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Essential metals—case study on iron

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Abstract

Iron is a vital element in life. Because of the insolubility of iron oxides and sulfides the implication is that dissolved iron was fairly abundant and that oxygen and sulfide were rare in the atmosphere and ocean. Iron and its compounds present as pollutants in the atmosphere can cause deleterious effects to humans, animals, and materials. Analyses of urban air samples show that the iron content averages $1.6 \mu\text{g}/\text{m}^3$, with the iron and steel industry probably the most likely source of emission. Iron is a natural component of soils and its concentration can be influenced by some industries. Iron concentration in surface water varies greatly, from 61 ppm to 2680 ppm. The disposition of iron in the human body is regulated by a complex mechanism to maintain homeostasis. Iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, as a result of formation of free radicals. Iron has the capacity to accept and donate electrons readily. The content of body iron is regulated primarily by absorption since humans have no physiological mechanism by which excess iron is excreted. Iron has been identified as a component of asbestos and other mineral and synthetic fibers. Inhalation of iron oxide fumes or dust by workers in the metal industries may result in deposition of iron particles in lungs, producing an X-ray appearance resembling silicosis. During the last decades efforts regarding dietary iron supply focused mostly on the prevention of deficiencies, especially during growth and pregnancy. The chemical form of the iron influences absorption, as do interrelationships with other dietary components.

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1. Introduction

Iron is a vital element in life. The major scientific and medical interest in iron is as an essential metal, but toxicological considerations are important in terms of accidental acute exposures and chronic iron overload.

With rare exceptions, virtually all live organisms are dependent on iron for survival. Despite the ubiquitous distribution and abundance of iron in the biosphere, iron-dependent life must contend with the paradoxical hazards of iron deficiency and iron overload, each with its serious or fatal consequences. Homeostatic mechanisms regulating the absorption, transport, storage, and mobilization of cellular iron are therefore of critical importance in iron metabolism, and a rich biology and chemistry underlie all of these mechanisms. A coherent understanding of that biology and chemistry is now rapidly emerging. The past decade have brought a

revolution to the understanding of the molecular events in iron metabolism. Of central importance has been the discovery of proteins carrying out functions previously suspected but not understood or, more interestingly, unsuspected and surprising. Parallel discoveries have delineated regulatory mechanisms controlling the expression of proteins long known—the transferrin receptor and ferritin—as well as proteins new to the scene of iron metabolism and its homeostatic control. These proteins include the iron-regulatory proteins (IRPs 1 and 2), a variety of ferrireductases in yeast and mammalian cells, membrane transporters (DMT1 and ferroportin-1), a multi-copper ferroxidase involved in iron export from cells (hephaestin), and regulators of mitochondrial iron balance (frataxin and MFT). Experimental models, making use of organisms from yeast through the zebrafish to rodents have asserted their power in explaining normal iron metabolism, as well as its genetic disorders and their underlying molecular defects. Iron absorption, formerly poorly understood, is now a fruitful subject for research and well on its way to

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detailed elucidation. The long-sought hemochromatosis gene has been found, and active research is underway to determine how its aberrant functioning results in disease that is easily controlled but lethal when untreated. A surprising connection between iron metabolism and Friedreich's ataxia has been discovered. It is no exaggeration to say that the new understanding of iron metabolism in health and disease has been explosive, and that recent history is likely to be prologue to what lies ahead (Aisen et al., 2001; Burke et al., 2001; Santos et al., 2000).

2. Iron in the environment

Tentative geochemical cycles for the prebiological Earth are developed by comparing the relative fluxes of oxygen, dissolved iron, and sulfide to the atmosphere and ocean. The flux of iron is found to exceed both the oxygen and the sulfide fluxes. Because of the insolubility of iron oxides and sulfides, the implication is that dissolved iron was fairly abundant but that oxygen and sulfide were rare in the atmosphere and ocean (Crichton and Pierre, 2001; Walker and Brimblecombe, 1985).

The advent of oxygen was catastrophic for most living organisms, and can be considered to be the first general irreversible pollution of the earth. Oxidation of iron results in insoluble Fe(III) and loss of bioavailability. A new iron biochemistry (Crichton and Pierre, 2001) became possible after the advent of oxygen, with the development of chelators of Fe(III), which rendered iron once again accessible, and with the control of the potential toxicity of iron by its storage in a water-soluble, nontoxic, bioavailable storage protein (ferritin).

2.1. Air

Iron and its compounds, present as pollutants in the atmosphere, can cause deleterious effects to humans, animals, and materials. Iron and iron oxides are known to produce a benign siderosis, and iron oxides have been implicated as a vehicle for transporting high concentrations of both carcinogens and sulfur dioxide deep into the lungs, thereby enhancing the activity of these pollutants. Iron oxides also cause damage by staining materials. Analyses of urban air samples show that the iron content averages $1.6 \mu\text{g}/\text{m}^3$, with the iron and steel industry the probable source of emission. Pollution from iron emission can be reduced by use of particulate control equipment. The economic costs of air pollution from iron have not been determined, but the costs of abatement for basic oxygen furnaces vary between 14% and 19% of total industrial plant costs. Adequate methods are available for the determination of iron in the ambient air (Nielsen et al., 1990; Gao et al., 1991; Sullivan, 1969).

