV, hemouvelin; HIF, hypoxia inducible factor; MAPK, mitogen-associated activated protein kinase; ERK, extracellular signal-regulated kinase. Reproduced with permission from Wallace [11].

(For figure see next page.)
is the only known mammalian iron exporter that facilitates and modulates cellular iron efflux [13]. The exchange of ferrous iron from ferroportin requires its reoxidation to ferric by the action of ceruloplasmin [14] or (in the intestine) its membrane-bound counterpart hephaestin [15]. Excellent reviews with greater detail are available for readers with a deeper interest [11, 16].

**Net Iron Absorption and Iron Recycling**

An average adult human loses about 1 mg of iron daily largely through the sloughing of epithelial cells in the intestine. This is a notably small amount when compared to the recommended doses for prevention and treatment of iron deficiency anemia (often 200 times as much or more). In contrast to this very small net intake, the amount of iron internally recycled in the body is considerably more; estimated to be 25–30 mg/day (Fig. 1) [16]. In biology, cycles with a high flux such as this offer the opportunity for exquisite control; if there is a regulatory gateway in the cycle, then closing this gateway has an immediate and profound effect on the concentration of the substrate concerned. As we will see below, this is how the acute phase reaction can elicit such a very rapid hypoferrremia. For many years the details of the sensors, regulators, and effectors of these processes remained obscure though it was assumed that there must be one or more hormonal regulators that can transmit information from iron-utilizing end organs (primarily the bone marrow) to the intestine.

**Hepcidin: The Master Regulator of Iron Metabolism**

Although many pathways and molecular mechanisms are involved in regulating iron metabolism, the current view is that these are all coordinated by the hepatic-derived peptide hepcidin which acts as the master regulator of iron. Hepcidin integrates diverse signals about iron need with counter-regulatory signals designed to suppress iron intake and recycling when there is a threat of infection [17]. The mature 25 amino acid bioactive form of hepcidin contains 8 cysteines linked by 4 disulfide bridges. Hepcidin–like molecules with high homology to human hepcidin are found in mammals, fish, reptiles, and amphibians, but not in birds or invertebrates.

Hepcidin was independently discovered in the early 2000s by 3 groups who were searching variously for novel antimicrobial peptides and for a liver-expressed iron-responsive gene [18–20]. Ganz and colleagues [19] in Los Angeles coined the name hepcidin to describe a hepatic-derived peptide with microbicidal properties. (Note that although the hepcidin molecule does itself possess some antimicrobial activity, this is rather weak compared to peptides such as defensins, and its primary contribution to innate immunity is via regulation of iron.) Experiments manipulating the hepcidin gene Hamp1 soon demonstrated that Hamp1 knockouts became iron load-ed and Hamp1 overexpression led to congenitally fatal iron deficiency [17]. Nemeth et al. [21] in the Ganz lab then showed that the molecular mechanism of hepcidin’s action was via binding to, and causing the internalization and degradation of, ferroportin.

Ferroportin is heavily expressed in 2 sites (on the basolateral membrane of enterocytes and on macrophages) and thereby regulates both iron absorption and tissue redistribution [17, 21] (Fig. 1). Hepcidin-mediated down-regulation of ferroportin on the enterocytes blocks iron egress causing a build-up of intracellular iron which in turn blocks further uptake by DMT1. Note that by exerting its control on the basolateral side of the cell it also blocks the uptake of iron released from heme by the action of heme oxygenase. Figure 2 shows that iron utilization by young anemic children is very efficient at low levels of hepcidin but is almost entirely blocked when hepcidin levels are high [22]. Ferroportin blockade in macrophages prevents iron recycling [21].

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**Fig. 4.** Hepcidin causes a rapid, sustained fall in serum iron. Mice received a single 50-μg intraperitoneal injection of hHep (human hepcidin). Reproduced with permission from Rivera et al. [26].
Hepcidin represents an exquisite example of evolution in response to the 2 opposing aspects of iron in biology; its obligate need for many physiological processes versus the threat it poses by encouraging pathogen growth. Existing data suggest that hepcidin balances the need for iron against the threat of infection [23]. Figure 3 (reproduced from Wallace [11]) and its accompanying legend gives a simplified summary of the mechanisms by which hepcidin achieves this balancing act. The figure summarizes current knowledge and numerous further details are sure to emerge.

In brief, the iron-responsive modulation derives signals from hepatic iron and transferrin saturation and pos-
possibly oxygen saturation through HIF1. The recently described bone marrow-derived hormone, erythroferrone, produced by young erythroblasts also downregulates hepcidin expression in response to active erythropoiesis [24]. Conversely, inflammation, mostly mediated through IL-6 but with alternative stimulation by IL-22 and type 1 interferon, upregulates hepcidin and thus blocks iron absorption and recycling [25]. Figure 4 illustrates the impressive speed and extent by which hepcidin can elicit an immunoprotective innate response [26].

Figure 5, reproduced from Drakesmith and Prentice [23], illustrates how differential levels of hepcidin expression change the iron concentration in various body compartments with likely consequences for the susceptibility to infection. Hepcidin-induced hypoferremia in circulating plasma protects against the acute threat of bacterial and yeast sepsis. Prolonged upregulation of hepcidin leads to the anemia of inflammation or of chronic infection [27], which in turn protects against the blood stage of malarial infection [28]. Similarly, hypoferremia in hepatocytes protects against the hepatic stages of malaria infection [29]. Intriguingly, though as yet unproven, we propose that iron lock-down in macrophages may be the reason that intracellular pathogens have selected macrophage phagosomes as their niche of choice [23].

