Essentiality of copper in humans[1–3]

Ricardo Uauy, Manuel Olivares, and Mauricio Gonzalez

ABSTRACT The biochemical basis for the essentiality of copper, the adequacy of the dietary copper supply, factors that condition deficiency, and the special conditions of copper nutriture in early infancy are reviewed. New biochemical and crystallographic evidence define copper as being necessary for structural and catalytic properties of cuproenzymes. Mechanisms responsible for the control of cuproprotein gene expression are not known in mammals; however, studies using yeast as a eukaryote model support the existence of a copper-dependent gene regulatory element. Diets in Western countries provide copper below or in the low range of the estimated safe and adequate daily dietary intake. Copper deficiency is usually the consequence of decreased copper stores at birth, inadequate dietary copper intake, poor absorption, elevated requirements induced by rapid growth, or increased copper losses. The most frequent clinical manifestations of copper deficiency are anemia, neutropenia, and bone abnormalities. Recommendations for dietary copper intake and total copper exposure, including that from potable water, should consider that copper is an essential nutrient with potential toxicity if the load exceeds tolerance. A range of safe intakes should be defined for the general population, including a lower safe intake and an upper safe intake, to prevent deficiency as well as toxicity for most of the population. *Am J Clin Nutr* 1998(suppl);67:952S–9S.

KEY WORDS Copper, copper nutrition, copper deficiency, copper requirements, toxicity, transcription factors, metal-responsive elements, oxidoreductase enzymes, superoxide dismutase, humans

INTRODUCTION

Copper is a transition metal with three oxidation states: Cu^0, Cu^{1+}, and Cu^{2+}. The cupric state is found most often in biological systems. A need for copper in higher animals has been suggested because hemocyanin, the oxygen-carrying molecule of invertebrates, was found to contain copper, but the potential essentiality of copper for humans was not recognized until 1928 when Hart et al (1) showed copper to be essential for erythropoiesis in rats fed a milk-based diet. They were able to correct the anemia by adding ash from animal or vegetable sources to the diet. They went on to show that the hydrogen sulfide precipitate from the ash, which contained copper sulfide, was responsible for the recovery. Similar findings in humans established the basis for essentiality (2, 3). This paper will review present knowledge on the essential nature of copper for humans, the biochemical basis for essentiality, the adequacy of the dietary copper supply, factors that condition deficiency, and the special conditions that affect copper nutriture in early infancy.

BIOCHEMICAL BASIS FOR ESSENTIALITY

Organisms as diverse as yeast and mammals share a requirement for the regulation of copper metabolism to ensure correct function of several copper-binding proteins. Recently, biochemical and molecular studies have provided new evidence for homeostatic cellular processes for copper, contributing to a better understanding of the molecular mechanisms involved in this regulation. Copper affects enzyme activity, both as a cofactor and as an allosteric component of several cuproenzymes. In addition, important aspects of the copper-dependent regulatory mechanisms in genetic expression of different target genes have been found using yeast as a eukaryotic model.

Role of copper in metalloproteins

Studies on the biochemical basis of copper essentiality have shown that an important number of copper-containing proteins display oxidative reductase activity (**Table 1**). Copper functions as an electron transfer intermediate in redox reactions. Copper has been shown to be an essential cofactor for catalytic activity in protein-lysine 6-oxidase (4, 5) and catechol oxidase (6, 7) (Table 1). In addition, crystallographic studies of Cu/Zn superoxide dismutase 1 (SOD1) (8), cytochrome-*c* oxidase (9, 10), and ceruloplasmin (11) confirmed that copper is an essential cofactor in oxidative and reductase enzymes (Table 1 and **Table 2**).

Cytochrome-*c* oxidase is an inner-mitochondrial–membrane protein complex that catalyzes the reduction of molecular oxygen to water and utilizes the free energy of this reaction to generate a transmembrane proton gradient during respiration.