The effects of ferric/ferrous chlorides and ferric oxide on emissions in Tongchuan coal combustion process were experimentally investigated using a thermogravimetric analyzer (Liu et al., 2000). The results show that all iron compounds used in the experiments, namely FeCl_3 , FeCl_2 and Fe_2O_3 can change the emissions of SO_2 and NO , but with different mechanisms and different magnitudes. FeCl_3 plays the role of both a catalyst and an absorbent in the conversions from coal-S to SO_2 and from coal-N to NO . The catalysis reduces the activation energy required for the formation of SO_2 and NO , whereas the rates of the reactions leading to SO_2 and NO are increased. The catalysis is concerned with the forms of coal S. Because of absorption, FeCl_3 reacts with SO_2 to produce $\text{Fe}_2(\text{SO}_4)_3$. Additionally, FeCl_3 can decrease the emissions of CO ; that is, the combustion characteristics can be changed. The effects of FeCl_2 on the emissions of SO_2 , NO , and CO are similar to those of FeCl_3 , but weaker. Fe_2O_3 can also decrease the SO_2 and NO emissions to some extent, but it has negligible influence on CO emission.

2.2. Soil

Iron is a natural component of soils, but its concentration can be influenced by some industries. It has been reported that urban soils showed different heavy metal characteristics (Yang et al., 2001). Apparent accumulations of Pb, Zn, Cu, and Ni were observed, of which Pb occurred mainly in conjunction with iron oxides, and Ni and Zn existed in residual forms. Cu showed the same importance of different chemical forms but for soluble forms.

2.3. Water

Iron concentration in surface water ranges for the most part from 61 to 2680 ppm (Abal et al., 2001; Trindade and Cavalheiro, 1990). The lithophilic elements Li, K, Rb, Mg, Ca, Ba, Al, La, Ti, V, Mn, Fe, Co, and Ni account for >90% of the elemental mass and do not show any change in their concentrations with time (Tuncer and Tuncel, 2001).

A study has reported that, due to agricultural activities and increased water extraction, ground water levels have generally decreased in large areas of the peaty lowlands in the Netherlands (Smolders and Roelofs, 1995). As a result, iron-containing seepage has decreased in many regions, while alkaline Rhine river water, which is rich in sulfates and poor in iron, has been used to compensate for the shortage of water. This has resulted in increased alkalinity and organic sediment breakdown. Increased sulfate reduction leads to iron sulfide precipitation and internal alkalinity generation. As a result of these processes, phosphate and ammonium levels in sediment pore water have

increased strongly. Release of these nutrients to the water layer has resulted in internal eutrophication of the peatland ecosystems. Furthermore, iron levels have decreased strongly as a result of decreased seepage and iron sulfide precipitation. As a result, sulfide accumulates in sediment pore water and reaches toxic levels. Furthermore, decreased iron levels appear (Smolders and Roelofs, 1995).

The direct and indirect effects of iron on the structure and function of lotic ecosystems have been reviewed (Fritz et al., 1975; Vuori, 1995). In addition to the mining of Fe-enriched ores, intensified forestry, peat production, and agricultural water runoff have increased the load of iron in many river ecosystems. The effects of iron on aquatic animals and their habitats are mainly indirect, although the direct toxic effects of Fe^{2+} are also important in some lotic habitats that receive Fe-enriched effluents particularly during cold seasons. Ferric hydroxide and Fe-humus precipitates, on both biological and other surfaces, indirectly affect lotic organisms by disturbing the normal metabolism and osmoregulation, and by changing the structure and quality of benthic habitats and food resources. The combined direct and indirect effects of iron contamination decrease the species diversity and abundance of periphyton, benthic invertebrates, and fishes. Sorption and co-precipitation of metals by Fe-oxides decrease the bioavailability (Fritz et al., 1975; Vuori, 1995).

The bioaccumulation of iron in the organs and tissues of the freshwater crab, *Potamonautes warreni* Calman, from three metal-polluted aquatic ecosystems was examined (Steenkamp et al., 1993). Differences in iron concentrations in water and sediment were related to environment variables. The highest iron concentrations in the crab occurred in the gills, suggesting this organ to be the prime site for the absorption and/or loss of iron to and from the aquatic environment. Despite the absence of a seasonal or gender-related tendency in iron concentrations in the various organs and tissues, appears to be an inverse relationship between size and the capacity of the crab to bioaccumulate iron.

