Gut 1997; **40**: 435–439

Gut

Leading article

Regulation of intestinal non-haem iron absorption

Iron is a precious metal in biological terms, having essential roles in oxygen transport, electron transfer and as a co-factor in many enzyme systems, including DNA synthesis. The biological importance of iron is underscored by the evolution of very complex mechanisms for its acquisition, utilisation and preservation in even the most primitive organisms. These systems have evolved because bioavailability of iron is limited and also because its biological activity is potentially toxic as seen, for example, in haemochromatosis. In humans, iron loss from the body is minimal[1] and the level of iron is controlled by regulating its absorption from the intestine.[2 3] Iron in food is available in two forms – haem iron (in muscle as myoglobin and haemoglobin) and non-haem iron (in vegetables, cereals and meat). Haem iron accounts for only 10–20% of dietary iron in industrialised countries but can contribute a disproportionate 30% of the absorbed iron due to its higher bioavailability. Non-haem iron accounts for 80% of dietary iron in industrialised countries and an even greater proportion worldwide or in vegetarians.[4] For this reason it is more important from a worldwide perspective and it is also more affected by other micronutrients and by regulatory mechanisms in the intestine.[5]

Non-haem iron absorption can be considered to occur in two phases—first, the assimilation of iron from the intestinal lumen into the epithelial cell and, second, the transfer or export of the assimilated iron to plasma. Both of these phases are known to be responsive to the level of body iron stores,[6] but the intracellular mechanisms influencing these phases and the mechanism by which the enterocyte is informed of body iron requirements are poorly understood.

Here, we draw together evidence from older physiological experiments and more recent studies using cell and molecular biology techniques to speculate how this recent information may be pertinent to how iron is taken up by enterocytes, how its transfer to plasma is controlled and, finally, how information about body iron stores or requirement is conveyed to enterocytes and by what mechanism this information can influence both of these processes

Dietary iron uptake

Many studies have demonstrated that the site of absorption is limited to the duodenum and upper jejunum,[7] although the process can be extended distally in certain states.[8] Non-haem iron forms insoluble complexes readily and it is believed that its reduction to a ferrous form is essential for membrane transport to occur.[9] Experimental and clinical observations have supported a role for gastric juice HCl[10] in this reduction process. More recently, it has been shown that ascorbic acid is concentrated in gastric juice.[11] As ascorbic acid forms soluble monomeric complexes with iron that prevent polymerisation and aid iron absorption, this has given rise to the intriguing prospect that iron, like vitamin B_{12}, has a gastric intrinsic factor to aid absorption.[12] In addition to the form of iron, the absorption of dietary iron is affected and often impeded by other micronutrients such as phytates and calcium.[5 13] Conrad *et al*[14] have also proposed a role for gastric mucin in iron absorption, and the taurocholate content of bile may be an additional factor.[15]

Once in the soluble ferrous state, iron can be assimilated by both active energy-dependent mechanisms and a passive low capacity process.[16–19] Several proteins involved in these mechanisms have been identified by a variety of methods,[8 20 21] but their mechanism of action is unknown. Recently, Raja *et al*[22] reported that duodenal mucosa has an inherent ability to effect a reduction in iron and correlated its uptake in biopsy samples to this reducing capacity. It is unclear whether this activity resides in one of the recently discovered iron binding proteins. Raja *et al* also showed that the reducing activity is increased in iron deficiency anaemia and in genetic haemochromatosis, both conditions in which iron absorption is increased. However, in anaemia of chronic disease, where iron absorption is decreased,[23] the reducing capacity is comparable with controls.

From these observations one might deduce that iron absorption can be increased at the luminal border when requirements are greater (for example, in iron deficiency states) but that other mechanisms that possibly involve impeding its transfer to plasma must be operating in situations where iron absorption is to decrease such as in anaemia of chronic disease or in the iron replete or overload state.