Three copper atoms are present in Cox: subunit I contains two copper atoms (Cu_A center) involved in the electron transfer from

[1] From the Institute of Nutrition and Food Technology, University of Chile, Santiago.

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[3] Address reprint requests to R Uauy, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Casilla 138-11, Santiago, Chile. E-mail: uauy@abello.seci.uchile.cl.

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TABLE 1
Function of cuproenzymes with oxidation and reduction activity in humans

Enzyme	Function
Cytochrome-*c* oxidase	Electron transport, terminal oxidase
Superoxide dismutase	Superoxide dismutation
Catechol oxidase	Synthesis of melanin
Protein-lysine 6-oxidase	Collagen and elastin cross-linking
Ceruloplasmin	Ferroxidase
Amine oxidases	Deamination of primary amines
Dopamine-β-monooxygenase	Dopamine→norepinephrine
Peptidylglycine monooxygenase	α-Amidation of neuropeptides

cytochrome-*c* to the heme a_3-Cu_B center; subunit II contains one copper atom (Cu_B center) that functions as the dioxygen-reducing unit (10, 12–15). Ceruloplasmin is a blue multicopper oxidase that contains > 65% of the copper found in serum in vertebrate species (16). The crystal structure of ceruloplasmin confirms the presence of six tightly bound copper ions, three of them forming type I copper centers involved in electron transfer processes. The other three copper ions are in a single trinuclear center, which is the oxygen-activating site during the catalytic cycle of the enzyme (11). SOD1 is localized in the cytoplasm, where it catalyzes the dismutation of superoxide anions. Modeling for the enzymatic mechanism–based crystallographic studies has indicated that during catalysis the copper in the active site is reduced with the substrate O_2^- to yield first O_2 and then H_2O_2 (8, 17). No metal can replace copper because only this metal ion confers catalytic activity to SOD1 (18). Together, these studies show that copper is a major component of catalytic centers of different redox enzymes and thus its presence is essential for normal physiologic function, such as cellular respiration, free radical defense, synthesis of melanin pigment, connective tissue biosynthesis, and cellular iron metabolism.

The importance of the relation between copper and the catalytic feature of redox enzymes is highlighted by studies of SOD mutants associated with familial amyotrophic lateral sclerosis (ALS). The discovery of genetic mutations of SOD1 underlying 20–25% of the inherited cases of ALS has generated increased interest in how copper affects SOD1 activity (19). The initial thought was that neuronal death was associated with decreased free radical dismutation because of the abnormal enzyme, yet subsequent experiments with SOD1 null mutants in yeast and transgenic mice suggested that the toxic effect of the SOD1 mutation in human ALS cases was in fact related to enhanced activity of some other function and not to lower SOD activity (20–22).

Hodgson and Fridovich's (23) discovery that SOD1 catalyzes the oxidation of substrates by hydrogen peroxide launched a series of experiments conducted by Wiedau-Pazos et al (24) using the formation of electron paramagnetic resonance–detectable hydroxyl adducts of a model substrate [dimethyl pyrroline-*N*-oxide (DMPO)] as an index of SOD1 peroxidase activity. The authors discovered that DMPO hydroxyl adduct formation is higher in *SOD1* mutant genes from ALS patients, expressed in yeast. Eliminating copper from either the normal or mutant enzyme abolished the peroxidation of DMPO by hydrogen peroxide. Restoring copper (Cu^{2+}) gradually had a proportional effect in enhancing peroxidase activity. The addition of copper-chelating agents slightly increased peroxidative activity of the normal enzyme while it markedly decreased the activity in the mutant. From this experiment the authors could not determine whether the chelators remove copper from the active site of the mutant enzyme or bind to the copper, inhibiting the reaction. To test the significance of these finding in preventing the neuronal apoptosis typical of ALS, they evaluated the effect of transfecting normal or mutated *SOD1* genes on the viability of temperature-sensitive nigral neural cell lines. They were able to show that copper chelators significantly increased the viability of the SOD1 mutant neural cells by 30–70% but did not modify viability in the normal cells. These studies suggest that copper is fundamental for activity of SOD1 and that altered function of this enzyme is related to the pathologic manifestation in ALS.