2.4. Plant concentrations

It is well known that plants concentrate chemicals, including metals, depending mainly on species and the chemical's concentration in the soil. The contents of copper, molybdenum, sulfur, zinc, selenium, iron, manganese, and the copper/molybdenum ratio were determined in different native plant species from a mountainous area of central southern Norway. The overall mean values and ranges (mg/kg dry wt.) were as follows: Cu, 6.0, 0.9–27.2; Mb, 0.25, 0.01–3.57; Zn, 77, 8–320; Se, 0.05, <0.01–0.32; Fe, 208, 15–2245; Mn, 338, 31–3784; S (g/100 g dry wt.), 0.20, 0.03–0.56; Cu/Mb, 79, 1–7955. Amounts of the individual elements showed

considerable variability, both between and within plant groups. Another example is that significant differences were found between peripheral parts of two taxa of lichens, with higher concentrations of Fe and Al in *Xanthoria*, and Cd and Zn in *Parmelia*. An interspecies comparison of several hundred Italian measurements confirmed the higher affinity of *Xanthoria* for Fe and Al. To enhance data quality in biomonitoring studies, one should analyze only peripheral parts of the lichens, and avoid the use of both *Parmelia* and *Xanthoria* when monitoring Cd and Zn (Nimis et al., 2001).

3. Toxicokinetics of iron

The disposition of iron in the human body is regulated by a complex mechanism for maintaining homeostasis. Generally, as 2–15% is absorbed from the gastrointestinal (GI) tract, whereas elimination of absorbed iron is only ~0.01% per day (percent body burden or amount absorbed). During childhood, pregnancy, or blood loss, the need for iron is increased and so is the absorption. Absorption occurs in two steps: absorption of ferrous ions from the intestinal lumen into the mucosal cells, and transfer from the mucosal cell to the plasma, where it is bound to transferrin for transfer to storage sites. Transferrin is a β_1 -globulin with a molecular weight of 75,000 and is produced in the liver. As ferrous ion is released into plasma, it becomes oxidized by oxygen in the presence of ferroxidase I, which is identical to ceruloplasmin. There are 3–5 g of iron in the body, about two-thirds of which is bound to hemoglobin, 10% in myoglobin and iron-containing enzymes, and the remainder is bound to the iron storage proteins ferritin and hemosiderin. Exposure to iron induces synthesis of apoferritin, which then binds ferrous ions. The ferrous ion becomes oxidized, probably by histidine and cysteine residues, and by carbonyl groups. Iron may be released slowly from ferritin by reducing agents such as ascorbic acid, cysteine, and reduced glutathione. Normally, excess ingested iron is excreted, but some remains within shed intestinal cells, in bile, and in urine, and in even smaller amounts in sweat, nails, and hair. Total iron excretion is usually ~0.5 mg/day.

With excess exposure to iron or iron overload, there may be a further increase in ferritin synthesis in hepatic parenchymal cells. In fact, the ability of the liver to synthesize ferritin exceeds the rate at which lysosomes can process iron for excretion. Lysosomes convert the protein from ferritin to hemosiderin, which then remains in situ. The formation of hemosiderin from ferritin is not well understood, but it seems to involve denaturation of the apoferritin molecule. With increasing iron loading, ferritin concentration appears to reach a maximum and a greater portion of iron is found in hemosiderin. Both

ferritin and hemosiderin are, in fact, storage sites for intracellular metal and are protective in that they maintain intracellular iron in bound form. A portion of the iron taken up by cells of the reticuloendothelial system enters a labile iron pool available for erythropoiesis, and part becomes stored as ferritin.

4. Health and disease: the roles of iron

Iron is vital for almost all living organisms, participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport.

Iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, as a result of the formation of free radicals. Disorders of iron metabolism are among the most common diseases of humans, encompassing a broad spectrum of diseases with diverse clinical manifestations ranging from anemia to iron overload and, possibly, to neurodegenerative conditions. Understanding iron regulation in the molecular level is critical in identifying the underlying causes for each disease and in providing proper diagnosis and treatments (Adamama-Moraitou et al., 2001; NRC, 1979; Evans and Halliwell, 2001; Kuvibidila et al., 2001; Lieu et al., 2001).

Iron has the capacity to accept and donate electrons readily. This capability makes it physiologically essential, as a useful component of cytochromes and oxygen-binding molecules. However, iron is also biochemically dangerous because it can damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, protein, and DNA. This threat is reduced in the healthy state where, because of the fine regulation of iron metabolism, there is never an appreciable concentration of “free iron”. With pathological conditions, however, iron metabolism and superoxide metabolism are clearly interactive. Each can exacerbate the toxicity of the other. Iron overload may amplify the damaging effects of superoxide overproduction in a very broad spectrum of inflammatory conditions, both acute and chronic. Furthermore, chronic oxidative stress may modulate iron uptake and storage, leading to a self-sustained and ever-increasing spiral of cytotoxic and mutagenic events. The iron chelator deferoxamine is able to chelate “free iron” even inside the cell. It is in routine clinical use to promote the excretion of an iron overload (Emerit et al., 2001), when phlebotomy is harmful, and the dosage varies between 2 and 10 g/day. In conditions in which deferoxamine is used to prevent iron-driven oxygen toxicity, such as acute or chronic inflammatory diseases with oxidative stress, the dosage can be considerably reduced and the addition of antioxidants could be useful.

