Animal models with enhanced erythropoiesis and iron absorption

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Abstract

The regulation of iron absorption is of considerable interest in mammals since excretion is minimal. Recent advances in iron metabolism have expounded the molecular mechanisms by which iron absorption is attuned to the physiological demands of the body. The pinnacle was the discovery and identification of hepcidin, a hepatic antimicrobial peptide that regulates absorption to maintain iron homeostasis. While the intricacies of its expression and regulation by HFE, transferrin receptor 2 and hemojuvelin are still speculative, hepcidin responsiveness has correlated negatively with iron absorption in different models and disorders of iron metabolism. Consequently, hepcidin expression is repressed to enhance iron absorption during stimulated erythropoiesis even in situations of elevated iron stores. Animal models have been crucial to the advances in understanding iron metabolism and the present review focuses on phenylhydrazine treated and hypotransferrinaemic rodents. These, respectively, experimental and genetic models of enhanced erythropoiesis highlight the shifting focus of iron absorption regulation from the marrow to the liver.

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1. Introduction

Iron deficiency is the most prevalent cause of anaemia, affecting 15% of the world’s population [1]. Mammalian iron homeostasis is essentially a balance of the iron metabolism of haemoproteins, erythropoiesis, reticuloendothelial and the storage compartments. Haemoglobin, myoglobin and other haemoproteins constitute 85% of the total body Fe content, comprising 50 and 40 mg/kg body weight in men and women, respectively. The dynamic turnover of circulatory Fe of about 30–35 mg/day [2] is associated with plasma transferrin. Of this, about 80% is in a closed circuit of Fe trafficking from erythrophagocytosis of senescent red blood cells by macrophages to the bone marrow for incorporation into new erythrocytes [3]. The remainder includes non-haem iron-containing enzymes, FeS clusters and storage forms, ferritin and haemosiderin [4]. Under normal physiological conditions, the balance of iron homeostasis is in a dynamic equilibrium between systemic tissues. Daily absorption of about 1 mg Fe replaces the limited excretion through normal blood losses, desquamation of senescent enterocytes, exfoliation of the skin, biliary and urinary excretion [5]. However, nutritional, physiological, pathological or mutational aberrations pose challenges to the body’s iron homeostatic mechanisms. These generally manifest as iron deficiency, overload or maldistribution disorders. Stimulation of erythropoiesis depends on adequate plasma iron and iron release from the reticuloendothelial system (RES). Although the underlying causes of iron limited anaemia are multifaceted, reduced iron supply and enhanced activity of haematopoietic cells are common prognostic features. The spectrum of these disorders range from classical nutritional deficiency anaemia to the body’s...
defence mechanism in the anaemia of chronic disease and a
number of diseases due to mutations of iron transport genes.

2. Regulation of iron absorption

Our understanding of iron absorption regulation has undergone a revolution in the last 4 years. Over the previous 64 years, since it was proposed that body iron levels were controlled by regulation of dietary iron absorption [6], progress in identifying the underlying mechanisms was slow (early work summarised in [7,8], later work covered by [9,10]). Even the identification of the haemochromatosis gene (Hfe, [11]), followed by the principle genes responsible for transporting non-haem iron (duodenal cytochrome b (Dcytb), divalent metal transporter 1 (DMT1) for iron uptake into enterocytes, ferroportin and hephaestin for iron export to plasma, reviewed in [12]) did not immediately solve the problem. Only the discovery of the regulatory peptide hepcidin [13–15] produced the breakthrough and with the discovery that hepcidin (produced in the liver) directly acts on and regulates the intestinal iron transporter ferroportin [16] we now have the basis for a mechanistic understanding of iron absorption regulation (Fig. 1).