Copper also acts as an allosteric component of enzymes, probably conferring an appropriate structure for catalytic activity. Protein-bound copper is required for the activity of copper amine oxidases in organisms ranging from yeasts to mammals (25–27). These enzymes catalyze the oxidation of various primary biogenic amines and belong to a new class of redox enzymes present in all eukaryotic organisms that contain a peptide-bound TPQ 3-(2,4,5-trihydroxyphenyl)-L-alanine (Topa) quinone cofactor (28). Topa quinone is generated by posttranslational modification of a specific tyrosyl residue in the highly conserved sequence Asn-Tyr-Asp/Glu (27, 28). In vitro studies of histamine oxidase indicate that the inactive precursor copper-Topa quinone can be activated by incubation with cupric ions, and that copper-reconstituted active enzyme contains the Topa quinone cofactor (27). This finding corroborates that an important aspect of the biogenesis of these enzymes is a self-catalytic mechanism that involves protein-bound copper and is necessary for complete functional amine oxidase (copper-containing) activity (28).

Copper as an essential component in gene expression

Metals represent a class of important effector molecules that regulate gene expression in eukaryotic organisms by activation and repression of gene transcription. Studies of copper-regulated transcription in fungi have provided major advancements in the identification of components and mechanisms of action of eukaryotic copper-responsive transcription factors (29). The role of copper in the function of copper-binding proteins has been shown for Ace1, Mac1, and Amt1, subsets of regulatory proteins that act physiologically as components of metal-responsive genetic switches (**Table 3**). Transcription factors can serve as sensors of intracellular copper concentrations (30) or display a regulatory role in a switching process. The mechanism of transcriptional regulation by Ace1 and Amt1 (**Figure 1**) involves copper-induced binding of the factor to a specific upstream activation sequence in the 5′- end of the metallothionein promoter (31, 32). The specific increase of Ace1 DNA-binding activity is achieved through the cooperative formation of a Cu(I)-cysteinyl-thiolate cluster, which provides the free energy of stabilization for the tertiary fold (33). Cup9 is another regulatory protein expressed in yeast, this protein acts as a transcriptional factor that regulates the expression of important copper homeostatic genes involved in intracellular partitioning of copper (34).

Metal-responsive elements (MREs) (Figure 1) have been found in all eukaryotic metallothionein promoters, which are composed of a series of imperfect repeats containing 13–15 base pairs (29). However, interesting differences have been found between the yeast and mammalian metallothionein promoter: *1*) MREs of the mammalian metallothionein promoter bear no obvi-

TABLE 2
Function of copper-binding proteins in humans

Physiologic role	Copper-binding proteins
Radical scavenging	Superoxide dismutase
	Metallothionein
	Ferroxidase I
Metal transport	Metallothionein
	Ferroxidase I
	Transcuprein
	Albumin
Ferroxidase activity	Ferroxidase I
	Ferroxidase II
Synthesis of adenosine and homocysteine	Adenosylhomocysteinase
Blood coagulation	Factors V and VIII

TABLE 3
Copper-dependent transcription factors

Transcription factor and target genes	Function
Mac1[1]	
MT	Cellular Cu storage and buffer
CTT1	Cytosolic catalase
FRE1	Membrane Cu/Fe reductase
Amt1[1]	
MTI, *MTIIa*, and *MTIIb*	Cellular Cu storage and buffer
SOD1	Superoxide dismutation
Ace 1[1]	
MT	Cellular Cu storage and buffer
SOD1	Superoxide dismutation
Cup9[2]	Cellular Cu partitioning

[1] Copper as allosteric component of transcription factors; the copper is absolutely required for DNA binding.
[2] Unknown target gene.

ous resemblance to the binding site for Ace1 or Amt1; *2*) in contrast with mammalian metallothionein, yeast metallothionein genes are transcriptionally activated mostly by copper; and *3*) several metallothionein promoters of mammalian origin, including mouse metallothionein I (MT-I) and human MT-II, contain other regulatory elements intercalated with the MREs (eg, SP1, AP-2, and AABS). These differences suggest that the copper-dependent transcription regulatory mechanism in mammalian metallothionein species involves a different set of DNA-binding proteins; the protein-protein interaction pattern between these proteins is probably required to form a functional complex of transcription to regulate the expression of metallothionein genes and other target genes. The significance of this difference is unknown but it may be related to different requirements in copper homeostasis in multicellular organisms.