The provision of sufficient available iron is necessary to ensure the optimal response to recombinant human erythropoietin (rHuEpo). Functional iron deficiency (a state in which iron supply is reduced to meet the demands for increased erythropoiesis) is the common cause of rHuEpo hyporesponsiveness in dialysis patients who have normal iron status, even when they are iron-overloaded. Iron supplementation is not justified for hyporesponsiveness in patients with iron overload because of the potential hazards of iron overload aggravated by intravenous iron therapy. Furthermore, *in vivo* studies indicated that the promising effect of intravenous iron medication to compensate for iron-deficient erythropoiesis is not observed in iron-overloaded hemodialysis (HD) patients. Ascorbic acid, a water-soluble antioxidant as well as a reducing agent, has a number of influences on iron metabolism. Recent research highlights that ascorbic acid can potentiate the mobilization of iron from inert tissue stores and facilitates the incorporation of iron into protoporphyrin in iron-overloaded HD patients being treated with rHuEpo (Tarnag et al., 2001).

Iron overload can be classified on the basis of various criteria: route of access of iron within the organism, predominant tissue site of iron accumulation, and cause of the overload. Excess iron can gain access by the enteral or parenteral route, as well as the placental route during fetal life. The different distribution of iron within parenchymal or reticuloendothelial storage areas indicates different pathogenic mechanisms of iron accumulation and has relevant implications in terms of organ damage and prognosis of the patients. Iron overload may be either primary, resulting from a deregulation of intestinal iron absorption as in hemochromatosis, or secondary to other congenital or acquired conditions. A diagnosis of iron overload can be suspected on the basis of clinical data, high transferrin saturation, and/or serum ferritin values. However, several hyperferritinemic conditions are not related to iron overload, but may be symptomatic of severe disorders (inflammations, neoplasia) or a deregulation of ferritin synthesis (hereditary hyperferritinemia–cataract syndrome), and iron overload secondary to aceruloplasminemia, and the recently described dysmetabolic-associated liver iron overload syndrome, are characterized by low or normal transferrin saturation levels. Liver biopsy is still very useful in the diagnostic approach to iron overload disorders, by defining the amount and the distribution of iron within the liver. The analysis of *HFE* gene mutations (residue substitutions C282Y and H63D) is a simple but important tool in the diagnostic workup of iron overload conditions (AUST; Piperno, 1998).

Excess body iron accumulates in heterogeneous patterns and through various mechanisms. A deranged iron turnover somehow relates to the altered physiological barrier for iron absorption in several defined

chronic anemias with ineffective erythropoiesis. Unexcretable excess iron acquired from transfusions provides a therapeutic challenge. Genetic defects of proteins that are essential for the transport of iron into and out of cells (transferrin and ceruloplasmin) deprive the erythron of the metal and cause its accumulation in other vital organs. The hemochromatosis alleles predictably contribute to an iron burden from other causes and commonly facilitate the expression of porphyria cutanea tarda; furthermore, their clinical expression may be accelerated by hereditary hemolytic anemias. Even minimal iron excess in liver disease may contribute to the hepatocellular injury from agents such as alcohol and viruses. Uniquely localized siderosis occurs in the lung and kidney, where iron cannot turn over and causes variable tissue damage. The most devastating iron overload disorder, neonatal hemochromatosis, is understood the least of all (Burke et al., 2001; Bottomley, 1998).

Iron supplementation successfully decreases an ACEI-induced cough, which may be related to the decrease of NO generation associated with the inhibition of NO synthase activity in bronchial epithelial cells (Lee et al., 2001).

5. Toxicity of iron

The body's content of iron is regulated primarily by absorption, because humans have no physiological mechanism by which excess iron is eliminated. This regulation, however, is not absolute. Many variables influence iron absorption, such as the content of diets, iron doses, and life-styles. In the past, nutrition programs for iron fortification and the ingestion of iron preparations have been widely instituted because of the seriousness of worldwide iron deficiency. Also, we now know that a significant number of asymptomatic people carry the hemochromatosis gene, *HFE*, indicating that these people have the potential to accumulate excess body iron in their lifetime. Excess body iron can be highly toxic. This toxicity involves many organs, lead to a variety of serious conditions, such as liver, heart, and lung diseases, as well as diabetes mellitus, hormonal abnormalities, and a dysfunctional immune system. The tissue damage associated with iron overload is believed to result primarily from free-radical reactions mediated by iron. Iron is an effective catalyst in free-radical reactions. The diseases associated with iron overload can be managed effectively or prevented. Therefore, early diagnosis of iron overload and appropriate therapy are critical. By providing the necessary data, clinical chemistry laboratories can play the pivotal role in the management of these health problems (Anderson, 1993; Fujiwara, 1989; Gutteridge et al., 2001; Hershko et al., 1993; Kang, 2001).

Acute iron toxicity is nearly always due to accidental ingestion of iron-containing medicines and most often occurs in children. As of 1970, there were ~2000 cases in the United States each year, generally among children aged 1–5 who ate ferrous sulfate tablets with sweetened coatings. A decrease in the frequency of this problem followed the use of “childproof” lids on prescription medicine. Severe toxicity occurs after the ingestion of >0.5 g of iron or 2.5 g of ferrous sulfate.

