Risk from Exposure to Metals: Deficits and Excesses (Cu, Fe, Mn, Al, Cr, B)

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Introduction

Adequate supply of metals by the diet is essential for proper functioning of all cells and tissues. This is true, in particular, for trace elements, mostly transition metal ions, which act as cofactors of many essential enzymes. To give some examples, Mn is the prosthetic group of the water-oxidizing enzyme in photosynthesis, Fe and Cu are involved in electron transporting chains of chloroplasts and mitochondria, and are essential for the activity of oxidases and oxygenases. The evolutionary recruitment of these metals to fulfill such a role in life is based on their redox properties, which means readiness to be reduced by reductants (as the highly reduced lipid and carbohydrate substrates deriving from the diet) and then reoxidized by oxygen. Redox cycles in living organisms are regulated in such a way as to make reoxidation by oxygen usable as a source of chemical energy to be stored in ‘energy-rich’ phosphate bonds. However, redox reactivity of this kind leads to risk of damage to cell and tissues, if these metals are not properly transported from the intestine to the sites of biosynthesis of the target enzyme. In fact three potentially harmful events may take place, if biological handling of metals is deranged from the proper track:

i) interference between different metabolic routes, for instance when one metal is present in excess over the others, with respect to physiological levels;

ii) production of potentially toxic free radicals from reaction of the reduced form of a transition metal with oxygen to give intermediately reduced and very reactive species like superoxide, \( \text{O}_2^− \), hydrogen peroxide, \( \text{H}_2\text{O}_2 \), and
risk from exposure to metals: deficits and excesses (Cu, Fe, Mn, Al, Cr, B)

Table 1. Metal-induced production of oxygen radicals

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
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<tr>
<td>(AH_2 + Me^n) → AH(^+) + Me(^{n+1}) + H(^+) 1)</td>
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<tr>
<td>(AH^+ + O_2) → A + O(^2^-) + H(^+) 2)</td>
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<tr>
<td>(Me^{n+1} + O_2) → (Me^n) + O(^2^-) 3)</td>
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<tr>
<td>(O_2^- + O_2^- + 2H^+) → H(_2)O(_2) + O(_2) 4)</td>
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<tr>
<td>(Me^n + O_2^-) → (Me^{n+1}) + O(_2) 5)</td>
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<tr>
<td>(Me^{n+1} + H_2O_2) → OH(^+) + OH(^-) + Me(^n) 6)</td>
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\(AH_2\) is any divalent-reducing compound; Me is any metal ion in the oxidized (n electrons) or reduced (n + 1 electrons) state.

hydroxyl radical OH\(^-\) (Table 1: this happens when metal oxidation occurs outside the proper sites on specialized proteins where oxygen is fully reduced to water by the concerted transfer of four electrons);

iii) binding to nucleophiles such as essential cell thiols (glutathione, SH side chains of enzyme-active sites and of membrane proteins, etc.).

In view of these considerations, the borderline between safe intake and toxicity is very subtle for metals of this kind. Furthermore, different tissues have different susceptibilities depending on inherent properties of their structural components and their metabolic and functional activities. Nevertheless, all the potentially dangerous effects brought about by metals in the living systems may be included, at least in part, within the concept of oxidative stress. Oxidative stress is the consequence of an imbalance between oxidant and antioxidant factors inside a definite compartment (Table 2). This may be due to either excess of oxidants, deficiency in defenses, or intrinsic higher vulnerability of the tissue or cell implicated in the process. Metals are peculiar in this context, because they may play a major role both as source of damage (e.g. excess of a given metal) and as source of impaired defense (e.g. deficiency of a metal prosthetic group in an antioxidant enzyme). As a matter of fact, the main defense molecules are metalloenzymes, either preventing the production of oxidizing species (such as cytochrome c oxidase, which permits the concerted reduction of oxygen by four electrons to water with minimal leakage of intermediate reduction products outside the mitochondrial membrane) or intercepting already formed radicals (such as Cu, Zn or Mn-superoxide dismutase for superoxide, catalase and peroxidases for hydrogen peroxide). Beside that, proper handling or sequestering of metal ions, under either high affinity (physiological transport) or low affinity (metal overload) regimes, are vital processes in preventing metal reactions with superoxide and hydrogen peroxide to yield the indiscriminate, high potential oxidant hydroxyl ion.