Import and export of plasma iron

All living cells require iron for growth. The iron is delivered into cells by plasma transferrin, a serum glycoprotein, and its specific receptor which resides on the cell surfaces.[24] The location of these receptors at the basolateral membrane on enterocytes[25] suggests that they are involved in acquisition of plasma iron by the cell for its growth and metabolism.[26] This is further supported by the relative abundance of these receptors on the proliferating cells in

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the crypts which have a high requirement for iron.[27-29] The transferrin receptors (TfR) on cell membranes undergo an endocytic cycle during which the pH in the endosome falls as a result of an ATPase mechanism so that iron is released from transferrin to the cell and the endosome with apotransferrin is returned to the cell surface.[24 30] Functionally, it is thought that this receptor mechanism cannot operate in reverse to transfer iron to plasma because of the effects of pH on the affinity of transferrin and apotransferrin for endocytic processing.[31] The process by which iron is transferred to plasma has been difficult to study and little is known about it. Plasma transferrin does not seem to be necessary for the process as iron absorption is increased when transferrin is deficient or absent,[32] and even when present, some iron appears in the portal circulation as non-transferrin bound iron.[33] Although not directly involved in iron absorption, it is possible that TfR plays an indirect role by conveying information about body iron stores to the cell.

Influence of information conveyed to crypt cells about body iron stores on the intestine

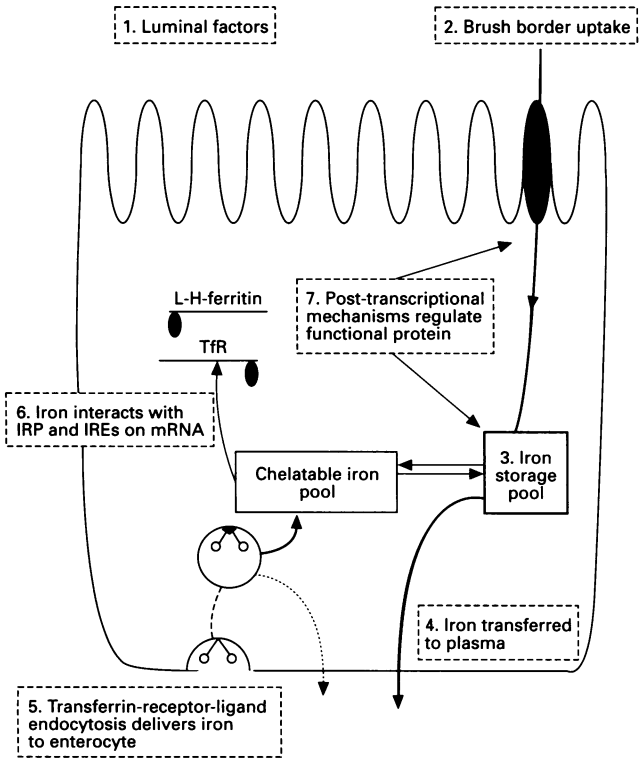
Transferrin receptor is abundantly expressed on all proliferating and growing cells putatively because of their high requirement for iron.[26 28] Thus, cells in the crypts of Lieberkuhn always express TfR and the endocytic mechanism may impart information about body iron stores based on plasma transferrin saturation. Transferrin has a single polypeptide chain organised into two domains each with a distinct iron binding site. Studies have shown that diferric transferrin has a much greater affinity for receptor-mediated endocytosis than monoferric transferrin.[27 34] Thus, the rate of delivery of iron to cells improves when body iron stores are increased, transferrin saturation is high and more diferric transferrin is circulating. Consequently, transferrin should be a good candidate for a "humoral" mediator to control iron absorption.[35] Several studies have reported the effects of transferrin saturation in relation to iron absorption from the perspective of transferrin accepting iron from intestinal cells.[36] None has given sufficient specific information to determine whether transferrin saturation is an important factor in conveying information to the intestine in such a way as to influence iron absorption. Such studies would need to allow for the lag time response in the intestine that may be occasioned by migration of crypt cells to functional sites at the tips of villi. Studies in situations where transferrin is deficient lend support to the proposition that transferrin and its receptor convey information about body iron stores. Atransferrinaemia is a rare congenital condition in which iron absorption is increased and iron overload ensues in a pattern similar to that seen in genetic haemochromatosis.[37] A similar condition occurs in the hpx mouse model where hypotransferrinaemia results in iron overload and iron absorption is increased.[38 39] This suggests that transferrin is not required for iron transfer to plasma but that it may be essential for conveying the information to enterocytes that body iron stores are replete. If transferrin is given to these patients or mice, there is evidence that iron redistribution can take place, but it is unclear whether iron absorption is controlled in this situation.[40]