Chronic iron toxicity or iron overload in adults is a common problem. There are three basic ways in which massive amounts of iron can accumulate in the body. The first is idiopathic hemochromatosis, which is due to abnormal absorption of iron from the GI tract. The condition may be genetic. A second possible cause of iron overload is excess dietary iron. The third circumstance in which iron overload may occur is from blood transfusions for some form of anemia and is sometimes referred to as transfusional siderosis. The body iron content is increased between 20 and 40 g. Most of the extra iron is hemosiderin. The greatest concentrations are in the parenchymal cells, the liver, pancreas, as well as in endocrine organs and the heart. Iron in reticuloendothelial cells (in the spleen) is greatest in transfusional siderosis and in the Bantu. Additional clinical effects may include disturbances in liver function, diabetes mellitus, and even endocrine disturbances and cardiovascular effects. At the cellular level, increased lipid peroxidation occurs with consequent membrane damage to mitochondria, microsomes, and other cellular organelles.

It has been reported (Fargion et al., 2001) that increased ferritin with normal transferrin saturation is frequently found in patients with hepatic steatosis, but it reflects iron overload only in those patients in whom it persists despite an appropriate diet. The simultaneous disorder of iron and glucose and/or lipid metabolism, in most of the cases associated with insulin resistance, is responsible for persistent hyperferritinemia and identifies patients at risk for nonalcoholic steatohepatitis.

The toxicity of an iron-carbohydrate complex was compared with that of several other iron compounds (Weaver et al., 1961). In addition to the iron-carbohydrate complex, the test substances included ferrous sulfate, ferrous gluconate, ferrous fumarate, a ferric choline citrate complex, an iron-polysaccharide complex, and tablets of ferroglycine sulfate complex. The iron-carbohydrate complex produced the least amount of gastric distress. In the subacute study, ferrous sulfate produced more emesis than did the iron-carbohydrate complex. No other signs of toxicity were observed, and no pathological lesions were found during autopsy. The authors concluded that the iron-carbohydrate complex is the least toxic of the iron compounds tested.

A protective factor in iron toxicity is metallothionein (MT), a low-molecular weight, cysteine-rich protein that

binds metals. The protective role of MT in Cd toxicity is well established, but its ability to protect against toxicity of other metals remains unclear. Some studies have suggested that MT is an important protein in the cellular defense against Cd toxicity and lethality, but it provides much less protection against the lethality of the other metals (Park et al., 2001).

6. Iron's contribution to toxicity of complexes of substances

Iron has been identified as a component of asbestos and other mineral and synthetic fibers. In biological systems, iron has been reported to interact with cell membranes, killing the cell or being internalized by it (Fubini and Mollo, 1995). Such reactions may generate iron-induced active oxygen species (AOS). The production of such species has been reported to be related to the cytotoxicity of certain inhaled fibers. The generation of iron-induced AOS has been found to be decreased by antioxidants and iron chelators. Possible mechanisms for the role of iron-induced free radicals in causing cell damage at the molecular level have been described and discussed. Both lipid peroxidation and DNA damage have been reported to be related to the presence of iron in fibers. The mobilization of iron from fibers by chelators has been found to be dependent upon the chemistry of the fiber and has been reported to differ for various fiber types, including different kinds of asbestos fibers. The iron-associated toxicity of a fiber has also been related to deposition of endogenous iron. The ultimate toxicity of iron-containing inhaled fibers reflects a series of complex and continuous modifications occurring at the surface of the fiber.

Environmental particles <10 μm average aerodynamic diameter (PM10) are associated with mortality, exacerbation of airways diseases, and loss of lung function. It has been suggested that PM10 particles, along with other pathogenic particles, generate free radicals at their surface in reactions involving iron, contributes to their pathogenicity (Gilmour et al., 1996; Smith et al., 1998). It has been suggested that in rats there is a synergistic action of iron and cadmium on manganese transport and retention, and that iron transport and retention are more sensitive to the iron concentration than to the presence of cadmium (Gruden and Munic, 1987).

6.1. Hemochromatosis

Measuring the transferrin saturation level is a cost-effective way to screen for suspected disease. Subsequent workup includes serum ferritin levels, hepatic enzyme levels, and *HFE* gene testing or liver biopsy. *HFE* gene testing can provide a definitive diagnosis in many

patients. Ironically, this diagnostic advance has led to some confusion regarding the criteria for diagnosis of hemochromatosis, with dependence on genetic testing instead of investigations for iron overload. Because many people who are homozygous for the C282Y mutation, or compound-heterozygous for the C282Y and H63D mutations, either do not express or only partially express the disease, it is essential to confirm a diagnosis of hemochromatosis on the basis of increased body iron stores. Liver biopsy remains the best method for confirmation and has an important role in the patient with either borderline iron overload or advanced disease. Persistent elevation of serum ferritin concentration in the absence of overt liver damage, inflammation or neoplasia, and estimation of mobilized body iron by repeated phlebotomy, are reasonable alternatives to liver biopsy. Although the precise definition of iron overload is still debated, a diagnosis of hemochromatosis cannot be made without demonstrating increased body iron stores (Bassett, 2001; Hash, 2001).