Brain is particularly susceptible to injuries caused by deficits or excesses of metal ions because it is extremely sensitive to oxidative stress. This is the consequence of a variety of factors like those listed in Table 3. The
**Table 2. Oxidative stress and antioxidant defense**

*Oxidative stress* is the product of imbalance in a three-compartment system

1. Source of reactive oxygen species
2. Defense molecules
3. Target tissue

*Antioxidant defense* can operate at the level of compartment 1 (prevention), 2 (interception), or 3 (repair) with different mechanisms (left) and molecules (right)

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<tbody>
<tr>
<td>Metal sequestration and transport</td>
<td>Noncatalytic (scavengers)</td>
<td>Phospholipid-glutathione peroxidase</td>
</tr>
<tr>
<td>Four electron oxidation</td>
<td>Catalytic (enzymes)</td>
<td>DNA-repair enzymes</td>
</tr>
<tr>
<td>Metallothionein, glutathione, ferritin, transferrin, ceruloplasmin</td>
<td>Vitamin E, vitamin C, urate, glutathione, bilirubin, flavonoids</td>
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<tr>
<td>ATP, ubiquinone, ferrooxidase</td>
<td>Superoxide dismutase, catalase, peroxidases</td>
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**Table 3. Factors determining brain vulnerability to oxidative stress with reference to the three compartments of Table 2**

1. *Excess of production*
   - High rate of oxidative metabolic activity
   - Endogenous generation of oxygen radicals by specific neurochemical reactions, *e.g.*, dopamine oxidation
   - Increased deposition of transition metals with age

2. *Deficit of defense*
   - Low levels of protective antioxidant enzymes with respect to other tissues like liver or blood

3. *Propensity of the target*
   - High concentration of readily oxidizable substrate, in particular, membrane lipid polyunsaturated fatty acids
   - High ratio of membrane surface area to cytoplasmic volume
   - Neuronal anatomical network vulnerable to disruption
   - Neuronal cells are nonreplicating
object of this article will be to survey recent data on risks for brain deriving from deficits or excesses of Cu, Fe, Al, Mn, Cr and B, in the light of the considerations outlined above. The matter will be mainly organized around the more and more clearly emerging relationships between metals and major neurodegenerative diseases, like Alzheimer’s disease, Parkinson’s disease, familial amyotrophic lateral sclerosis, prion disease. Furthermore, some cases of neurodegeneration derive from well-established genetic mutations affecting metal transport and thus they represent models for the effects on brain of severe disturbances of metal metabolism. This is the case of Menkes’ and Wilson’s disease for copper, and of aceruloplasminemia and Friedreich’s ataxia for iron. Altogether, these clinical syndromes are very useful for the identification of molecular mechanisms underlying the risks of brain exposure to deficits or excesses of metals.

**Copper**

The importance of balanced dietary supply and unperturbed homeostasis of copper for brain is well elucidated by the symptoms of Menkes’ disease and Wilson’s disease, two inherited disorders of copper metabolism resulting from genetically determined loss of function of very similar ATPases localized to the trans-Golgi network of the cell, which are involved in copper transport [1]. The key importance of the two diseases to understand how deficits or excesses of copper affect the brain, resides in the tissue-specific expression of the two ATPases implicated in either disease, although they utilize a common structural frame and an identical mechanism of action. In *Menkes’ disease* it is the copper-transferring system of the placenta, gastrointestinal tract and blood-brain barrier which is lost, with consequent copper deficiency in the tissues. The clinical symptoms are thus related to loss of function of essential copper enzymes. In an animal model for the disease (the mottled-brindled mouse) we have recently shown [2] that copper deficiency leads to specific decrease in brain of cytochrome c oxidase and superoxide dismutase activities. In addition, a dramatic change of markers for apoptosis was detected in brain: loss of the antioxidant Bcl2 protein, which also plays a fundamental role in brain development and morphogenesis, release of cytochrome c from the mitochondria and depletion of ATP. Histological analysis of brain revealed a high percentage of apoptotic cells in the neocortex and the hippocampus. These results clearly show that copper deficit may cause mitochondrial damage in the brain consequent to inactivation of cytochrome c oxidase, the copper-dependent enzyme responsible for mitochondrial function. In turn, this event may trigger leakage of oxygen radicals out of damaged mitochondria, leading to neurodegeneration via oxidative stress-mediated apoptosis.

