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The molecular basis of copper and iron interactions

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The intimate relationship between Fe and Cu in human nutrition has been recognised for many years. The best-characterised link is provided by caeruloplasmin, a multiCu-binding protein that acts as a serum ferrioxidase and is essential for the mobilisation of Fe from storage tissues. Decreased Cu status has been shown to reduce holo-caeruloplasmin production and impair ferrioxidase activity, leading, in a number of cases, to decreased tissue Fe release and the generation of anaemia that is responsive to dietary supplementation with Cu but not Fe. Dietary Fe absorption also requires the presence of a multiCu ferrioxidase. Hephaestin, a caeruloplasmin homologue, works in concert with the IREG1 transporter to permit Fe efflux from enterocytes for loading onto transferrin. The essential role of hephaestin in this process has been recognised from studies in the sex-linked anaemic (*sla*) mouse, in which Fe efflux is markedly impaired as a result of a mutation in the hephaestin gene that results in a truncated and non-functional version of the protein. There is emerging evidence that a number of other components of the intestinal Fe transport pathway are also Cu sensitive. Divalent metal transporter 1 (DMT1), the Fe transporter located at the apical membrane of enterocytes, is also a physiologically-relevant Cu transporter, suggesting that these two metals may compete with each other for uptake into the duodenal enterocytes. Furthermore, expression of both DMT1 and the basolateral Fe-efflux transporter IREG1 can be regulated by Cu, suggesting that the Fe–Cu relationship may be more complex than first thought.

Cu: Fe: Anaemia: Caeruloplasmin: DMT1

Copper status and body iron metabolism

The intimate relationship between Cu and Fe metabolism has been recognised for many years. In fact, Cu was identified as an 'anti-anaemic' factor as far back as 1928, when studies demonstrated that Cu could facilitate Hb formation (Hart *et al.* 1928). Indeed, the Cu–Fe connection had been acknowledged for at least 100 years previous to this finding (for an extensive review, see Fox, 2003), but it is only relatively recently that the molecular basis for the biological interactions between these two metals has begun to be understood. The discovery of caeruloplasmin, the Cu-dependent ferrioxidase, formed the initial bridge between Fe utilisation and Cu status. However, in recent years it has become apparent that Cu–Fe interactions occur at the dietary and intestinal level. The molecular mechanisms underlying these interactions suggest that the Cu–Fe relationship may be more complex than it was first thought.

Iron utilisation

Body Fe content is 3–5 g (approximately 50 mg/kg body weight), of which approximately 70% is present in the circulating erythrocytes, 20% is stored as ferritin and haemosiderin in the liver, 5% is incorporated into myoglobin in muscle and 5% is bound or utilised by various enzymes (e.g. the cytochromes). Fe delivered to the tissues for metabolic utilisation or storage is carried in the circulation by transferrin, which binds to specific receptors on the cell surface allowing transferrin-bound Fe to be internalised via endocytosis. In the erythroid precursor newly-acquired Fe is delivered to the mitochondria for incorporation into haem, whereas in the liver Fe is directed for long-term storage in the cytosolic protein ferritin (for review, see Andrews, 1999, 2000).

The majority of metabolic Fe turnover in the body is accounted for by the continual synthesis and destruction of erythrocytes. The typical lifespan of an erythrocyte is

Abbreviations: Ctr, Cu transporter 1; DMT1, divalent metal transporter; IRE, Fe-responsive element; *sla*, sex-linked anaemia.

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120 d. After this time period, senescent erythrocytes are engulfed by cells of the reticulo-endothelial system (a combination of splenic macrophages and the Kupffer cells in the liver) and the Fe contained within Hb is recovered by the action of haem oxygenase. This liberated Fe can be either re-utilised for new erythrocyte production or can be delivered to the liver for long-term storage. From the total body load of 3–5 g only approximately 1 mg Fe/d is lost through blood loss, desquamation of cells lining the gastro-intestinal and urinary tracts, and skin (there are no defined excretory mechanisms for the disposal of excess body Fe). Thus, to maintain normal homeostatic Fe balance dietary Fe absorption must match endogenous Fe losses.