6.2. Brain damage

The brain shares with other organs the need for a constant and readily available supply of iron and has a similar array of proteins available to it for iron transport, storage, and regulation. However, unlike other organs, the brain places demands on iron availability that are regional, cellular, and age sensitive. Failure to meet these demands for iron with an adequate supply in a timely manner, can result in persistent neurological and cognitive dysfunction. Consequently, the brain has developed mechanisms to maintain a continuous supply of iron. However, in a number of common neurodegenerative disorders, there appears to be an excess accumulation of iron in the brain, which suggests a loss of the homeostatic mechanisms responsible for regulating iron in the brain. As a result of a loss in iron homeostasis, the brain becomes vulnerable to iron-induced oxidative stress. Oxidative stress is a confounding variable in understanding the cell death that may result directly from a specific disease and is a contributing influence in the disease process. The underlying pathogenic event in oxidative stress is cellular iron mismanagement (Gorell et al., 1999; Liu et al., 2001; Qian and Wang, 1980; Thompson et al., 2001).

Accumulations of iron are often detected in the brains of people suffering from neurodegenerative diseases; yet it is often not known whether such accumulations contribute directly to disease progression. The identification of the genes mutated in two such disorders suggests that errors in iron metabolism do indeed have a key role (Rouault, 2001). It was reported that patients with chronic fatigue syndrome had significantly

increased serum aluminum and decreased iron compared with controls (van Rensburg et al., 2001).

6.3. Iron and atherosclerosis

During the past decade, considerable evidence has supported the role of oxidative stress in the development of atherosclerosis and related cardiovascular diseases (Lieu et al., 2001; Emerit et al., 2001; Chau, 2000). The oxidation of low-density lipoprotein (LDL) and lipid is believed to be one of the crucial events leading to plaque formation in vasculature. It has been hypothesized that iron-mediated oxidation is involved in this process. Supporting this idea, several epidemiological studies have shown that the level of body iron stores is positively correlated with the incidence of coronary heart disease in human populations. However, some studies have yielded conflicting results. Recently, studies conducted in our laboratory and others have demonstrated that iron deposition is prominent in human atherosclerotic lesions. The iron deposits appear to colocalize with ceroid, which is an end product of extensively oxidized lipid and protein complex, in lesions, providing histological evidence to support the iron hypothesis. Additional experiments in animals have further revealed that the severity of atherosclerosis can be markedly influenced by iron overload or deficiency. Collectively, these data suggest a strong basis of pathology that supports the detrimental role of iron in vascular damage and progression of the disease (Chau, 2000).

6.4. Iron and cancer

Iron can induce free radicals that cause DNA double-strand breaks and oncogene activation (Reizenstein, 1991; Whysner and Wang, 2001), as suggested by four epidemiological studies that found a higher cancer risk in patients with larger iron stores than in those with smaller ones. In addition to its effect on carcinogenesis, iron can also maintain the growth of malignant cells as well as growth of pathogens. Breast cancer cells, for instance, display 5–15 times more transferrin receptors than normal breast tissue. Iron-carrying transferrin is in fact a growth factor. Hyposideremia in patients with cancer or infection is not a paraphenomenon but a functioning defense mechanism “nutritional immunity”.

Metal ions, specifically iron, are necessary for the production of hydroxyl radicals. Iron has not received much attention in discussions of estrogen-induced carcinogenesis and human hormone-associated cancer. An elevated dietary iron intake enhances the incidence of carcinogen-induced mammary cancer in rats and estrogen-induced kidney tumors in Syrian hamsters. Estrogen administration increases iron accumulation in hamsters and facilitates iron uptake by cells in culture.

In humans, elevated body iron storage has been shown to increase the risk of several cancers, including breast cancer. The involvement of iron in hormone-associated cancer in humans suggests routes for preventing cancer by regulating metal ion metabolism and interfering with iron accumulation in tissues (Liehr and Jones, 2001).

An overabundance of iron in various body tissues is associated with increased risk of neoplasia at the site of deposition of the metal. Thus some persons who have inhaled excess iron and whose alveolar macrophages are overloaded with the metal are at increased risk of developing cancers of the larynx, bronchi, lungs, and mediastinum (Weinberg, 1993). No carcinogenic effect could be demonstrated for the iron oxides in intra-tracheal instillation and intraperitoneal injection in rats (Steinhoff et al., 1991).

6.5. Occupational diseases due to iron exposure

Inhalation of iron oxide fumes or dust by workers in the metal industries may result in deposition of iron particles in the lungs, producing an X-ray appearance resembling silicosis. These effects are seen in hematite miners, iron and steel workers, and arc welders. Hematite is the most important iron ore (mainly Fe_2O_3). A report on autopsies of hematite miners noted an increase in lung cancer, as well as tuberculosis and interstitial fibrosis. The etiology of the lung cancer may be related to concomitant influences such as cigarettes or other workplace carcinogens. Hematite miners are also exposed to silica and other minerals, as well as radioactive materials; other iron workers have exposure to polycyclic hydrocarbons. Dose levels of iron among iron workers developing pneumoconiosis have been reported to exceed 10 mg Fe/m^3 .