Prior to 2001, extensive work had identified several physiological factors that regulate iron absorption. The quantitatively most important, but least studied, is the developmental regulator, which oversees the close matching of iron absorption rates to the demands of growth. In mice (and rats), this means iron absorption reaches a maximal rate at the time of maximal growth declining by about 4-fold to early adulthood. One interesting hypothesis about the cause of haemochromatosis was that normal iron absorption regulation includes a mechanism to down-regulate iron absorption as an animal grows to adulthood and that haemochromatosis was caused by a failure in the inhibitory mechanism [17]. We now can partly reconcile this down-regulation with the production of hepcidin which seems to increase during maturation [18]. Another iron absorption regulatory factor is hypoxia [19], a condition that is also associated with enhanced erythropoiesis. Several lines of evidence, however, suggest that the increased iron absorption occurs independently of any erythropoietic response. These include the time course of the increased iron absorption, which responds before significant changes in plasma iron turnover [20], reticulocyte levels [21], or plasma iron [20,22] although another study has shown a drop in plasma iron coincident with increased absorption at 8 h of hypoxia [23]. The rise in iron absorption closely parallels the rise in plasma erythropoietin levels [21]. Further evidence is the finding that iron absorption can respond to hypoxia even when the bone marrow is destroyed by radiation [21,24,25] or when erythropoietin levels are reduced by partial nephrectomy plus splenectomy [21,24,25]. Finally, injection of erythropoietin in normoxic mice to generate a similar erythropoietic response as seen in hypoxic mice produces little or no increase in iron absorption [21,25]. The hypoxic response also tends to increase iron stores [26–28] and plasma iron levels [22,26], and is not blocked by prior iron loading [23,29], hence is independent of the stores regulator. Thus, hypoxia qualifies as an independent regulator of iron absorption.

Other regulatory factors for iron absorption include pregnancy (increased requirements in last trimester [30]), gender (virgin female rodents have higher iron stores than males [31]) and inflammation. The last of these also seems to involve hepcidin, which dramatically increases in inflammatory conditions [32] and is a major factor in anaemia of chronic diseases [33]. However, the majority of investigations of iron absorption regulation have focussed on the key relationship between iron supply to the erythron, iron stores and iron absorption. These are closely integrated as red cell iron is by far the largest iron compartment in the body, with iron stores being the next largest in iron replete mammals. Finch [9] enunciated the concept of ‘regulators’ of iron absorption, focussing on the erythroid and stores regulators. The stores regulator was established from many studies that show iron absorption changing when iron stores are altered independently of changes in haemoglobin
levels or erythroid activity. On the other hand, the erythroid regulator was established from studies showing increased iron absorption in the absence of anaemia (and therefore no hypoxia) when erythroid activity was increased, e.g., by erythropoietin [34]. This occurred even after correction of absorption for changes in iron stores, and supported many earlier studies in man and animals (references in [34]) hence an independent erythroid regulator was hypothesized [9]. Increased erythropoiesis is important as the iron overload associated with some common hereditary anaemias (especially thalassaemias) is a significant health problem in humans [35] while a derangement in the stores regulator leading to pathological expansion of iron stores (even while red cell production and turnover remains normal) seems to underlie most cases of genetic haemochromatosis. Hepcidin seems to provide a final common pathway for both these regulators being responsive to increased erythropoiesis and iron stores [32] and therefore seems to be important in both these conditions. We still require further data to map all the known factors that regulate iron absorption to specific genes or regulatory pathways, however, this will likely be forthcoming in the near future.

Much of the work described above has been shown to operate in man as well as rodents, however, work with rodent models has been essential to the elucidation of iron absorption regulation [36]. The present review is aimed at describing an important set of rodent models for the study of iron absorption, namely those with experimentally or genetically enhanced rates of erythropoiesis, in particular, we describe in detail phenylhydrazine treatment and hypotransferrinaemia, the most extensively studied of these models.

3. Phenylhydrazine-induced haemolytic anaemia

Hereditary or acquired haemolytic anaemia in humans results from reduced life span or destruction of red blood cells and a failure of the bone marrow compensatory responses. Haemolysis of the red blood cells reduces the efficiency of oxygen delivery which stimulates increased erythropoiesis and because of the deficit in the Fe supply and haemoglobin levels, the haemopoietic cells are numerically and morphologically abnormal. Other features include spherocytosis, polychromasia, red-cell reticulocytosis, an increase in urinary urobilinogen and porphyrins [37]. These disorders are associated with ineffective erythropoiesis and are generally characterized by sustained enhancement of erythroid activity that promotes increased gastrointestinal iron absorption and ultimately leads to tissue iron overload. This model of enhanced erythropoietic activity, increased iron absorption and tissue iron overload is induced in experimental animals by the administration of phenylhydrazine (PHZ). It represents a situation where the triad of haem catabolism, anabolism and iron absorption is interactive in balancing systemic iron homeostasis. PHZ induces haemolysis by a reaction of the oxidized drug with ferrihaemoglobin to form ferrihaemochrome. This is often accompanied by the production of reactive oxidants that cause oxidative denaturation of oxyhaemoglobin and generate Heinz bodies [38]. PHZ also reacts with Hb to cause the dismutation of O$_2$ and produces H$_2$O$_2$ within the erythrocyte [39]. Alklylation of haem to form N-phenylhaem and oxidation of Hb affect red cell membrane integrity resulting in extravascular haemolysis [40]. These products aggregate as senescent antigen that are recognized by autologous immunoglobulins [41], thus inducing removal of cells from the blood by phagocytosis, manifesting a huge haemochatharsis.