In *Wilson’s disease*, on the other hand, the deficient ATPase, which has a 55% amino acid identity with respect to that typical of the Menkes’
disease, is expressed predominantly in liver and transports copper into the hepatocytic-biliary secretory pathway for incorporation into ceruloplasmin, the protein that contains 95% of plasma copper. This defect results in copper accumulation in the hepatocytes, liver cell necrosis and leakage of copper in the plasma. The disease thus represents a model for intoxication by copper excesses. These are, in fact, taken up by extrahepatic tissues, leading to copper deposition in the basal ganglia and neuronal loss. Therefore, both deficits and excesses of copper induce neurodegeneration and very likely because of increased oxidative stress in both cases. According to the models provided by the two genetic diseases just described, lack of copper may lead to neurodegeneration because of the inactivation of cytochrome oxidase and mitochondrial function, and consequent increased superoxide production in the respiratory chain. Excesses of copper will create conditions that are favorable to superoxide-yielding redox cycle with oxygen and to thiol binding in proteins and membranes.

Deficits of copper can affect brain also because they may interfere with iron homeostasis. Ceruloplasmin, the copper protein of the plasma, is also an enzyme with very efficient ferroxidase activity [3]. It is able to oxidize Fe(II) to Fe(III) conveying four electrons to oxygen in a single step: thus, water is produced, instead of superoxide and hydrogen peroxide, and iron can enter its transport and deposit pathway via incorporation into transferrin as ferric iron. Ceruloplasmin is heavily affected by copper inadequate supply to its sites of synthesis. If copper is not incorporated into the protein at the rate of the protein synthesis, ceruloplasmin is rapidly degraded. This actually occurs in Wilson's disease, in which the absence of ceruloplasmin activity in the plasma is a diagnostic marker. In the light of the role of ferroxidase in the transport of iron, the neurological symptoms of this disease can also be consequence of altered iron homeostasis. In fact, marked accumulation of iron in neuroglia and neurons, as well as in hepatocytes, islets of Langerhans and reticuloendothelial cells are observed in individuals with aceruloplasminemia, an autosomal recessive disease linked to mutations of the ceruloplasmin gene [4]. The predominant clinical symptoms in these patients are neurological and, also in this case, it is reasonable to assume that accumulation of iron, consequent to the absence of the ferroxidase activity, leads to overproduction of oxygen free radicals, leading to neuronal loss and neurodegeneration.

Neurodegenerative diseases that are characterized by late onset in the life offer a very interesting matter of analysis as far as risks related to copper are concerned, insofar that aberrant copper chemistry rather than excessive or deficient metal availability seems to be implicated as a causative factor of neurodegeneration, again strongly associated to overproduction of oxygen free radicals.

The familial form of amyotrophic lateral sclerosis (FALS) is a very special and much studied example of this possibility [4]. The disease has a late onset and leads to death within 3 years since the early symptoms. The target of
neurodegeneration is quite specific, because only motor neurons are affected, with consequent progressive impairment of all muscular activities. In most cases the disease shows no genetic or familial origin; however, 10% of the cases are familial (FALS) and 20% of FALS have an autosomal dominant mutation of the gene encoding Cu, Zn-superoxide dismutase. In spite of this intricate etiopathological pattern, the phenomenology of the disease is identical in all sporadic and familial cases. This has stimulated a great deal of interest in studying the relationships between the enzyme involved in the few genetically determined cases and the neurodegeneration typical of the disease. Cu, Zn-superoxide dismutase [5] is a very efficient enzyme, considered to be a primary defense barrier in oxidative stress since it catalyzes the disproportionation of two molecules of superoxide into dioxygen and hydrogen peroxide at a rate very near to the diffusion-controlled limit. The site of enzyme activity resides on the copper, which is involved in a redox cycle with the superoxide substrate, but can also cycle with the peroxide product. The two cycles (A and B) are shown in Table 4. While cycle A will remove O$_2^-$ from any potentially harmful interaction with metals or other radicals to generate more potent oxidants like OH$^-$ (see Table 1), cycle B becomes a generator of the potent oxidant OH$^-$, and envisages a paradoxical pro-oxidant activity of Cu, Zn-superoxide dismutase. However, cycle B can be effective only under a certain set of circumstances, because it is much less kinetically favored than cycle A unless in the presence of large excesses of H$_2$O$_2$ or in the presence of enzyme variants with abnormal affinity for H$_2$O$_2$. The latter case may be due to either distorted copper coordination or altered geometry of the active site channel. Some of the FALS-typical enzyme variants have been purified and some of them indeed showed an increased propensity for the reaction with H$_2$O$_2$ in a kinetic assay [6]. Interestingly enough, such mutants, which are associated to the most severe cases of FALS, are fully active. These results stimulated the hypothesis that neurodegeneration in FALS is related to increased oxidative stress, however not due to loss of function by Cu, Zn-superoxide dismutase, but to a gain of a new, pro-oxidant function by the enzyme.