Pulmonary siderosis results from inhalation of iron dust or fumes. It falls into the group of pneumoconioses in which the pulmonary reaction is minimal despite a heavy dust load. Because fibrosis is not caused by inhalation of iron dust, the clinical course is benign and pulmonary function tests and blood gases are within normal limits. Pulmonary siderosis is currently considered to be a simple pneumoconiosis with a good prognosis. Some recent studies suggest the possibility of a more serious outcome with fibrosis even in the absence of any associated silicosis.

The incidence of siderosis among iron workers, turners, and grinders has been reviewed. X-ray changes in the lungs of electric arc welders may be caused by inhalation of iron oxide particles; the X-ray changes are manifested by opacity of the films without any evidence of pulmonary fibrosis or congestion. Cases of siderosis or benign pneumoconiosis have been reported among grinders of bearings made of chrome-vanadium and chrome-molybdenum tool steel containing 98% iron, ~2% alloys mentioned above, and 0.2% silica. The

details of the metal processes are given in several studies, which also outline the composition of metal dust generated in the industry. The frequent monitoring of personnel, enforcing preventive steps, and reducing the concentrations of metal dust in the work environment will help to reduce the incidence of siderosis among metal workers (Buckell et al., 1946; Lasfargues et al., 1991; Peltier et al., 1984; Rubin and Bruderman, 1992).

7. Iron in food

During recent decades, efforts regarding dietary iron supply focused mostly on the prevention of deficiencies, especially during growth and pregnancy (Schumann, 2001). Correspondingly, homeostatic mechanisms increase intestinal iron absorption in iron deficiency, but its downregulation at high intake levels seems insufficient to prevent accumulation of high iron stores at high intake. There is no regulated iron excretion in overload. Excess of pharmaceutical iron may cause toxicity, and therapeutic doses may cause GI side effects. Chronic iron excess, for example in primary and secondary hemochromatosis, may lead to hepatic fibrosis, diabetes mellitus, and cardiac failure (Bassett, 2001; Hash, 2001; Schumann, 2001). Chronic intake of 50–100 mg Fe/day of highly bioavailable iron with home-brewed beer in sub-Saharan Africans lead to cirrhosis and diabetes. Applying a safety factor of 2 would lead to an upper safe level of 25–50 mg Fe/day for this endpoint of conventional iron toxicity. However, beyond this kind of damage, iron is known to catalyze the generation of hydroxyl radicals from superoxide anions and to increase oxidative stress, which, in turn, increases free-iron concentration. This self-amplifying process may cause damage to lipid membranes and proteins, showing the relationship between radical generation and organ damage after ischemia-reperfusion events on the one hand, and available free iron in clinical and experimental settings on the other. Correspondingly, epidemiological studies as well as observations in heterozygotes for hereditary hemochromatosis suggest that the risk of atherosclerosis and acute myocardial infarction is related to body iron stores, although there is conflicting epidemiological evidence as well. The most recent and best controlled studies, however, support the hypothesis that iron stores are related to cardiovascular risk. Iron-amplified oxidative stress may also increase DNA damage, activate precarcinogens, and support tumor cell growth. This is supported by experimental, clinical, and epidemiological observations. As a result of these mechanisms, high iron stores may present a health hazard. Although this has not been finally proven, available evidence strongly recommends against increasing iron intake beyond physiological requirements. To

avoid iron deficiency symptoms, however, care must be taken to meet recommended daily intake.

8. Estimation of the bioavailability of iron

Factors influencing the bioavailability of iron, lead, and cadmium through GI absorption are here reviewed. Bioavailability of iron is known to be influenced by factors present in the lumen of the gut, in intestinal mucosal cells, and in tissues remote from the gut. Iron absorption increases as the quantity ingested increases. The chemical form of the iron influences absorption, as do interrelationships with other dietary components. Interrelationships between metals influence bioavailability at luminal and mucosal phases. Secretions into the gut lumen affect iron absorption. Absorption phases for any metal can be divided into uptake into the mucosal cell, transfer through the cell, and movement into plasma. A substance within the mucosal cell seems necessary for iron absorption. Mechanisms controlling release of iron to plasma are not well understood. The amount of body iron influences iron absorption. Little is known about the bioavailability of iron, and even less is known about mechanisms influencing availability of other metals (Ragan, 1983).