Transferrin receptors seem to be regulated in enterocytes[29 41] in the same way as in other cells.[42 43] When cells are replete, TfR is down regulated to limit further uptake of plasma iron. Concomitantly, production of ferritin, the iron storage protein, is increased[41] and this is known to correlate with iron absorption.[44 45] The observation that there is a gradient of TfR expression along the crypt tip–villus axis which apparently mirrors absorptive behaviour[25 29] may give a clue to the mechanism by which enterocytes respond to information about body iron stores. To understand how this may occur, some knowledge of cellular iron metabolism is required.

Regulation of the availability of iron

Iron in free solution is toxic to biological systems and particularly to membrane structures where it has been implicated in the generation and propagation of free radicals.[46] Thus, iron is maintained in a non-toxic form bound to the polymeric protein ferritin. This is a complex protein found in all cells in even the most primitive of organisms and has an enormous capacity to store iron.[47] The ferritin polymer (FN) is made up of 24 subunits composed of either heavy (HF) or light chain (LF) ferritin. In certain tissues or in particular conditions of iron loading, one or other of these subunits may predominate in the FN shell giving rise to a family of isoferritins.[47 48] There is some evidence that isoferritins have different iron uptake or retaining properties – L-rich ferritin takes up iron more slowly but retains it more avidly than H-rich ferritin.[49] H-rich ferritin may have a higher capacity for rapid iron uptake.[48] This has given rise to the idea that H-rich ferritin may have a housekeeping function in cells where iron availability is labile, whereas L-rich ferritin primarily has a longer term storage function. The tissue distribution of isoferritin (for example, L-rich in liver; H-rich in heart or reticuloendothelial cells)[49-51] and the ability to change a tissue's isoferritin profile in response to iron excess or deficiency[49 52] are consistent with this concept.

L- and H-ferritin are encoded by different genes on separate chromosomes. Their initial transcription is coordinated and the amount of L- and H-ferritin mRNA transcribed may be set constitutively in cells according to the iron milieu in which they find themselves.[45 48 49] To respond rapidly to changes in iron availability, a post-transcriptional mechanism has evolved to allow ferritin to become rapidly available without having redundant protein in the cells.[53] This means that there is abundant ferritin mRNA in cells, the translation of which is repressed until iron is present. Thus, as cellular iron availability is increased, ferritin synthesis also increases to ensure adequate storage and "detoxifying" capacity in the cell. The molecular regulation of ferritin expression is elegantly linked to the reciprocal molecular regulation of TfR expression which controls entry of iron from plasma into most cells in the body (Figure).[54] The essence of such control is a sequence of mRNA which folds into a stem-loop structure and confers "iron-responsiveness" to protein expression.[55] These iron-responsive elements (IREs) interact with a protein (iron-responsive protein; IRP) and can result in repression of mRNA translation by impeding ribosomal attachment or enhance translation by protecting the poly-A tail against degradation according to their position in the RNA transcripts. In the presence of iron the affinity of the IRP for IREs is weakened, leading to down regulation of TfR because its mRNA is degraded and increased ferritin synthesis because "dormant" or repressed ferritin mRNA can now be translated. Other key proteins involved in iron metabolism, such as transferrin and 5'-amino-levulinate synthase, are regulated in a similar way,[56 57] and it is possible that membrane proteins involved in iron transport also respond to iron concentrations through control of IREs. This mechanism for controlling iron availability to cells from plasma probably also operates in the intestinal cell where the reciprocal expression of these proteins in relation to body iron load has also been reported.[25 29 45]