A remarkable feature of copper-dependent transcription factors (Ace1, Amt1, and Mac1) is their association with the expression of other genes related with several physiologic processes. The SOD1 promoter contains a single Ace1 binding site that functions in vivo to coregulate SOD1 and metallothionein transcription in response to copper (35, 36). In addition, Mac1 has been found to regulate the transcription of two target genes: *FRE1* (encoding a component of plasma membrane associated with both Cu(II) and Fe(III) reduction) and *CTT1* (encoding the cytosolic catalase) (37). Yeast phenotypes of Mac1 loss-of-function mutants can be suppressed by added copper or iron, for respiration-deficient mutants, the phenotype could be rescued only by copper. These studies indicate that the absence of copper in yeast produces drastic effects in cellular processes such as proliferation, growth, and metabolic activity. These effects are related to dysfunction of copper-responsive transcription factors, suggesting a major role of this metal in eukaryotic cellular physiology.

There is little information regarding metal-regulated transcription units and their copper-dependent activity in higher eukaryotes. The mouse metallothionein promoter is the most thoroughly studied of the metal-regulated transcription systems in metazoan organisms, and nuclear factors that bind to a metallothionein gene-metal control sequence have been reported (38). A zinc-finger transcription factor, MTF-I, that binds MREs has been cloned in mice (39) and its activity is postulated to be controlled by a metal-sensitive inhibitor (40). Interestingly, transient transfection assays of heterologous chloramphenicol acetyltransferase constructs showed that human MT2A, MT1X, and MT1H promoters can respond to metals, including zinc and copper (41). More studies of the mechanisms of copper-regulated transcription in mammalian species are necessary, particularly at the cellular and molecular levels, connecting copper-dependent transcription factors and different physiologic cellular processes.

ESSENTIALITY OF COPPER FOR HUMANS

Infants with typical features of copper deficiency (anemia refractory to iron treatment and low plasma concentrations of copper were first reported in the United Kingdom in 1956 (42). Copper was considered the likely cause for the anemia, but it was not until a series of controlled case studies of copper deficiency in infants recovering from malnutrition was reported by Cordano et al in 1964 (43) that the full spectrum of copper deficiency was shown. Subsequent reports during the 1970s of acquired copper deficiency in low-birth-weight neonates and in infants and children receiving copper-free total parenteral nutrition served to clearly define copper as an essential nutrient for human infants. Concomitantly, tissue copper deficiency as a result of a rare inherited defect of copper transport, Menkes syndrome, was also recognized (44). Copper deficiency is more commonly an acquired condition induced by the imbalance between need and dietary copper supply. We will first address the acquired conditions leading to copper deficiency and then briefly refer to the inherited metabolic defect.

Factors conditioning copper deficiency in humans

Copper deficiency is more frequent in preterm infants, especially those with very-low birth weights, because of their reduced copper stores at birth given the smaller relative size of the liver and higher requirements determined by their high growth rate compared with full-term infants (45–49). Infants fed cow milk–based diets exclusively are more prone to develop copper deficiency than infants who are breast-fed because of the low copper content of cow milk and limited absorption of this mineral in cow milk (50, 51). In contrast, breast-fed infants absorb more copper, which may be due to the lower casein content of human milk or to factors associated with human milk that enhance copper absorption (52, 53). In developing countries, where infant feeding is often based on cow milk enriched with a high concentration of refined carbo-