The relative biological values for rats of the iron from seven elemental iron powders (produced by electrolysis, reduction with hydrogen, carbon monoxide, and desiccated ammonia, and carbonyl process) were compared with the *in vitro* solubility of the iron powders in 0.2% (w/v) hydrochloric acid for periods of 5–90 min. The values obtained for percentage of solubility in 10 min were within the fiducial limits of the individual values of six iron samples; the exception was one preparation of carbonyl iron whose solubility, but not relative biological value, was as high as that of electrolytic iron. These data indicate that dissolution rates were good predictors of bioavailability of some types of elemental iron powders. There was good agreement between specific surface areas and particle size distributions of discrete fractions containing fine particles of comparable sizes (7–10 μm) of electrolytic, hydrogen, and carbon monoxide-reduced iron, and whole preparations of carbonyl iron, but these measurements were not satisfactory criteria for predicting bioavailability (Motzok et al., 1978).

Eight laboratories conducted a hemoglobin repletion test for the estimation of the bioavailability of iron from four sources, using depleted male albino rats (Fritz et al., 1975). Ferrous sulfate was used as the reference standard. Ferric orthophosphate was found to have a relative biological value of 11 (range 6–22), an old sample of hydrogen-reduced iron 27 (range 15–41), and ferric citrate 96 (range 75–125). Good results were obtained with a simplified basal diet prepared without

ingredients that had previously contributed variable quantities of iron. There was no apparent advantage in using the change in hemoglobin during the repletion period instead of the final hemoglobin value as the criterion of response to iron supplements. Several statistical treatments of the data yielded similar conclusions regarding relative biological values of the iron sources.

Nutritional iron deficiency (ID) is caused by an intake of dietary iron insufficient to cover physiological iron requirements. Studies on iron absorption from whole diets have examined relationships between dietary iron bioavailability/absorption, iron losses, and amounts of stored iron. New insights have been obtained into regulation of iron absorption and expected rates of changes of iron stores or hemoglobin iron deficits when bioavailability or iron content of the diet has been modified and when losses of iron occur (Hallberg, 2001). The effects of ID are probably related to age, up to ~20 years, explaining some of the earlier controversies. Difficulties in establishing the prevalence of mild ID have been outlined, as has the degree of underestimation of the prevalence of mild ID when using multiple diagnostic criteria. It seems that contemporary low-energy life-styles are a common denominator for the current high prevalence not only of ID but also of obesity, diabetes, and osteoporosis.

A potential risk of interactions between micronutrients affecting absorption and bioavailability must be considered in any supplementation or fortification strategy. At levels of essential micronutrients present in foods, most micronutrients appear to utilize specific absorptive mechanisms and not be vulnerable to interactions. In aqueous solutions and at higher intake levels competition between elements with similar chemical characteristics and uptake by nonregulated processes can take place. These interactions have clearly been demonstrated in experimental absorption studies and to some extent have been confirmed in supplementation studies. Negative effects of iron supplementation on indices of zinc and copper status and of zinc supplementation on iron and copper status have been reported. In contrast, a negative effect of calcium on iron absorption has not been confirmed in long-term supplementation studies. Ascorbic acid has a strong iron absorption-promoting potential, and in iron-deficient populations ascorbic acid supplementation improves iron status. Thus, ascorbic acid supplements or an increased intake of foods rich in ascorbic acid foods could have important public health implications, especially in populations subsisting on a diet based mainly on plant food. The effect of poor status of a given micronutrient on absorption and utilization of other micronutrients should also be considered while developing strategies to improve micronutrient status in a population. Awareness of these interactions, combined

with a balanced evaluation of the dietary intake of the population with regard to substances promoting and inhibiting absorption and the risk for multiple deficiencies, could lead to more effective strategies to improve micronutrient status (Bhaskaram, 2001; Madhavan Nair, 2001; Nielsen et al., 1990; Sandstrom, 2001). Micronutrient efficiency can affect growth, cognition, immune homeostasis, and reproductive performance (Bhan et al., 2001; Seshadri, 2001; Verhoeff et al., 2001).

Fortification of salt with iron has been developed by the National Institute of Nutrition (NIN) as a strategy for the control of iron-deficiency anemia (IDA) in India, similar to iodization of salt for control of iodine-deficiency disorders (IDD). Stability of the iron-fortified salt (IFS), its bioavailability, and organoleptic evaluation of food items containing the IFS have been confirmed. Acceptability and effectiveness of the IFS in schoolchildren and in multicentric community trials have been shown. With the introduction of universal iodization of salt as a national policy in 1988, NIN has developed a formulation for double fortification (DFS) of salt with iodine and iron. The stability of the nutrients under laboratory conditions along with their bioavailability were found to be good but to vary with the quality of salt used. The DFS has been evaluated in controlled trials in tribal communities and in residential schoolchildren. Overall, in these trials, DFS effectively controlled iodine deficiency, but a clear impact on reducing anemia was not shown. In residential schoolchildren, increased urinary excretion of iodine as well as reduced anemia were observed. The quality of salt has been found to be an important determinant of the stability of iodine in DFS. Further evaluation of this potentially important intervention is in progress (Sivakumar et al., 2001; Souphanthong et al., 2000).

Some possible strategies to enhance micronutrient content and bioavailability of diets in developing countries include dietary diversification and modification (Friel et al., 2001; Gibson and Hotz, 2001).

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