Key steps in iron absorption.
1. Luminal factors affecting iron uptake include the valence state of iron, other micronutrients such as phytate and calcium, gastric juice HCl and ascorbic acid, mucin, and bile.
2. Brush border proteins with reducing or transport properties and possibly fatty acids translocate iron to cytoplasm.
3. An intracellular iron storage pool exchanges iron as required with other cellular compartments. It is unclear whether iron derived from plasma and from the gut lumen is dealt with in the same way by this pool in enterocytes or whether iron from these pathways is kept separate. Both plasma-derived and diet-derived iron are incorporated into ferritin.
4. The mechanism for iron transfer to plasma is poorly understood. Although transferrin may normally play some role in transporting iron in the portal circulation, it is not essential for this process.
5. Iron is taken up by enterocytes from plasma by a receptor–ligand endocytic mechanism. The endosome becomes acidified by an ATPase mechanism, iron is released to the cell and apotransferrin is recycled to the plasma surface. Iron delivery is determined by density of receptor expression and iron saturation of plasma transferrin.
6. Iron supplied to cells controls the expression of proteins such as transferrin receptor and ferritin. The mRNA for these and some other iron-related proteins contain a sequence of nucleotides which form a stem-loop structure and confer iron-responsiveness to the translation of the mRNA (thus called iron-responsive elements: IRE) by binding a protein (iron-responsive protein: IRP) which seems to be a cytoplasmic aconitase.
7. The ferritin heteropolymer is composed of subunits which have different iron uptake and retaining properties. Thus, the isoferritin produced could play a major role in determining iron availability to the cell and to the transfer/export mechanism on the plasma membrane.
8. Another dimension in the control is the migration of cells from proliferative sites in the crypts to the tips of villi. It is conceivable that a constitutive level of protein, including ferritin, expression is set by transcriptional means in the crypts and modification of this profile is limited by time and post-transcriptional mechanisms when the cells are in functional positions at the tips of villi.

Control of iron absorption

Since the classic studies of Hahn *et al*[58] and Granick[59] demonstrated increased iron absorption in anaemia, which is reversible when iron stores are replete, many investigators have attempted to delineate the mechanisms that control iron absorption. The concept that has received most attention over the years is that of "mucosal block" in which intestinal cells had a storage capacity for dietary iron that was transferred to the body according to need. The candidate protein most favoured to achieve this process was ferritin.[59 60] When it was shown that ferritin synthesis continued in the intestine even in the presence of iron deficiency,[61 62] the prospect of its having a regulatory effect on absorption was largely discounted. However, elucidation of the molecular control of ferritin expression[54] may explain why ferritin synthesis occurs even in the presence of an iron deficiency state[63] and further consideration of ferritin as the candidate protein in a "mucosal block" or a retentive type mechanism is warranted. There is no direct correlation between iron absorption and serum iron or serum iron proteins although there is good correlation with mucosal iron content and body iron stores.[64–66] It is well recognised that acute changes in body iron status, whether overload or deficiency, are not reflected by changes in iron absorption for a period of two to three days.[67 68] This lag response time probably correlates with the migration time for proliferating cells in the crypts of Lieberkuhn to differentiate and migrate into functional positions on the villi.[69] Thus, the luminal epithelial cells may be pre-programmed in the crypts to respond to dietary iron challenges. It is possible that some of the pre-programming involves synthesis of proteins that have no function until the cells reach the villus tip. Some of the programming should involve the setting of a constitutive L:H ferritin transcript ratio. If body iron stores are replete and plasma transferrin is saturated, then TfR is down regulated in enterocytes early in their migration up the villi.[29] In crypt cells a constitutive ratio of L:H ferritin transcripts could be set such that when the functional cell is exposed to dietary iron two to three days later, an L-rich isoferritin may be produced[49 52] that would retain iron for longer and thereby impede its transfer to plasma. The mucosal iron would be lost when the cell desquamates as proposed by Crosby.[60] In an iron deficient state, TfR may be persistently expressed on enterocytes, constitutive ferritin transcription favours a lower L:H ratio and when those cells are challenged with dietary iron, an H-rich ferritin protein is synthesised that can give up iron easily according to need and iron transfer to plasma is less impeded. Functional enterocytes at the tips of villi have only a limited lifespan and the translational response of ferritin according to a pre-set L:H transcript ratio may therefore supersede any de novo transcription of ferritin which may take some hours to peak.[70]

Two other lines of evidence support such an hypothesis.