3.1. Haematological indices

Haemolytic anaemia and hyperplastic erythropoiesis induced by intraperitoneal administration of PHZ is associated with an initial destruction of about 20% of the circulatory red cell mass each day. Pronounced anaemia seen 3–5 days after the administration of PHZ is characterized by decreased haematocrit, Hb, red cell count and mean corpuscular volume [42,43]. As erythropoietin (EPO) production is increased [44,45], erythroid marrow expands, and both blood flow and erythroid progenitor cells iron uptake is increased [45]. Proliferation of the erythroid marrow results in increased reticulocytes in circulation. Moreover, PHZ anaemia is associated with hyperferreraemia, increased total iron binding capacity (TIBC) and a 3- to 4-fold increase in iron turnover [46,47]. Increased haemopoietic iron demands create an imbalance between marrow iron needs and supply resulting in elevated RBC protoporphyrin [48,49]. Iron loss is extremely low in PHZ-treated animals [50], therefore increased absorption, erycyto catabolism and reticuloendothelial circulation result in increased serum iron. Erythrophagocytosis of haemolysed erythrocytes contributes to elevated serum iron and this iron is more readily available for haemopoietic stem cells for Hb production than that derived from iron stores [9,48]. While transferrin receptor (TFR1) in the bone marrow, blood and spleen of rats increased about 4-fold [51], serum diferric transferrin was significantly reduced [52] in PHZ-treated rats. The initial decrease in diferric transferrin preceded a decrease in hepcidin expression (discussed later) and was thereafter elevated on days 6 and 7 of PHZ treatment [52].

3.2. Splenic and hepatic iron metabolism in PHZ-treated animals

Extramedullary haemopoiesis and increased erythropagocytosis are features exhibited in the spleen during acute haemolysis induced by PHZ. In consequence, morphological changes include splenomegaly and congestion of haemosiderin deposits [46]. Catabolism of haemolysed red blood cells is associated with increased expression and activity of haem oxygenase 1 [53] suggesting increased capacity for degradation of Hb products in the spleen. Splenic iron level was therefore increased in rats after PHZ treatment [54]. DMT1 and TFR1 mRNA expressions were increased in the spleen of PHZ-treated mice [45,55]. Increased splenic erythropagocytosis results in a net flux of iron into the circulation from haemolysed red blood cells. While the mechanism of iron exchange between macrophages and transferrin is still undefined, ferroportin mRNA increased in the spleen of PHZ-treated mice [45],
consistent with efflux of iron being mediated by this protein. Hepatic non-haem iron levels increased significantly after PHZ treatment and this was sustained 7 days after haemolysis [52]. Liver iron accumulates due to increased absorption driven by haematopoietic activity in PHZ-treated animals. Liver iron accumulates initially due to haemolysis, although later on, absorbed iron may also be diverted to the liver. Saturation of serum transferrin has been shown to preferentially divert absorbed iron to the hepatocyte [56,57]. Iron deposition in the liver could in addition to the TFR/DMT1 routes, be due to influx of non-transferrin bound iron (NTBI). Furthermore, as PHZ-induced haemolysis is stressful and produces haem-derived toxic reactants, the expressions of acute phase proteins hemopexin, haptoglobin and CD163 in the hepatocytes [58,59] and the kidneys [60,61] are increased. These organs mop up the haem-derived products from circulation. The sequestration of Hb iron in the liver of the transferrin-saturated animal is analogous to patients manifesting ineffective erythropoiesis and hepatocyte iron overload [61]. Anaemias, such as thalassaemia, sideroblastic and some dyserythropoietic anaemias, are characterized by high intestinal iron absorption most probably due to enhanced erythropoiesis. However, efficient iron recycling typifies hereditary spherocytosis with comparable iron turnover as the conditions above [44] to maintain a form of compensated haematological indices. Compensation and normality also ensue during chronic hereditary or drug-induced haemolytic anaemia (Table 1) [62].