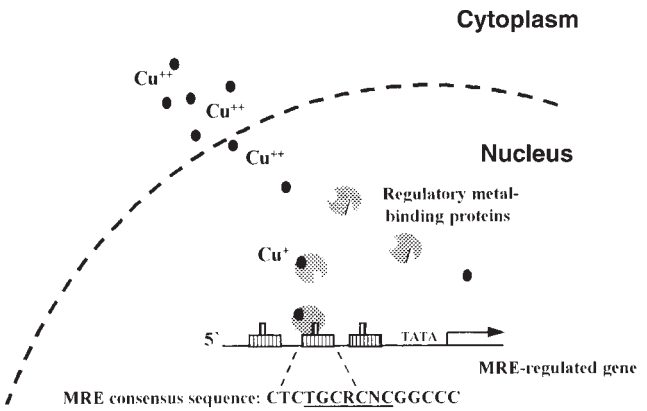

FIGURE 1. Model for eukaryotic copper-regulated transcription. Copper ions enter the nucleus and bind to regulatory metal-binding proteins (Ace1, Mac1, and Amt1). Binding of copper affects the tertiary structure of these proteins, which become activated to interact with specific upstream sequence metal-responsive elements (MREs) in the 5′-end of MRE-regulated genes. R, a purine nucleoside; N, a nucleoside.

hydrates, copper deficiency may be more prevalent because fructose and other refined sugars lower copper absorption.

Copper deficiency has been reported in subjects with malabsorption syndromes—such as celiac disease, tropical and nontropical sprue, cystic fibrosis, and short-bowel syndrome—resulting from intestinal resection (54, 55). Increased gastrointestinal copper losses usually explain the occurrence of copper deficiency in malabsorption syndromes. Copper deficiency should be suspected in infants with prolonged or recurrent diarrheal episodes, abnormal bile loss, intestinal resections, or loss of intestinal contents from intestinal fistula (54, 56). High oral intakes of zinc and iron decrease copper absorption and may predispose to copper deficiency (54, 57). This phenomenon is used as a therapeutic strategy in Wilson disease, in which a high zinc intake (40–50 mg/d) has been shown to lower copper absorption. Copper deficiency also has been documented in subjects receiving penicillamine, or other cation-chelating agents, or high doses of oral alkalis, which enhance copper losses (54).

During total parenteral nutrition, insufficient copper supply will occur unless subjects are supplemented with trace elements, including copper. In subjects with impaired biliary secretion or with cholestasis, copper supplementation should be reduced to compensate for the limited losses. In patients with impaired liver function, copper supplementation should be withheld unless biochemical signs of copper deficit are documented. Enteral alimentation products also need to contain adequate copper to meet the needs of individual patients (58–61).

On the basis of published information, the most common cause of copper deficiency is an insufficient copper supply during the nutritional recovery of malnourished children (62). Several factors are frequently associated with copper deficiency in these infants: low birth weight, short duration of breast-feeding, cow milk consumption, consumption of highly refined carbohydrate-based diets, increased losses of nutrients as a result of diarrheal disease, and frequent infections. During nutritional recovery, these infants grow at 5–10 times the normal rate for their age, thus increasing the requirements imposed by growth. This is yet another risk factor contributing to the 30–40% prevalence of biochemical signs of copper deficiency reported in these children.

Castillo-Durán et al (56) evaluated the magnitude of copper losses in 14 infants during an acute diarrheal episode requiring hospitalization. The results were compared with those obtained in 15 matched control infants. Fecal losses in the diarrhea group were two times greater than those in the control group. The diarrhea group was in negative copper balance up to 7 d after hospital admission. Copper losses were directly related to fecal weight. Furthermore, Rodriguez et al (55) compared the copper status of 19 children with chronic diarrhea with that of two control groups (19 healthy and 11 malnourished children). Plasma copper concentrations were 30% lower and hair copper content decreased three to fourfold in the chronic diarrhea group relative to the control groups. Low copper intakes during recovery from diarrhea may further limit copper nutriture.