Copper utilisation

Cu released from the enterocyte travels in the portal blood, bound mainly to albumin and histidine, to the liver. Newly-acquired Cu is rapidly targetted towards a number of Cu-dependent enzymes through the action of several intracellular chaperone proteins (for review, see Puig & Thiele, 2002). Three of these chaperones have been well characterised and are known to distribute Cu to distinct cellular compartments. Cu chaperone for superoxide dismutase 1 delivers Cu for incorporation into the cytoplasmic Cu–Zn-dependent superoxide dismutase, an essential component of the cellular antioxidant protection network. Cyclooxygenase 17 transports Cu specifically to the mitochondria for insertion into the integral membrane enzyme cytochrome c oxidase. Human ATX1 homologue facilitates the movement of Cu into the secretory transgolgi network, where it can bind to the Cu-transporting ATPases (ATP7A and ATP7B, the proteins mutated in Menkes disease and Wilson disease respectively), or can be incorporated into a number of other cupro-proteins, including the blood clotting factors V and VIII, tyrosinase and lysyl oxidase. In the liver a major proportion of the 'new' Cu is loaded onto caeruloplasmin (via a human ATX1 homologue/ATP7B-dependent process) before its release into the systemic circulation. Interestingly, while caeruloplasmin-bound Cu accounts for approximately 95% of the total serum Cu, it appears that caeruloplasmin is not the Cu transport protein (in the same way that transferrin transports Fe) employed for delivery of Cu to the tissues for metabolic purposes, since acaeruloplasminaemic patients (who have low levels or the absence of serum caeruloplasmin) have a normal tissue Cu content (Miyajima *et al.* 1987; Harris *et al.* 1998).

Unlike Fe, there is no physiological store of Cu and body levels are therefore maintained by balancing dietary absorption, distribution and utilisation, with biliary excretion of excess Cu. The liver is the main organ involved in the redistribution of Cu to various tissues for incorporation into the cupro-proteins and enzymes. In addition, the liver also controls the excretion of excess Cu into the bile. In Wilson disease, patients accumulate Cu within the liver and also exhibit an increase in the formation of apo-caeruloplasmin (i.e. caeruloplasmin lacking Cu). Thus, the pathology of this disease illustrates the central role of the normal Wilson ATPase (ATP7B) in regulating body Cu metabolism, delivering Cu to caeruloplasmin so that it can perform its essential metabolic function (see p. 565) and targetting excess Cu to the biliary canalicular membrane for expulsion from the body in the bile (Mercer, 2001).

The role of caeruloplasmin in iron metabolism

Anaemias resulting from Cu or Fe deficiency display remarkably similar haematological features (Smith & Medlicott, 1944; Cartwright *et al.* 1956), leading to the suggestion that a common pathway exists in the aetiology of these diseases. At an early stage it was suggested that the common factor in disease progression is a Cu-dependent catalytic process. The search for Cu-stimulated enzymic activity in serum has ultimately led to the isolation of a multiCu-binding protein with serum oxidase activity termed caeruloplasmin (Holmberg & Laurell, 1948). Caeruloplasmin has subsequently been shown to act as a ferrioxidase, converting Fe^{2+} to Fe^{3+} (Curzon & O'Reilly, 1960), and to increase the rate of loading of Fe onto transferrin (Osaki *et al.* 1966). Furthermore, the addition of caeruloplasmin (and apo-transferrin) to perfused liver preparations markedly stimulates Fe efflux, suggesting that caeruloplasmin is a crucial factor for the mobilisation of Fe from the body stores for its metabolic utilisation (Osaki & Johnson, 1969). More recently, the key role of caeruloplasmin in Fe metabolism has been confirmed in studies on human patients and mice displaying disrupted caeruloplasmin production. Acaeruloplasminaemia is an autosomal recessive disorder that results in imbalances in body Fe metabolism, with symptoms that include high serum ferritin (indicative of high tissue Fe levels) and mild anaemia (Miyajima *et al.* 1987). The lack of caeruloplasmin as the cause of these symptoms has been confirmed in a knock-out mouse model of the human disease (Harris *et al.* 1999). Interestingly, infusion of caeruloplasmin into knock-out mice induces the release of Fe from the storage tissues.