Results recently obtained in our laboratory are in line with this hypothesis. We have set up a human neuroblastoma cell model where FALS-typical Cu, Zn-superoxide dismutase mutants were coexpressed with the endogenous wild-type enzyme. Such transfected cells are more sensitive to increased oxidative stress generated by redox cycling drugs like paraquat [7] or by nitric oxide donors [8]. In both cases, this higher susceptibility was counteracted by treatment of these cells with the copper-chelating agent D-penicillamine. Actually D-penicillamine decreased the formation of oxidizing radicals by a recombinant FALS mutant in an in vitro isolated system [9] and its administration extended the survival of transgenic mice expressing a FALS mutant [10]. In conclusion, neurodegeneration in FALS appears as a model for risk of exposure to copper under conditions where aberrant copper chemistry may
occur for improper handling of the metals by its physiological carriers even though its concentration limits are normal. In such circumstances, copper can become available in the cell to harmful reactions that generate oxidative stress.

The argument of improper copper binding as possible mediator of brain injury is also relevant to another late-onset neurodegenerative disease, namely Alzheimer’s disease. There is wide consensus indicating increased oxidative stress in the brain of Alzheimer patients, and large body of evidence is in favor of the involvement of metal ions as the major cause of free radical overproduction [for a review, see 11]. In this disease there are also familial and sporadic forms, both characterized by the proteolytic fragmentation of the transmembrane amyloid precursor protein with consequent accumulation of amyloid β protein (Aβ) in amyloid plaques. Metals can participate in this process at two distinct levels, i.e. increasing the aggregation of Aβ and mediating its neurotoxicity. This is a very important point since Aβ is present as a soluble protein in normal fluid and tissues, and tends to form aggregates also in healthy aged individuals, although the aggregates forming in Alzheimer brain are much less soluble. It should also be kept in mind that Zn, Fe and Cu are more concentrated than normal in the neuropil of Alzheimer patients and are further concentrated in the core and periphery of plaques [12]. A plausible hypothesis would be that exposure to excessive amounts of these metals or predisposition to their selective accumulation are risk factors for the onset of the disease. In the light of these facts, it is considerably important that copper induces dramatic aggregation of Aβ under conditions of physiological acidosis [13]. Furthermore, Aβ is able to bind and reduce copper; Cu(I) can be reoxidized by H₂O₂ to produce OH⁻ radicals, possibly contributing to the further fragmentation of the amyloid precursor protein to toxic peptides [14], which have been shown to enhance metal-catalyzed free radical production [15].

A comparable model for risk exposure to copper seems to be operative in prion disease. Also in this case, copper has been shown to bind to an octapeptide in the amino-terminal region of the normal cellular prion protein [16]. The disease is associated to a conformational transition of the normal cellular form of the protein to the prion disease-specific isoform,
characterized by a higher content of $\beta$ structure and increased proteolytic resistance [17]. This purely conformationally-mutated form of a normal protein essential for proper brain function is considered to be the infectious agent (prion) of spongiform encephalopathies (prion disease). Copper has been shown to stabilize the conformation of the infectious form in experiments of reversible denaturation [18] suggesting that the metal may play an at least complementary role in the etiology of prion disease.

Iron

Adventitious or loosely bound iron is the principal culprit in the metal-driven radical-producing redox cycles with oxygen taking place in tissues. We have already seen that the inherited disease aceruloplasminemia severely affects iron metabolism, since lack of ferroxidase inhibits fast and safe oxidation of Fe(II) to Fe(III). It has been shown that ceruloplasmin is expressed on the surface of mammalian astrocytes as a glycosylphosphatidylinositol-anchored form [19]. The absence of the protein leads to accumulation of reduced iron in the brain which is ready to participate in oxidant-induced tissue damage, as shown by the marked increase of plasma lipid peroxidation in aceruloplasminemic patients [3]. However, it cannot be neglected that iron is essential for respiration (it is the prosthetic group of iron-sulfur proteins, cytochromes and cytochrome oxidase in mitochondria) and its inability to bind efficiently to transferrin may affect iron transfer to respiratory enzymes. Thus the disturbance of iron transfer in aceruloplasminemia represents a useful model for risks faced by brain in both iron loading or iron deficiency. In fact either direct radical production via redox cycling by adventitious iron or mitochondrial damage ultimately result in oxidative stress, apoptosis and neurodegeneration.