3.3. Iron absorption and regulation during PHZ-induced haemolytic anaemia

Increased gastrointestinal absorption of iron is well documented during haemolysis in experimental animals [64,65] and humans [61]. Enhanced absorption of iron is associated with acute enlargement of the reticuloocyte pool due to stimulated erythropoiesis [22], which ultimately leads to tissue iron overload [47,54]. Increased iron absorption in the presence of elevated hepatic stores defines the importance of the erythroid drive overriding iron storage in the regulation of iron absorption [9]. Iron absorption in PHZ-treated animals increased about 2-fold 3–5 days after treatment and thereafter declined to the control level by the 7th day [52]. Both uptake and transfer processes were enhanced during this adaptation [45]. An earlier study had demonstrated both morphological and physiological adaptation of the brush border of the duodenum of PHZ-treated animals that favoured increased iron absorption [64]. This was the development of an expanded absorptive surface area coupled with an enhanced electrical driving force in the epithelia of the enterocytes. Recent studies [45,52] have further shown that iron transport genes were up-regulated when mice and rats were treated with PHZ. Moreover, immunohistochemical staining in these mice revealed no morphological changes in the duodenum but an enhanced expression of the proteins involved in iron absorption [45,52].

The time-course of iron absorption due to stimulated erythropoiesis induced by PHZ correlated significantly with the expression of Deytb, DMT1 and ferroportin. Consequently, the signal effecting increased iron absorption in this model enhanced the expression and activities of these proteins. Moreover, the time lag before the increase in iron absorption that was apparent after the administration of PHZ was the time required for the body to detect the need for more iron rather than that due to maturation, migration and modulation of iron transport genes [54]. Hepcidin, a liver synthesized antimicrobial peptide discussed above as a negative regulator of iron absorption, was actually down-regulated during haemolysis induced by PHZ [32,44,54]. The attenuation of hepcidin production when erythropoiesis is iron restricted is also evident in Trf

Table 1
Quantitative representation of Fe metabolism during chronic haemolytic condition in man (mg; adapted from [63])

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Haemolytic anaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron absorption</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>2500</td>
<td>1500</td>
</tr>
<tr>
<td>Plasma iron</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fe loss</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Body stores</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>RE, RBC destruction</td>
<td>20</td>
<td>150</td>
</tr>
<tr>
<td>Bone marrow, RBC produc</td>
<td>20</td>
<td>150</td>
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The hepatocyte is now recognized as the central control co-ordinator of the body’s iron homeostasis [71,72]. Rat liver transplantation studies pointed to this function [73] but the determining factor was elusive at the time. Unlike the other forms of iron overload disorders, Trf

The interplay of Hfe, TFR1, TFR2 and differe-transferrin in a signaling cascade regulating hepcidin expression has been discussed extensively by Frazer et al. [71]. The
hypothesis does not include hemojuvelin, a molecule that might be inactivated along with hepcidin expression [75,76] in juvenile haemochromatosis. Interestingly, the interaction of ferroportin and these molecules, before the discovery of hepcidin, was hypothesized as being in the macrophages and the duodenum [77]. Ferroportin (now known as the target protein of hepcidin action) and basolateral efflux of iron have indeed been speculated as rate limiting in the regulation of intestinal absorption of iron [78–80] however this only seems to be the case in certain physiological states [81]. A decrease in dffic ferr transferrin preceded the decrease in hepatic hepcidin level when erythropoietic demands were enhanced by PHZ-induced haemolysis [52]. Thus iron deficiency was sensed in the absence of dffic ferr transferrin, Hfe is proposed to bind to TFR1, and its signal to induce hepatic hepcidin expression thus abrogated [82].

The roles of serum transferrin and transferrin saturation in the regulation of iron absorption have earlier been investigated [83–85] even though all attention, again, was focused on the gastrointestinal tract. This is particularly relevant to Trf hyp/hpx mice model and it is discussed further in the second part of this review. Stimulated haemopoietic activity is enhanced in PHZ-treated animals by increased EPO production [44] and EPO injection has been shown to correlate indirectly with decreased hepatic hepcidin expression [86]. It is known that EPO does not exert a direct effect on the intestine [25] and its effect on hepcidin expression in the hepatocyte is also not known [87]. EPO is however a potent component of the erythropoietic regulator of intestinal absorption of iron which represses hepatic hepcidin expression to cause enhanced iron absorption in the gut. The independence of this regulator from iron stores was also confirmed in PHZ-treated mice, as the inhibitory effect of PHZ on hepatic hepcidin expression was not obliterated when these mice were loaded with iron dextran [32]. The complexities and the detailed molecular regulation of hepcidin expression in drug-induced haemolytic anaemia and other models of iron absorption await elucidation.

