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 multi-Cu-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 multi-Cu 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 anaemia (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...
Cu released from the enterocyte travels in the portal blood, bound mainly to albumin and histidine, to the liver. Newly-acquired Cu is rapidly targeted 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 mitochondrial 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. 1999).

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 targeting 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. Acaeruloplasminae 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 (Askhwith 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 (Copper & Davies, 1988). Furthermore,
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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 Fe intake is of non-haem-Fe. Both haem- and non-haem-Fe are 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\(^{2+}\) 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 (Ctrl1; 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
Cu uptake is increased (Linder et al. 1999). Recent studies have revealed that hephaestin is expressed in the intestine (Vulpe et al. 2001) and that the mutation present in the sla mice would lead to protein misfolding and reduced ferroxidase activity (Syed et al. 2002). It is unclear whether this ferroxidase 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 (Frazier 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. 1998). 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 Ctrl1 (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 Ctrl1 and DMT1 in various model systems.

Human Ctrl1 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 Ctrl1 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 Ctrl1 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 Ctrl1 protein trafficking between the plasma membrane and intracellular compartments (Petris et al. 2003). Interestingly, generation of Ctrl1 knock-out mice has revealed that in heterozygous animals (homozygous Ctrl1 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 Ctrl1 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 Ctrl1; 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 200000 live births) and Wilson disease (an autosomal recessive disease with a frequency of between one in 35000 and one in 100000 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 vs. 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 multCu-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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References