While iron transport across the cell membrane is bound to the ferroxidase activity of ceruplasmin, studies on yeast suggest that the human homolog, frataxin, of a yeast mitochondrial protein involved in iron transport, would also act, with a mechanism yet unclear, in the mitochondrial iron transport. Actually knockout yeast lacking the frataxin homolog [20] accumulate iron in their mitochondria, and this leads to respiratory impairment, owing to damage in the mitochondrial genome. Strong analogies between yeast and human copper transport have already been ascertained [21] and there is no reason they do not work also for iron. If this is true, we have a molecular key to rationalize brain dysfunction in Friedreich's ataxia, an autosomal recessive degenerative disease recently linked to frataxin deficiency. Symptoms of this disease are largely neurological but also include hypertrophic cardiomyopathy and diabetes. Frataxin is localized in mitochondrial membranes and therefore mitochondrial iron overload and consequent mitochondrial damage should be the key molecular markers of the disease. Iron overload has been actually
observed in heart biopsies of Friedreich’s patients and increased iron has been
detected in their dentate nucleus by pulsed NMR [22]. It is also relevant that
heart mitochondria of Friedreich’s patient have a typical loss of activity of iron
sulfur proteins, which are particularly sensitive to oxy-radical damage [23].
One of the inactivated proteins is the enzyme aconitase which is also a sensor
for iron homeostasis in the cell, so that its inactivation closes a vicious circle
that may amplify iron overload.

As in the case of copper, iron is implicated in metal-related oxidative stress
in major adult-onset neurodegenerative diseases as well, however with some
mechanism of their own, which may help in understanding specific risks
associated to prolonged exposure of brain to disregulated iron homeostasis.
Iron accumulates specifically in Alzheimer lesions [24] and in a form that
differential staining identified in part as Fe(II), the reducing species ready
to react with H₂O₂ to give OH⁻ radical (see Table 1). Iron may be reduced
by Aβ, as reported for copper [14], or, alternatively, it may stay partially
reduced at the steady state for a relative decrease of ferroxidase activity
in the lesions [25]. The lesion-bound iron could be readily removed with
deferoxamine, indicating that it is likely to be adventitious iron. While in
Alzheimer lesions its source may well be degradation of heme by heme
oxygenase, an enzyme which has been shown to localize in amyloid plaques
and to be chronically induced in Alzheimer’s disease [24], elevated dietary
iron availability in healthy aged persons cannot be ruled out as a risk factor
for the onset of the disease.

Another source of iron toxicity may be operative in Parkinson’s disease, a
late-onset brain disease in which neurodegeneration affects dopaminergic
neurons in the substantia nigra. Iron accumulates in astrocytes of the
substantia nigra in old rats and, at the same time, there is a decrease of
reduced glutathione, the major low-molecular-weight free radical scavenger
of the cell (Table 2). These data indicate that aging predisposes the substantia
nigra to oxidative stress and that oxidation actually occurs. However in
Parkinson’s disease a specific source of redox-active iron has been identified
in ferritin, the protein responsible for iron storage in a redox-inert form.
Iron can be released from ferritin by 6-hydroxydopamine [26], a derivative of
dopamine implicated in Parkinson’s disease, which is easily oxidizable by O₂
to give superoxide. This will be a tissue-specific mechanism for iron-mediated
oxidative stress and a further indication of how circumstantial events, as
dopaminergic excitation or, more in general, augmented catechol production
by the hormonal network, may lead to increased risk of metal availability in a
potentially neurotoxic form. In the case of Parkinson’s disease, like in that of
Alzheimer’s disease, there are also genetic factors that contribute to the onset
of the disease, in particular genetically linked mitochondrial disfunctions.
They will concur in amplifying oxidative stress, supporting again the view that
environmental and endogenous (sex, age, familial inheritance) factors play a
synergistic role in determining brain sensitivity to metal exposure and that
risk assessment should be made for each individual or population in the light of the multifactorial nature of the process.