It appears that this link between Cu and Fe is not limited to man, but is also evident in lower eukaryotic species. Genetic studies of Fe metabolism in the yeast *Saccharomyces cerevisiae* have shown that the Cu-binding protein Fet3, which has sequence homology to caeruloplasmin, is required for high-affinity Fe uptake (Askwith *et al.* 1994; Dancis *et al.* 1994). Like caeruloplasmin, Fet3 has ferrioxidase activity, suggesting that oxidation and reduction of Fe are crucial to its movement across biological membranes.

Diet–gene interactions: regulation of intestinal iron and copper absorption

There is good evidence for a role for Cu in intestinal Fe absorption (Fig. 1). Studies in Cu-deficient animals have revealed that while uptake of Fe appears normal, efflux from the enterocytes is impaired (Lee *et al.* 1968). This decrease in the ability of the intestinal epithelium to release Fe acquired from the diet is not linked to the reduced ferrioxidase activity of caeruloplasmin associated with Cu deficiency, since Fe transport is not influenced by the addition of caeruloplasmin to intestinal preparations from Cu-deficient rats (Coppen & Davies, 1988). Furthermore,

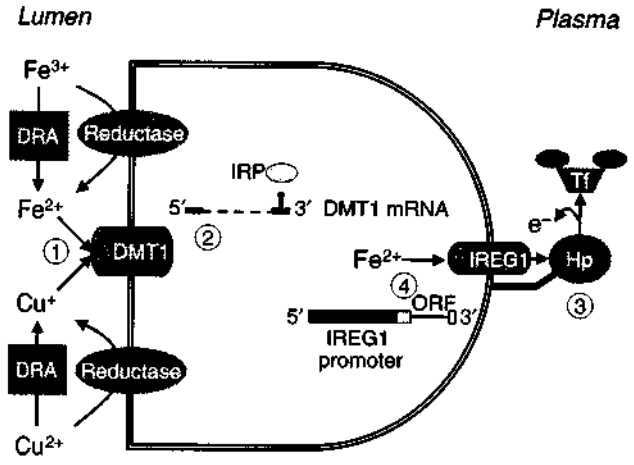

Fig. 1. Copper–iron interactions in the intestine. There are several potential mechanisms by which copper can alter the intestinal absorption of iron. (1) Recent data suggest that copper and iron compete for uptake into duodenal enterocytes via divalent metal transporter (DMT1). (2) Copper specifically regulates the expression of the iron-responsive element (IRE)-containing isoform of DMT1, possibly by modulating the RNA-binding activity of cytosolic iron regulatory protein (IRP). (3) Efflux of iron out of enterocytes is dependent on the presence of hephaestin, a caeruloplasmin homologue that acts as a multicopper ferrioxidase to facilitate the loading of iron onto transferrin. (4) Copper exposure increases the RNA and protein expression of the efflux transporter IREG1 with a concomitant increase in iron export from the cell. IREG1 regulation may occur at the level of the IREG1 promoter through interactions with an as yet uncharacterised copper-dependent transcription factor. Tf, transferrin; Hp, hephaestin; DRA, dietary reducing agents; ORF, open reading frame.

no impairment of intestinal Fe transport is evident in caeruloplasmin knock-out mice (Harris *et al.* 1999).

Mechanisms involved in dietary iron absorption

Most Western diets contain a mixture of haem-Fe (found exclusively in animal tissues) and non-haem-Fe (found extensively in cereals and vegetables, but also in meat). Haem-Fe accounts for approximately 5–10% of the daily Fe intake in industrialised countries (Hallberg, 1981), whereas in vegetarian diets and in developing countries the haem-Fe intake is negligible. The main form of Fe in all diets is non-haem-Fe. Both haem- and non-haem-Fe are absorbed in the duodenum (the proximal region of the small intestine), through independent mechanisms. The processes involved in the uptake of haem are not clearly understood, but it is thought to be absorbed intact through an uncharacterised membrane transporter. Inside the enterocyte the Fe contained with the haem-porphyrin ring is excised by the action of haem oxygenase and it enters a common pool along with the non-haem-Fe.