4. Hypotransferrinaemic mice—the mouse model with highest sustained iron absorption and highest recorded spontaneous tissue iron loading

The hypotransferrinaemic mouse (trf hyp/hpx ), originally characterized by Bernstein [88], has been a very useful animal model for the study of iron metabolism. The mice have a point mutation in a transferrin mRNA splice donor site [89] that leads to incorrect splicing [90] with almost complete loss of transferrin synthesis [89]. Homozygotes have less than 2% of normal transferrin levels [88] with a resultant severe hypochromic anaemia and concomitant parenchymal iron overload with contrasting low iron levels in spleen and bone marrow [88,91,92]. The pattern of tissue iron overload resembles the haemochromatosis phenotype but the latter does not have concomitant anaemia [88,92]. In mice, the homozygous hypotransferrinaemic phenotype has much higher levels of tissue and body iron loading than is seen with homozygous Hfe knockout (KO) or the C282Y mutation (Fig. 2). On the other hand, heterozygous trfhpx/hpx mice are effectively normal. Part of the explanation for the mildness of the trf hyp/hpx phenotype is a partial compensation of transferrin turnover such that the steady state level of transferrin in the heterozygote is 75% of normal [26,93].

Trf hyp/hpx mice die within 2 weeks of birth unless life-sparing injections of transferrin (or plasma, or serum) are given [88]. The mice can live for near normal life spans if given approx. 1 mg of transferrin per week [88,94]. This is sufficient to maintain transferrin levels at <2% of normal for most of their life, yet they live with a reduced body size, cardiomegaly, splenomegaly, increased iron stores and a chronic anaemia [88,94]. If transferrin injections are stopped, the mice die with massive iron overload after a few months [89].

The phenotype of the mice illustrates several features of iron metabolism. The almost complete absence of transferrin results in drastically reduced incorporation of iron into red cells, demonstrating the high level of dependence of these cells on the transferrin-TFR1-DMT1 iron uptake system. In contrast, some other tissues have near normal iron levels (e.g., brain, [95]), while a select group of cells are massively iron overloaded.

![Graph showing total body iron increase in mice with iron metabolism gene mutations](image)
especially hepatocytes, pancreatic acinar cells and, to a lesser extent, muscle cells [94]. This latter finding suggests that many tissues, especially those that develop iron overload [96–98] can take up iron via NTBI pathways.

Trfpx/hpx mice have the highest reported level of iron absorption of the hereditary mouse models, presumably due to the complete absence of hepcidin expression ([33,99], Fig. 3). Even hepcidin knock-out mice do not seem to reach the levels of tissue iron seen in trfpx/hpx mice ([14,94], Fig. 2), although no direct, background strain and diet-matched comparison has been made. A recent report of Hemojuvelin KO mice (Hjv−/−, [100]) suggests they match the trfpx/hpx mouse in iron loading, although little data were reported on body weights and haemoglobin levels in these mice except that they appeared normal.

This extremely high level of absorption in trfpx/hpx mice was used by McKie et al. to identify several genes implicated in iron absorption including Dcytb [101], Ireg1 (ferroportin, [102]) and HCP1 [103].

Fig. 3 shows the dramatic decrease in liver hepcidin mRNA and Fig. 4 the increase in iron absorption genes in trfpx/hpx mice. Comparison of trfpx/hpx mice with thalassaemic mice and erythropoietin (EPO) deficient mice illustrates the complexity of iron absorption regulation. EPO deficient mice have similar haemoglobin levels (without the enhanced erythropoiesis) to the trfpx/hpx mouse [104], yet iron absorption is increased many times more in the trfpx/hpx mouse. Clearly, factors other than anaemia are important. The thalassaemic mice additionally have increased plasma iron turnover, considered a stimulant for iron absorption, while EPO-deficient mice do not. Thalassaemic mice, however, still have far lower iron absorption rates than those seen in trfpx/hpx mice [99,105]. The anaemia in beta thalassaemic mice is less severe than trfpx/hpx mice, however, anaemia alone in mice does not increase iron absorption greatly. Hepcidin levels are decreased in beta thalassaemic mice [106]. Hepcidin levels have not yet been studied in EPO-deficient mice but a similar inherited mild anaemia in mice without enhancement of erythroid iron uptake has been studied recently (haemoglobin deficit mice, hbd [107]). Like EPO-deficient mice, hbd mice have raised plasma iron but no increase in net absorption of iron. Hbd mice had a small increase in hepcidin levels, in line with the increased plasma iron [107] and relatively unchanged iron absorption.