INFLAMMATORY DISEASES AND NITRIC OXIDE MECHANISMS

The anaemia of chronic inflammatory disease is characterised by a normocytic, normochromic blood profile with reduced serum iron, reduced erythropoiesis, raised serum ferritin, abundant iron stores in marrow, and reduced iron absorption. Experimental studies have implicated cytokines, especially interleukin 1β, tumour necrosis factor α and interferon γ in mediating this response,[23] possibly through apoferritin sequestration of iron.[71] Evidence is emerging that nitric oxide may play a central role in this process as an end-effector of cytokine activity. Nitric oxide has a strong affinity for iron and interferes with the homeostatic mechanism involving IREs on mRNA sequences,[72] resulting in increased expression of TfR and increased ferritin synthesis, thereby distorting the normal reciprocal relation between ferritin and TfR protein expression.[73] Thus, ferritin expression in the intestinal mucosa is reversibly increased at a time when iron absorption is known to be reduced,[74–76] further suggesting that ferritin is a sump or reservoir controlling iron absorption.

GENETIC HAEMOCHROMATOSIS

In this condition, iron absorption continues at a level inappropriate for the increased amount of body iron stores.[6 64] The intestinal L:H ferritin ratio is lower in haemochromatosis than would be expected,[45 77] possibly

allowing proportionately more iron to transfer to plasma. The fact that several iron proteins[29 40 45] and iron uptake mechanisms[2 64 78] are demonstrably inappropriate though still all apparently coordinately regulated in the intestine suggests that there is a common regulatory defect. One such example may involve the ATP protein which regulates acidification of endocytic vesicles containing transferrin and its receptor. The specific consequence of a defective endocytic vesicle ATPase in enterocytes, in contrast to other cell types which have altrenative means of importing iron,[31] would be to signal an apparent shortage of body iron stores resulting in the observed increased enterocyte expression of iron transport proteins and relatively reduced ferritin synthesis. The physiological result of such a defect would be akin to having no transferrin,[37 39] and indeed the pathological sequelae of atransferrinaemia are very similar to those seen in genetic haemochromatosis.[40] One could also anticipate a number of different mutations in such a protein that could cause varying degrees of functional deficit resulting in variable clinical expression of the disease[79-81] akin to the situation found with other defective ATP linked proteins such as occurs in cystic fibrosis[82] or Wilson's disease.[83]

In conclusion, it seems that the mechanisms of assimilating and harnessing iron that have evolved over millions of years in primitive bacterial cells may operate in tandem to control iron absorption in the intestine. The uptake pump has a background activity level which can be increased if more iron is required. A reservoir or sump capacity for iron may reside in the molecular regulation of ferritin which is responsive to body iron stores and can operate to impede iron transfer to plasma. Cytokines and nitric oxide may operate through this system to reduce the availability of iron during inflammation. The three dimensional architecture of the intestinal villus adds a further degree of complexity, perhaps contributing a built-in lag time to achieve steady state control of iron absorption. Recent observations in these areas can be used to reinterpret previous physiological observations and suggest exciting possibilities for further study.

Note added in proof

Since this article was written, a Cys-282-Tyr mutation in the HLA H region of chromosome 6 has been discovered in association with genetic haemochromatosis.[84] The precise role of HLA H in iron metabolism is not known. It encodes a membrane protein thought to be a binding site for β_2-microglobulin and, interestingly, a β_2-microglobulin-aemia, like atransferrinaemia, has been associated with iron overload in mice.[85] Transferrin and its receptor are endocytosed in clathrin-coated pits which are known to interact with endocytosed vessicles containing HLA proteins.[86] A trafficking fault involving β_2-microglobulin or transferrin could have the same consequences as the faulty ATP protein which we have used as an example earlier and result in excessive and inappropriate iron absorption because information about body iron stores would not be conveyed to the mechanisms controlling its absorption.

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