Dietary non-haem-Fe is largely present in its less soluble and non-absorbable Fe^{3+} form and must therefore be reduced to Fe^{2+} before it becomes bioavailable. This reduction is achieved by the actions of both the dietary reducing agents (e.g. ascorbic acid) and the gut's endogenous reducing activity in the form of a recently-characterised ferric reductase duodenal cytochrome b that resides on the apical membrane of duodenal enterocytes (McKie *et al.* 2001). Fe^{2+} generated by these reducing mechanisms can be transported into the cell by divalent metal transporter 1 (DMT1; Fleming *et al.* 1997; Gunshin *et al.* 1997), which is also present on the luminal membrane of duodenal enterocytes (Trinder *et al.* 2000). Uptake of Fe^{2+} by this transporter is driven by an inwardly-directed H^+ gradient (Gunshin *et al.* 1997; Tandy *et al.* 2000).

At this stage there are two possible fates for the absorbed dietary Fe. If the body stores are adequate, Fe can be re-oxidised to Fe^{3+} and stored in the enterocytes as ferritin. Fe stored as ferritin is lost into the intestinal lumen when the enterocytes are sloughed off at the villus tip and may exit the body in the faeces. If there is a metabolic requirement for Fe, it will enter a labile intracellular pool and be processed for transport out of the cell via a basolateral membrane export protein known variably as IREG1, ferroportin 1 or MTP1 (Abboud & Haile, 2000; Donovan *et al.* 2000; McKie *et al.* 2000). Fe^{2+} leaving the enterocyte is immediately oxidised to Fe^{3+} by a ferrioxidase, hephaestin (Vulpe *et al.* 1999), and loaded onto transferrin for onward transport in the blood. Each transferrin molecule has the capacity to carry two Fe^{3+}.

Mechanisms involved in dietary copper absorption

The human diet is thought to provide between 0·6 and 1·6 mg Cu/d, most of which is highly bioavailable (≤70% is absorbed; Linder & Hazegh-Azam, 1996). Dietary Cu is thought to be largely present as Cu^{2+}. However, Cu is a redox metal that can rapidly change its oxidation state between +1 and +2, and the reducing environment of the small intestinal lumen, together with endogenous copper reductase activity (Knopfel & Solioz, 2002) on the apical surface of enterocytes, favours the existence of the Cu^+ state. Interestingly, recent data have confirmed that Cu^+ is likely to be the species transported (Arredondo *et al.* 2003; Tennant *et al.* 2004). The nature of the Cu uptake pathway is still open to debate, but two candidate transporters exist in enterocytes, human Cu transporter 1 (Ctr1; homologous to a family of yeast Cu transporters) and DMT1 (for review, see Sharp, 2003).

Absorbed Cu is immediately bound by a number of intracellular chaperones that direct Cu to specific cellular sites. In order for Cu to be released from enterocytes one of these chaperones, human ATX1 homologue, delivers Cu to the transgolgi network where it is loaded onto the Menkes ATPase (ATP7A protein). Cu-loaded Menkes ATPase translocates to the basolateral membrane of the enterocyte, releasing its Cu into the portal circulation where it is bound by histidine and albumin for delivery to the liver (Peña *et al.* 1999).