**Other Metals (Mn, Al, Cr, B)**

It has been long known that chronic exposure to *manganese* (manganism) produces extrapyramidal syndromes similar to Parkinson's disease and also behavioral disturbances and cognitive problems. Currently, subclinical neuropsychological changes may be imputed to exposure to Mn-containing compounds present in gasoline as additives. Although specific mechanisms for Mn neurotoxicity have been suggested, as, for instance, induction of nitric oxide synthase [27], resulting in potentiated NO production by microglia, the ultimate consequence will be as usual an increased oxidative stress. NO, in fact, may have a role in initiating and amplifying the oxy-radical production cascade because it reacts very rapidly with $O_2^-$ to give peroxynitrite and the OH$^-$ radical. This role may be crucial in the case of Mn because this metal cannot be involved in a redox activity like that of Fe or Cu, being much more kinetically sluggish. Activation of NO synthase would not require redox activity since the enzyme, which is a heme protein, contains an additional metal-binding site that modulates the enzyme activity. A direct involvement of Mn in the production of reactive oxygen species is also ruled out by the finding that, in an animal model, it actually protected nigrostriatal neurons from oxidative stress in an iron-induced model of Parkinsonism [28]. This protection has been explained in term of competition between a redox-inert and a redox-active metal in brain tissue, and would represent a model for mutual interference between metabolic routes of different metals, to be always taken in consideration in cases of multiple exposure. This is clearly the case for the effects of aluminum in the Alzheimer disease [29]. The presence of aluminum in Alzheimer-typical lesions has been usually found in association to the presence of iron and there is epidemiological evidence that risk of developing Alzheimer's disease is higher where aluminum concentration in drinking water is >100 µg/l. Other aluminum-induced brain injuries have been reported, in particular dementia, associated to dialysis treatment. Aluminum is not essential for any living organism and its uptake is due to its presence in water because of industrial pollution. As in the case of Mn, enhancement of free radical production by aluminum has been reported, in spite of lack of efficient redox cycling. It may be due to its capability of binding phosphates and thus disrupting the mitochondrial energy-producing machinery, but there is also evidence that aluminum loading of neuroblastoma cells [29] increases the accumulation rate and total content of iron. Since the two metals share the same transport system, aluminum loading may up-regulate the iron transport system as well. However, it is clear that aluminum also disregulates the sensing of iron by the cell, since iron accumulation in the presence of aluminum is not able to suppress iron.
uptake. This may be the consequence of effects on the transferrin-independent iron uptake or on aconitase, the iron-responsive element binding protein [23]. These effects can be mediated by direct reaction of aluminum with oxygen or phosphate groups of proteins, which is also responsible for the induction by aluminum of neurofibrillar tangles, the intraneural counterpart of extraneural amyloid plaques in Alzheimer’s disease. The consequent iron accumulation gives rise to the cascade of events typical of metal-mediated oxidative stress.

The effects of manganese and aluminum that we have just outlined belong to the field of toxicology and of the increasing risk connected with technological pollution of soil and water. Manganese, on the other hand, is an essential nutrient, performing among other functions, a crucial role as prosthetic group of manganese superoxide dismutase, a key antioxidant enzyme in the mitochondria. Deficits of manganese have not been described in humans, although it may become a potential risk due to subclinical deficiency of trace elements brought about by current trends in food consumption (vegetarian diets, increasing uniformity of food supply, etc.). In this context it is interesting to mention that genetic inactivation of the mitochondrial Mn-containing superoxide dismutase produced in mice a novel neurological phenotype with spongiform degeneration of the brain, which was successfully cured with the administration of a superoxide dismutase-mimetic manganese compound [30]. This result addresses the point of subclinical deficits of other microelements in humans. Total parenteral nutrition often gives new hints and insight concerning this problem. Cases of chromium deficiency, rescued by chromium readministration, were analyzed and shown to produce not only the expected symptoms related to insulin resistance, but also nerve and brain disorders associated to impaired energy utilization. It is now ascertained that chromium intake in westernized diets is suboptimal and requires supplementation of 10–20 µg for average patients undergoing total parenteral nutrition. The mechanism by which chromium sustains brain function is not known and is difficult to discriminate from its insulin-sensitizing effect. Modulation of brain function by chromium would simply reflect modulation of brain function by insulin. Thus, diabetes leads to changes of hypothalamic functions similar to those observed in normal aging. In fact, the positive effect of chromium on energy utilization can be easily explained with optimization of glucose metabolism by insulin. This will prevent formation of free radical production by mitochondria, and eventually slow down aging processes. As a matter of fact, chromium picolinate has been shown to increase median and maximal lifespan in rodents [31].

Energy utilization requires also boron. Boron deprivation results in decreased brain electrical activity and poorer motor performances, attention and memory. On the other hand, boron toxicity results in behavioral depression, hypotonicity, ataxia. Considering the affinity of B for oxygen ligands, it is conceivable that B may interfere with the metabolism of other elements, like Ca, Cu, Mg, Mo and P, and that it may interfere in the processes
of energy production. Either excesses or deficits will in this way result in enhancement of oxidative stress for brain, like in the cases of Mn or Cr.