As discussed above iron absorption is regulated by iron stores, erythropoietin and hypoxia. The latter two alone seem to be unable to enhance iron absorption to the levels seen in trfpx/hpx mice where iron stores are increased (thus tending to decrease iron absorption) [105], thus it seems another factor is important in trfpx/hpx mice.

Perhaps, the iron deficiency in the bone marrow of trfpx/hpx mice is important. This raises the question of how this is communicated from bone marrow to the liver, where hepcidin levels are so low, despite the high iron stores there. Another obvious factor is the absence of circulating diferric transferrin. This has been suggested to be a factor that regulates hepcidin synthesis, possible acting partially via TFR2, absence of which seems to lead to iron overload [71,108,109].

In 1999, we investigated the role of transferrin in regulating iron absorption in trfpx/hpx mice by correcting the chronic anaemia with daily transferrin injections for three weeks from weaning [85]. This was an attempt to ‘normalise’ the trfpx/hpx mice as much as possible. The transferrin injections were then stopped allowing transferrin to disappear from the circulation (the half life of transferrin in mice is only about 1 day). Iron absorption was measured before and 7 days after cessation of the injections. It was found that haemoglobin levels had not changed as the half life of red cells in mice is 10 days or more [110]. Iron stores were also unchanged, yet iron absorption was significantly increased by the reduced levels of transferrin. This study preceded the discovery of hepcidin hence no measurements of that were made but presumably levels of this key regulator had fallen as transferrin levels fell.
Can we reproduce the enhanced iron absorption seen in trfhpx/hpx mice with experimental treatments of genetically normal mice? Experimentally enhanced erythropoiesis by chemical haemolysis, as described in detail above, produces only mild increases in iron absorption. Hypoxia can, in some mouse strains, produce a large response [21], however hypotransferrinaemia has a greater effect than hypoxia when compared in the same mouse strain [26,99,105]. Iron deficiency in mice usually involves dietary iron restriction, whereas trfhpx/hpx mice have high iron absorption rates when fed diets with normal iron levels. Alteration in dietary iron level can, by itself, alter iron absorption by a local mechanism at the level of the gut [78].

All this is tantalizing evidence that hepcidin may not quite provide the complete story of systemic iron absorption regulation. Clearly, hepcidin is of major importance, as shown by comparison of HjvKO, hepcidinKO and trfhpx/hpx mice. Until further work on iron absorption in hepCidinKO mice, or a careful comparison of trfhpx/hpx and hepCidinKO or HvKO mice is performed on the same genetic background and diets, it remains possible that iron absorption is not maximally enhanced simply by loss of hepcidin. Perhaps, the absence of diferric transferrin in the plasma can also directly affect the gut. Many studies failed to provide evidence for such an effect [84], although these were all acute studies. Perhaps, there is an additional signal from the bone marrow to the gut such as amino-laevulinic acid [67,111]. Perhaps, intestinal epithelial cells can respond directly to hypoxia as well as via hepcidin. Most cells seem able to respond to hypoxia [112] and cultured epithelial cells also have a hypoxic response [113] and can regulate iron transport in response to cytokines [114,115] which are altered in hypoxia. We have also found indirect evidence for a local responsiveness to IL-6 in the duodenum of mice [116]. Finally, local responsiveness of the gut to ingested iron is also important [78] and implies a degree of local regulation of iron absorption by the gut in response to enteroctye iron levels.

In conclusion, the discovery of hepcidin and its mechanism of action has provided a potential answer to the mystery of iron absorption regulation, especially in well-studied models like Trfhpx/hpx mice and PH2-treated animals where the liver has moved to centre stage. Further careful studies can deliver a complete description of the regulatory mechanism(s).

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References