The role of hephaestin in intestinal iron efflux

The understanding of the link between intestinal Fe absorption and Cu status has been greatly enhanced by studies carried out in sex-linked anaemic (*sla*) mice. The *sla* phenotype is characterised by normal Fe absorption from the diet but defective transfer of Fe into the plasma. The chromosomal region containing the *sla* locus has

subsequently been mapped (Anderson *et al.* 1998) and a candidate gene identified that is mutated in the *sla* mice. The gene encodes the protein hephaestin (Vulpe *et al.* 1999), a caeruloplasmin homologue that is widely expressed in intestinal tissue (Vulpe *et al.* 1999; Frazer *et al.* 2001; Rolfs *et al.* 2002). Recent studies have revealed that hephaestin exhibits marked ferrioxidase activity (Attieh *et al.* 2002) and that the mutation present in the *sla* mice would lead to protein misfolding and reduced ferrioxidase activity (Syed *et al.* 2002). It is unclear whether this ferrioxidase activity represents the main or the only mode of action of hephaestin in modulating Fe absorption. The initial predictions were that hephaestin would interact with IREG1 at the basolateral membrane to oxidise Fe, leaving the enterocytes for loading onto transferrin. However, recent immunohistochemical studies have cast some doubt on this hypothesis, demonstrating that hephaestin is localised largely within intracellular structures (Frazer *et al.* 2001).

Copper-dependent regulation of intestinal metal transporter expression

Emerging evidence suggests that hephaestin is not the only level at which intestinal Fe absorption can be regulated by Cu status. In Cu-deficient rats there is a decrease in ferritin protein levels in enterocytes that leads to a reduced mucosal non-haem-Fe content (Thomas & Oates, 2003). In addition, in Caco-2 cells, a well-established model of polarised intestinal epithelial cells, induction of Cu deficiency stimulates Fe uptake across the apical membrane (Zerounian & Linder, 2002). This finding is in contrast to those of previous animal studies, which show no effect of Cu deficiency on the uptake step in Fe absorption (Lee *et al.* 1968). Interestingly, when Fe deficiency is induced in Caco-2 cells Cu uptake is increased (Linder *et al.* 2003) and, furthermore, when these cells are exposed to high Cu levels Fe uptake is markedly decreased (Tennant *et al.* 2002), suggesting that the absorption of these two metals may be closely related. Subsequent studies have shown that there is direct competition between Cu and Fe for uptake across the apical membrane (Tandy *et al.* 2000; Tennant *et al.* 2002; Arredondo *et al.* 2003). From the evidence available, therefore, it seems reasonable to suggest that dietary Cu and Fe utilise a common uptake pathway to enter intestinal epithelial cells. However, the nature of this common uptake mechanism is the subject of some debate. Two possible Cu transport mechanisms have been identified in intestinal cells, human Ctr1 (Lee *et al.* 2000) and DMT1 (Gunshin *et al.* 1997), but the relative roles of these two transport proteins in overall Cu transport are unclear. Cu absorption and excretion are tightly regulated to maintain a relatively constant body Cu content (Turnlund *et al.* 1989, 1998). In light of these findings a number of research groups have studied the effects of Cu loading or deficiency on the expression of Ctr1 and DMT1 in various model systems.

Human Ctr1 belongs to a family of high-affinity Cu transporters found in a diverse range of organisms from mammals to yeast and plants (for review, see Sharp, 2003). In yeast Ctr1 is tightly regulated at the transcriptional level by the Cu content of the local environment (Dancis *et al.* 1994). However, the mammalian homologues are not regulated following dietary Cu restriction in rats (Lee *et al.* 2000) or Cu loading in human Caco-2 cells (Tennant *et al.* 2002). Furthermore, endogenous Ctr1 protein expressed at the plasma membrane of Caco-2 cells (it is unclear whether it is localised to the apical or basolateral membrane) is not modified following exposure to Cu (Klomp *et al.* 2002), although there is some evidence, derived from studies on transfected cell lines, for Cu-dependent Ctr1 protein trafficking between the plasma membrane and intracellular compartments (Petris *et al.* 2003). Interestingly, generation of Ctr1 knock-out mice has revealed that in heterozygous animals (homozygous Ctr1 deletion is lethal) brain and splenic Cu levels are reduced by at least 50% whereas gut, liver and kidney Cu levels are not different from those of wild-type control animals (Kuo *et al.* 2001; Lee *et al.* 2001). Taken together, these findings suggest that Ctr1 may not be the major intestinal Cu transporter, given how tightly absorption is regulated. In addition, there is no evidence for Cu–Fe interactions via Ctr1; only Ag, another monovalent metal, competes markedly with Cu for uptake via this transporter (Lee *et al.* 2002).