Manifestations of copper deficiency in humans

Clinically evident copper deficiency is relatively infrequent in humans. The most common clinical manifestations of copper deficiency are anemia, neutropenia, and bone abnormalities, including fractures (54, 58, 62–64). Hematologic changes are characterized by the existence of hypochromic, normocytic, or macrocytic anemia, accompanied by a reduced reticulocyte count, hypoferremia, neutropenia, and thrombocytopenia (54, 62, 63–65). In a small proportion of cases there is microcytic anemia (54). Cytologic examinations of bone marrow reveal megaloblastic changes and vacuolization of the erythroid and myeloid progenitors. There is also an arrest of the maturation of myeloid precursors and the appearance of ringed sideroblasts (54, 62, 65). These alterations are unresponsive to iron therapy but are readily corrected by copper supplementation (66, 67). The prevailing view at this time is that anemia in copper deficiency is due to defective iron mobilization resulting from reduced ceruloplasmin activity (62, 64, 68).

Bone abnormalities are common in copper deficiency in low-birth-weight infants and in young children (58, 62, 64). These abnormalities, which mimic the changes observed in scurvy, include osteoporosis, fractures of the long bones and ribs, epiphyseal separation, fraying and cupping of the metaphyses with spur formation, and subperiostal new bone formation (62, 64). Less frequent manifestations of copper deficiency are hypopigmentation of the hair and hypotonia (62, 64), impaired growth (69), increased incidence of infections (70), and alterations of phagocytic capacity of the neutrophils (71). In addition, abnormalities of cholesterol and glucose metabolism have been reported, but are less well established (72–74). Prevalence of cardiovascular disease has been linked to high amounts of zinc and low amounts of copper in the diet, yet this hypothesis has not been validated (75).

Castillo-Durán et al showed that copper deficiency is associated with an increased incidence of infection (70) and impaired weight gain in infants recovering from malnutrition (69). The initial randomized, controlled trial included 27 infants recovering from protein-energy malnutrition: 13 received 80 μg $Cu \cdot kg^{-1} \cdot d^{-1}$ given as a supplement for 3 mo whereas 14 matched infants received a placebo. Plasma copper and ceruloplasmin dropped in the placebo group, 30% of whom had low plasma copper concentrations, whereas concentrations rose in the supplemented group during the rapid growth phase of recovery. The mean number of upper respiratory infections, days with fever, and febrile episodes per child per month were similar in both groups. However, seven infants in the placebo group had clinical

evidence of severe lower respiratory infection (mainly pneumonia) compared with only one subject in the copper-supplemented group ($P < 0.025$) (70). In a separate case-control study, 11 infants identified as copper deficient on the basis of low plasma copper and low ceruloplasmin concentrations and 10 matched copper-sufficient infants at a similar stage of their nutritional recovery were supplemented with 80 $\mu g\ Cu \cdot kg^{-1} \cdot d^{-1}$ as copper sulfate for 30 d. Daily weight gain and daily energy intake were significantly higher in the copper-deficient group than in the copper-sufficient group shortly after supplementation (69).

Copper deficiency is associated with altered immunity in humans (71, 76, 77). Heresi et al (71) studied 19 hypocupremic infants before and after 1 mo of copper supplementation. The phagocytic activity of polymorphonuclear leukocytes increased by 30% after copper supplementation whereas immunoglobulins remained unchanged. Kelley et al (77) described a decrease in the proliferation of peripheral blood mononuclear cells cultured with different mitogens in 11 men receiving a low-copper diet.

An increased concentration of total cholesterol and LDL cholesterol and a reduction of HDL cholesterol were observed in subjects fed an experimental diet low in copper (72, 73). A low copper intake was also shown to diminish glucose tolerance (74), alter cardiac rhythm and the electrocardiogram, and modify the hypertensive response to a hand-grip test (75). However, other studies have not validated the results of changes in cholesterol and glucose metabolism.