Conclusions

The availability of very general models for metal deficiency and toxicity, as those provided by genetically determined inherited disease or late-onset chronic disease with genetic predisposition, allows to outline common mechanisms for the complex nutritional relationships between metal ions and brain. Although each metal has its own metabolic routes and specificity of reactions, the intrinsic peculiarity of the central nervous system (Table 3) makes the insurgence of oxidative stress a likely ultimate step in processes leading to neurodegeneration from both sides of metal deficiency and overload. This is fairly evident for copper, where the Menkes and Wilson diseases represent very clear models for deficits and excesses, respectively, leading to oxidative stress and apoptosis along two distinct pathways. Deficits of copper lead to impairment of copper enzymes and thus antioxidative defense in both the preventive (cytochrome oxidase) and interceptive (superoxide dismutase) modes (Table 2). Excesses of copper lead to uncontrolled metal reactions with oxygen and overproduction of free radicals. Rise of radicals and loss of defense, together with intrinsic weakness of the target (Table 3), synergically concur to make brain extremely sensitive to derangement of metal homeostasis. The rationale provided by copper-dependent pathologies can be applied to all other metals considered, both essential nutrients and toxic pollutants, and allows to approach a strategy of damage prevention by dietary control as well as damage treatment by antioxidant therapy.

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References


**Discussion**

*Dr. Uauy:* You’ve revealed the complex actions of metals in the living organism, how we may have too little or too much, and how genetic polymorphisms affect the situation. ALS may be an extreme example, where the enzyme concerned is excessively active in its physiologic role but also becomes active in a peroxidative role. In an aging population there may be increasing mishandling of metals. How do we resolve the problem of giving too little or too much?

*Dr. Rotilio:* Well, I can’t solve that problem but I can give you some hints about which way we need to go. As I said, we have undertaken studies in Europe to see how much copper is supplied by the diet, and how much fortification can be given without risk of copper toxicity. These studies must continue because the proper control of copper availability is essential in elderly people, not only in relation to normal brain function but also because we now know that Alzheimer’s disease and Parkinson’s disease are sensitive to iron and copper. Other chronic diseases of the brain can also be exacerbated by minimal imbalances of copper and iron above the level of the physiological barrier, and in the case of copper even below it. So I see the problem as an exacerbation of a trend towards copper and iron accumulation in brain. There are diseases or mild forms of disease where proteins are formed that bind those metals and so act to generate an oxidative state. So the solution is to obtain much more data on the nutritional requirement for these metals.

*Dr. Rosenberg:* You showed how these genetic defects, like Menkes’ disease and defects in iron transport, can result in some specific cellular problems that could lead to damage, apoptosis and neurodegeneration. What do we know about the heterozygote state in these conditions? Is there any evidence that there is modification of function in heterozygous individuals? They would be much more prevalent in the population, and that might influence the handling of these metals on a large scale, particularly as people get older.

*Dr. Rotilio:* It’s a good point. Not much is known about the function of heterozygotes in these conditions.

*Dr. Bunout:* The gastrointestinal tract is extremely sensitive to a high copper intake, and severe symptoms can occur. Do you think it would actually be possible to achieve an excessive intake in a normal individual, sufficient to be of epidemiological importance?

*Dr. Rotilio:* In our study we did not see an increase in any of the molecular markers, even supplying quite a large amount of copper (up to 10 mg a day, the normal RDA being 1–2 mg). It appears that there are no molecular markers that can be followed in the normal population at these levels of intake, which is why we went to genetic disease as a model. So far, we haven’t any evidence for harmful effects in the normal population.

*Dr. Cole:* Are there data to show that either transgenic or nutritional manipulation of the level of the binding proteins can reduce the amount of free metal and have a protective effect?

*Dr. Rotilio:* There is a specific protein which could help a great deal in sequestering copper when it is present in excess, and that is metallothionein. Unfortunately, while
the induction of metallothionein has been very well studied in yeast, this is not the case for the human. As you probably know, when we do postmortem analyses of tissues for metallothionein, we only find zinc and cadmium bound to it. It seems that copper has no access to metallothionein under normal circumstances, or even in overexposed people, as there are no cases on record in which postmortem analysis has shown copper-loaded metallothionein. So I would prefer an approach using chelating agents, which are very useful, for instance, in Wilson’s disease, where the hepatic cirrhosis is partially alleviated by chelating agents. After that I would go for genetic manipulation of copper-sequestering and copper-binding proteins.