What is the role of DMT1 in intestinal Cu absorption? Recent work highlights the existence of competition between Cu and Fe for transport via DMT1 (Tandy *et al.* 2000; Tennant *et al.* 2002; Arredondo *et al.* 2003). Moreover, elegant studies using antisense technology to decrease endogenous DMT1 expression in Caco-2 cells reveal a concomitant decrease in both Fe and Cu uptake (Arredondo *et al.* 2003). If DMT1 is a physiologically-relevant Cu transporter it could be predicted that its expression should be modified by dietary Cu load. In Cu-deficient rats there is no change in DMT1 expression (Thomas & Oates, 2003). However, when Caco-2 cells are exposed to high Cu levels DMT1 protein and mRNA expression are markedly decreased (Tennant *et al.* 2002). Interestingly, in these studies the effects of Cu on DMT1 expression are restricted to the Fe-responsive element (IRE)-containing isoforms. This pattern of expression is identical with that observed following treatment of these cells with Fe (Yamaji *et al.* 2002), adding weight to the hypothesis that DMT1 is the major intestinal uptake transporter for both Cu and Fe and, moreover, suggesting that DMT1 regulation by these metals may occur via a common mechanism. Furthermore, it would appear that the molecular information required for these metal-mediated effects on DMT1 must reside at the level of the IRE in the 3′ untranslated region, since the IRE and non-IRE variants contain the same 5′ promoter region (Lee *et al.* 1998). Recent data, demonstrating that Cu (and several other divalent metals) can decrease Fe regulatory protein–IRE binding, possibly by replacing the labile fourth position Fe in the Fe–S cluster of Fe regulatory protein 1, support this hypothesis (Oshiro *et al.* 2002).

Interestingly, in Caco-2 cells treated with Cu the efflux of Fe from the cell into the basolateral medium is increased, and this increase is paralleled by an increase in IREG1 protein and mRNA expression (Tennant *et al.* 2002). Based on these studies it is believed that the following

coordinate events may occur at the apical and basolateral surfaces of intestinal epithelial cells to permit regulated absorption of Cu without unduly impairing Fe transport. At the apical surface there is direct competition between Cu and Fe for transport via DMT1. Furthermore, when dietary Cu levels are elevated the expression of the IRE-containing isoform of DMT1 is decreased. The combined effect of these first two stages is to decrease both Fe and Cu uptake into the enterocyte. In order not to compromise Fe status it is believed that basolateral Fe efflux is up regulated. IREG1 levels are increased by exposure of cells to high Cu (possibly as a result of transcriptional regulation of the gene) leading to increased efflux of Fe from the cell. Importantly, when total transepithelial Fe flux is measured in these studies there is no difference between Cu-treated and control cells, indicating that this basolateral step may be part of a homeostatic mechanism to ensure an adequate supply of Fe for body metabolism.

Public health issues concerning copper and iron

The available experimental evidence suggests that there is a close relationship between the metabolic roles of Cu and Fe in man (for reviews, see also Fairweather-Tait, 2004; Gambling, 2004). There are clear public health issues associated with imbalances in the nutritional supply of Fe, since Fe-deficiency anaemia is the most common nutritional disorder, affecting up to two billion of the population worldwide. In the UK alone it is estimated that the annual cost to the National Health Service of Fe deficiency is £25×10^6. In contrast, there is little evidence for major health problems associated with dietary Cu deficiency or overload. Only a few cases of chronic Cu deficiency have been cited in the literature since the first reported incidence (Cordano *et al.* 1964). Most recent cases have been associated with chronic malnutrition, and occasionally Cu deficiency has been seen in infants fed a cow's milk diet (Levy *et al.* 1985). Dietary Cu overload is not observed in the general population because of the tightly-regulated relationship between dietary absorption and biliary excretion of excess body Cu. Imbalance in Cu status is more commonly seen (although the incidence is still rare) in the inborn errors of Cu metabolism, Menkes disease (an X-linked recessive disorder that results in body Cu deficiency and affects one in 200 000 live births) and Wilson disease (an autosomal recessive disease with a frequency of between one in 35 000 and one in 100 000 that leads to Cu loading of the liver and brain).