The role of copper deficit in altered neurodevelopment has been postulated on the basis of the high copper content of the brain, especially of the basal ganglia. The existence of a prenatal critical phase in central nervous system development, during which copper deficiency can cause damage to the central nervous system, has been suggested (64). This could explain the severe mental deficiency associated with prenatal copper tissue deficit found in Menkes syndrome whereas postnatal acquired nutritional copper deficiency is not accompanied by neurologic abnormalities.

REGULATION OF COPPER NUTRITURE IN EARLY LIFE

The fetus is fully dependent on the maternal copper supply and during pregnancy, copper and ceruloplasmin serum concentrations rise significantly (63, 78). Pregnancy is associated with increased copper retention, which may be due in part to decreased biliary copper excretion induced by hormonal changes typical during pregnancy (78). Serum copper after the first trimester of pregnancy rises from a mean of ≈15.7 μmol/L (≈100 μg/dL) to double this value during the last trimester. Maternal concentrations during the latter half of gestation are five to seven times concentrations measured in the cord.

The fetus accumulates copper at a mean rate of 50 $\mu g \cdot kg^{-1} \cdot d^{-1}$ principally over the latter half of pregnancy; >50% of the copper is stored in the liver, mainly in metallothionein (47). Fetal serum copper and ceruloplasmin are low and remain stable for most of the third trimester, despite active accretion in fetal tissues. The increase in fetal liver stores is due both to increased liver size and to a higher copper concentration per unit of liver weight (47). The brain is the second site for copper accumulation during fetal life; in Menkes syndrome, the brain copper content is extremely low and the basal ganglia and cerebellum are particularly affected. Copper uptake into the fetal compartment is dependent on placental carrier-mediated copper transport from ceruloplasmin (79, 80). Other copper-binding complexes such as albumin or histidine-bound copper can also contribute to the carrier-mediated system (81). By the end of gestation, a full-term fetus will have accumulated close to 15 mg Cu, 9 mg of which will be in the liver. After birth, the concentration of copper in the liver drops steadily because the copper supply from the diet rarely meets the requirements for rapid growth typical of this stage. In addition, biliary secretion increases steadily during early life, augmenting fecal copper losses. Plasma copper and ceruloplasmin rise after birth, reaching adult values by 6 mo of age (82, 83). Mean serum copper on day 7 ranges from 4.7 to 7.9 μmol/L (30 to 50 μg/dL), depending on gestational age, and ceruloplasmin concentrations range from 3.1 to 4.7 μmol/L (20 to 30 mg/dL) accordingly.

Traditional mineral balance studies conducted in preterm or full-term infants fed cow milk–based diets or unfortified pasteurized human milk indicated that most infants were in negative copper balance or at best in marginally positive balance (48, 84). These observations, coupled with the lack of an effect of copper supplementation in determining a faster rise in serum copper and ceruloplasmin after birth, suggested that infants do not absorb copper well. Changes in intake during the first months of life up to four times the estimated requirements do not affect copper or ceruloplasmin concentrations (85). More recent studies in infants fed modern artificial formula or unpasteurized human milk, which used a combination of chemical-balance and stable-isotope-tracer (^{65}Cu) methods, indicated that preterm infants can absorb copper; moreover, if sufficient copper is provided they can retain enough copper to meet the requirements imposed by growth. Infants fed preterm human milk absorb close to 60% of their intake whereas those receiving premature formula will absorb only 15% (51) (**Figure 2**, A); the respective values for true ^{65}Cu absorption are 67% and 39%, respectively (51) (Figure 2, B). Fecal copper losses are higher with increasing intake whereas percentage absorption decreases (51) (Figure 2, A and B). The interpretation of these results is confounded by the fact that human milk provides much less copper and is associated with significantly higher copper absorption. The absolute retention of copper in infants fed human milk approached the expected retention based on in utero accretion data. The excretion of copper in the feces was directly correlated with fat and nitrogen fecal losses; thus, if fat is malabsorbed, copper is lost (51). This finding may explain some of the differences between results of modern studies of copper balance and those of older studies using pasteurized human milk or old artificial formulas, both of which are associated with higher fecal fat losses.