**Dr. Cole:** My question was more directed towards the iron-binding proteins. But my other question is whether there are normal polymorphisms that influence the affinity or coordination of these metals and the generation of ROS. False mutations seem to be everywhere, indicating that a number of different allelic variations could influence that coordination.

**Dr. Rotilio:** I understand your point but I have no answer.

**Dr. Uauy:** In terms of regulation, the first step is gastrointestinal absorption. We know that this can vary for iron from 1 to 15 or 20%. It’s downregulated when you have excess and upregulated when you have deficiency. So absorption is one level of regulation. In terms of transport and storage, it’s interesting that you have redundancy. For example, aceruloplasminemia causes no problem with copper, because copper is taken up and distributed by albumin and by small molecular weight tripeptides and whatever; the problem is linked to the ferroxidase activity of ceruloplasmin. So you have a defect on a copper transporter that affects iron mobilization, and you have anemia resistant to iron therapy because iron cannot be moved out of the tissues. At the cellular level, and for each of the metals, you have several binding proteins and several storage proteins. So in general I think the system is well protected unless critical mutations occur that cause problems with free radicals. However, in general, metals do not circulate as free ions. They are all bound, as obviously any free metal ion would trigger ROS formation. So cells and organisms are highly protected at whole body level, at organ level, and at cellular level. Now whether the heterogeneity in transporters and the heterogeneity in binders can provide populations at risk, I don’t think we know at present. It may be that there are specific groups in the population who are at risk of excess.

**Dr. Rotilio:** There is no sense in talking about free metal ions – they don’t really occur. But there are some associations that may carry a higher risk. For example, I’m not sure that copper-albumin is totally insensitive to oxidative stress, and it’s not a very well protected way of transporting copper. And ceruloplasmin likewise, because there is a lot of loosely bound copper there as well. So the point is to foresee challenges for these transporters – not all the transport systems are safe. Epidemiologically we can say that metals like copper and iron very rarely cause toxicity, so in general their handling seems to be safe.

**Dr. Møller:** In some countries it has become popular to take zinc supplements. Do you think that such additional zinc might interact with copper molecules or with enzymatic mechanisms and that this could be dangerous?

**Dr. Rotilio:** This is a very important point. Zinc is not redox-active like iron, so it will not generate free radicals or cause oxidative stress directly; it’s even considered to be a protective agent. However, I believe that the cases of zinc toxicosis that have been described are certainly caused by interference with the copper transporter or even the iron transporter, though the copper transporter is much more susceptible – perhaps because the first step in copper transport is passive rather than actively driven, so there could be a lot of competition with other metals. The specific actively driven
systems are beyond the first passage through the intestinal membrane, but at the gate there is real risk of competition between metals.

Dr. Uauy: I’d like to comment because that’s a highly relevant question. Both iron and zinc interfere with copper absorption. The upper level of zinc at the present time is defined by the adverse effect it has on copper absorption. So 40 mg of zinc, which is almost twice the RDA, may interfere with copper absorption. This property is used beneficially for patients with Wilson’s disease, to block the copper absorption. The concept is that if you’re going to provide iron, you also need to provide zinc and copper in the supplement.

Dr. Rosenberg: I think it’s useful that we call attention to these metal interactions, but the problem is not confined to trace metal interactions. For example, interactions will occur under certain circumstances following supplementation with calcium and other minerals. The absorption of iron can be affected by large amounts of calcium, and so forth.

Dr. Grantham-MacGregor: There’s a lot of agitation about whether we should supplement young children with iron, knowing that most populations will not be anemic. Is there any possible harm in it?

Dr. Rotilio: I personally – and this is the point of view of a nutritional biochemist, not a nutritional clinician – am against any kind of fortification with metals, because my personal scientific experience makes me think that the results can be unpredictable. The world of metals is so highly complex that you cannot even predict whether an interaction will take place.

Dr. Uauy: Some countries in which anemia is no longer prevalent have actually stopped fortification, for example Sweden. Any time you fortify you have to look at the risk/benefit equation. There are obviously potential risks. In the case of iron, hemochromatosis is probably one of the most prevalent genetic disorders in existence, and whether the heterozygotes are subjected to potential iron overload becomes a crucial issue. As populations move from deficiency to normality, fortification needs to be re-examined. We may be protecting children from anemia but equally we may be overloading the bulk of the population. In fact the adult or the elderly population not only will not benefit, but may have problems. It’s an open question at present.