**New Insights into the Causes of Iron Deficiency in Low-Income Countries**

Prior to this century, almost all research into the etiology of iron deficiency anemia concentrated on dietary factors and was based around the premise, as mentioned above, that humans are inherently badly designed to absorb iron especially from cereal-based diets. The discovery of the hepcidin-ferroportin axis effectively turns this assumption on its head and requires a thorough re-examination of the key etiological factors driving deficiency. Key to this conclusion is the finding that most known gene defects affecting iron status lead to iron overload, not deficiency, even in populations on a low iron intake [11]. The one exception relates to defects in the TMPRSS6 gene which normally suppresses hepcidin gene expression. Rare defects in this gene result in hepcidin overexpression and so-called iron-refractory iron deficiency anemia (IRIDA) [30]. These insights reveal that instead of struggling to absorb sufficient iron, the normal physiological state in humans is one of tonic suppression of iron absorption in order to maintain body iron homeostasis in the absence of any pathways for secretion of an excess.

We have extensively studied populations with high levels of anemia (pregnant women and young children in rural areas of Gambia and Kenya) and have been forced to re-evaluate many of our preconceived notions. First, in cross-sectional studies in both populations and using structural equation modelling we showed that hepcidin levels were, as predicted on the basis of the known biology of hepcidin, predicted by a combination of iron status, inflammation, and infection [31, 32].

We then used receiver-operator characteristic analysis to determine hepcidin cutoffs that best define iron deficiency anemia and the anemia of inflammation [33, 34]. In children the defined threshold (5.5 ng/mL, using the Bachem ELISA) also distinguished iron absorbers from nonabsorbers [33]. With support from the Bill & Melinda Gates Foundation, we have recently completed 2 randomized controlled trials in Gambian pregnant women and children under the umbrella of the HIGH Consortium (Hepcidin and Iron in Global Health) [35, 36]. These trials are testing whether it would be possible to develop a point-of-care diagnostic for hepcidin-guided iron administration on the principle that a hepcidin level below 5.5 ng/mL in children (or 2.5 ng/mL in pregnant women [34]) would signal that the subject was “safe and ready to receive iron.” These trials will shortly report full results.

Using longitudinal hepcidin measurements (weekly for 12 weeks) in over 400 participants from the children’s trial [35] we have shown that each child maintains a remarkably consistent level of hepcidin over the 3 months of study (albeit gradually rising in response to the iron supplementation). Fifty percent of the children consistently maintain their hepcidin above the 5.5 ng/mL, indicating that they are blocking iron absorption (Prentice et al., manuscript in preparation). Unsurprisingly, these high hepcidin levels are associated with raised CRP and AGP (markers of inflammation), but remarkably we found that hepcidin was upregulated even at very low levels of inflammation. This has important implications for the prevention and treatment of iron deficiency and its associated anemia as we discuss in our conclusions below.

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Prospects and Possible Applications for Hepcidin Agonists and Antagonists

Pharmaceutical companies are investing considerable resources in the potential therapeutic applications that could be derived from manipulating hepcidin [37]. For instance, iron overload could potentially be modulated by artificially enhancing hepcidin. To this end, Ganz and his team have synthesized a series of mini-hepcidins; short peptides that maintain hepcidin action, are re-engineered to be stable, and could potentially be orally active [38, 39]. In the global health context mini-hepcidin might be able to prevent the iron overload that occurs even in untransfused beta-thalassemia (due to the hepcidin-suppressing action of excess erythroferrone caused by the ineffective erythropoiesis that generates excess erythroblasts) [40]. Conversely, there is great interest in the possibility that hepcidin antagonists could be used to prevent or treat the anemia of chronic disease and anemias associated with treatments such as cancer chemotherapy [37]. Clinical trials are underway for both hepcidin agonists and antagonists and it is likely that these will become commonly used therapeutic agents in the mid-term future.

Conclusions: Implications of the New Molecular Insights

It goes without saying that the design and implementation of preventative or therapeutic interventions to combat disorders of iron metabolism (most commonly iron deficiency anemia in a global context) are greatly aided by a clearer understanding of the precise mechanisms regulating iron metabolism. As described above, the most important implication of these discoveries is the realization that humans have evolved to expend at least as much physiological effort in excluding dietary iron as in acquiring it. This has been driven by the hazardous nature of iron; both in causing oxidative damage and in promoting the growth of microorganisms. There is clear evidence that a moderate degree of iron deficiency anemia is highly protective against malaria [28] and likely protective against a range of bacterial and fungal pathogens [23] and possibly viruses [41]. This has resulted in the evolution of exquisite systems for chaperoning iron and regulating its intake and organ distribution. Against this background, our former attempts to counteract iron deficiency by using very high nonphysiological doses of highly absorbable iron given without food seem clumsy to say the least and have almost certainly caused a great deal of iatrogenic harm. An analogy would be that we have been trying to use a sledgehammer to break down a closed door rather than learning how to pick the lock. Our new understanding that even low level inflammation blocks iron absorption via the hepcidin/ferroportin axis throws the spotlight on the need to eliminate infections and the consequent inflammation. This will require so-called nutrition-sensitive interventions around improvements in hygiene and infection control and it is quite possible that such interventions could be more important in poor populations than nutrition-specific interventions around iron.

Disclosure Statement

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