In summary, modern copper balance studies show that copper balance in early life is affected by the type of feeding and the amount of copper supplied. If insufficient copper is supplied or if the copper supplied is poorly absorbed, deficiency may occur. Infants adjust copper absorption, reducing absorption at higher intakes and increasing absorption at lower intakes.

The elevated hepatic copper stores present at birth and the low ceruloplasmin concentrations early in life suggest that infants cannot adjust to high copper intakes. Some have suggested that even minimal copper loads may be associated with liver toxicity, on the basis of the rare occurrence of Indian childhood cirrhosis and idiopathic copper toxicosis; for idiopathic copper toxicosis, the incidence is estimated to range from 1 in 100 000 to 1 in 1 000 000. According to these findings, copper should be eliminated from the diet early in life. This is in sharp contrast with the common occurrence of biochemical signs of copper deficiency when copper is not added to infants' diets and the frequent

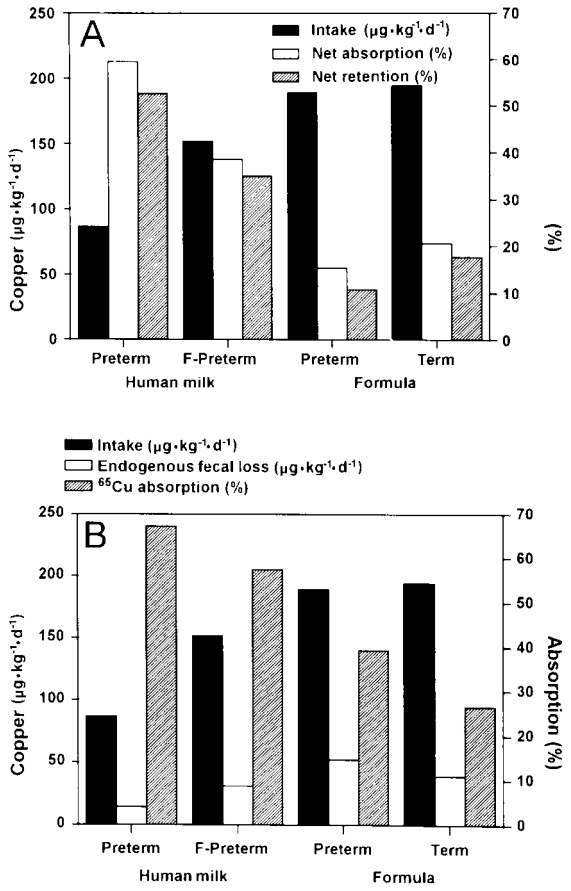

FIGURE 2. Effect of copper intake and type of infant feeding on mass copper balance (A) and on ^{65}Cu absorption and endogenous copper losses (B) in very-low-birth-weight infants. Data derived from reference 52. F-Preterm, fortified with a powdered protein-mineral supplement.

reports of clinical copper deficiency in low-birth-weight infants and malnourished children given low-copper diets. The available evidence suggests that indeed most infants are at risk of copper deficit unless copper is provided. Low birth weight and protein-energy malnutrition affect a sizable proportion of children throughout the world. It has been estimated that > 10% of children are born with low birth weight and that 20% of the children in the world are underweight. Thus, the risk of deficiency must be weighed against the risk of potential toxicity, which affects an extremely few selected individuals who most likely carry a genetic predisposition for copper accumulation.

Recommendations for infant feeding and total copper exposure, including copper in potable water, should take into account that copper is an essential nutrient and that toxicity is possible if the copper load exceeds tolerance. A range of safe intakes should be defined for the general population, including a lower safe intake and an upper safe intake, to ensure that for most of the population both deficiency and toxicity are prevented. When setting guideline values for populations, the special needs of genetically susceptible individuals should be considered only if there is public health significance.

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