More important from a public health perspective are marginal changes in Cu status. Typical Western diets provide between 0·6 and 1·6 mg Cu daily (Linder & Hazeg-Azam, 1996). The UK reference nutrient intake for Cu is 1·2 mg/d (Department of Health, 1991), suggesting that in many cases the diet supplies inadequate amounts of Cu and that marginal Cu deficiency may be prevalent in the UK population. In light of the plethora of Cu-dependent processes in the body, it is clear that a better understanding of Cu status is important; however, the assessment of marginal Cu deficiency remains extremely problematic because of the lack of suitable biomarkers (Milne, 1998). Changes in the most commonly measured variables, i.e. plasma Cu and caeruloplasmin levels, are only observed in chronic Cu deficiency, and both can vary independently of status as they are responsive to acute-phase infection. It is unclear whether in the general population marginal Cu deficiency would alter the ferrioxidase activity of caeruloplasmin sufficiently to have a deleterious effect on Fe mobilisation for utilisation in erythrocyte synthesis. However, in population groups predisposed to the development of Fe deficiency for dietary or other reasons (e.g. teenage females) even a minor reduction in caeruloplasmin activity may be a contributing factor to the progressive development of the Fe-deficient state.

To gain a better understanding of body Cu status a number of other potential biomarkers have been assessed including Cu–Zn superoxide dismutase, whose activity is reduced by severe Cu restriction but also varies with exercise. In addition, cytochrome c oxidase activity in platelets and leucocytes is relatively sensitive to changes in Cu status, but the measurements are laborious and not suitable for large epidemiological studies (Milne, 1998). Recent interest has focused on changes in the activity of other Cu-dependent enzymes, including peptidylglycine α-amidating mono-oxygenase (Prohaska *et al.* 1997; Faila, 1999) and plasma diamine oxidase (Kehoe *et al.* 2000). In addition, it has been suggested that various immune system markers may also act as physiological indicators of marginal Cu status (Bonham *et al.* 2002). These 'new' biomarkers require further evaluation, but it is possible that one or more of them may hold the key to unravelling the mysteries of body Cu status.

Could the relative levels of Fe and Cu in the diet contribute to the interactions observed between these two metals? At first glance this type of relationship appears unlikely, since the dietary Fe (approximately 10 mg/d) far exceeds Cu intake (≤1·6 mg/d). However, on close inspection, because Cu is vastly more bioavailable than Fe (70% for Cu *v.* 10% for Fe), absorption of these metals is essentially the same, i.e. 1 mg/d. Given the evidence from cell and molecular studies indicating that Fe and Cu employ the same transport mechanism to access enterocytes, i.e. DMT1 (Tennant *et al.* 2002; Arredondo *et al.* 2003), it is possible to envisage that increases in the dietary intake of Fe or Cu, perhaps coupled to the presence or absence of other dietary factors that alter their bioavailability, could have an important impact on the absorption of the other metal.

Conclusions

It is clear from the experimental evidence available that there is a close relationship between the biology of Cu and Fe. Cu deficiency alters body Fe metabolism via effects on the ferrioxidase activity of caeruloplasmin, a multiCu-binding protein that contains 95% of the Cu present in the serum, which is essential for Fe release from tissues. A second ferrioxidase, hephaestin, is implicated in Fe efflux from the intestine, although its full role and its regulation in response to changes in Cu status remain to be fully elucidated. Cellular and molecular data suggest that Cu and Fe do interact at the intestinal level, possibly through competition for transport into enterocytes via DMT1.

In addition, emerging evidence indicates that these nutritionally-essential dietary trace metals can regulate a number of key genes involved in intestinal metal absorption, suggesting that the special relationship between Fe and Cu may be more intimate than first believed.

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