Copper as an essential nutrient[1,2]

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ABSTRACT Animal and human studies have shown that copper is involved in the function of several enzymes. Studies have also shown that copper is required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism, myocardial contractility, and brain development. Copper deficiency can result in the expression of an inherited defect such as Menkes syndrome or in an acquired condition. Acquired deficiency is mainly a pathology of infants; however, it has been diagnosed also in children and adults. Most cases of copper deficiency have been described in malnourished children. The most constant clinical manifestations of acquired copper deficiency are anemia, neutropenia, and bone abnormalities. Other, less frequent manifestations are hypopigmentation of the hair, hypotonia, impaired growth, increased incidence of infections, alterations of phagocytic capacity of the neutrophils, abnormalities of cholesterol and glucose metabolism, and cardiovascular alterations. Measurements of serum copper and ceruloplasmin concentrations are currently used to evaluate copper status. These indexes are diminished in severe to moderate copper deficiency; however, they are less sensitive to marginal copper deficiency. Erythrocyte superoxide dismutase and platelet cytochrome *c* activities may be more promising indexes for evaluating marginal copper deficiency. *Am J Clin Nutr* 1996;63:791S–6S.

KEY WORDS Copper deficiency, anemia, neutropenia, infection, growth

INTRODUCTION

Copper is essential for the survival of plants and animals. Animal and human studies have shown that copper is involved in the function of several enzymes (1–4). The essentiality of copper for humans was first shown during the 1960s in malnourished children from Peru (5). These children had an anemia refractory to iron therapy, neutropenia, and bone abnormalities that were responsive to copper supplementation. Further studies confirmed these findings and established that copper was required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol metabolism, myocardial contractility, glucose metabolism, and brain development (6). Major alterations in mental development are also observed in Menkes syndrome, which is a genetic syndrome in which alterations in copper absorption and transport lead to early death (7).

DIETARY SOURCES

Concentrations of copper in food are highly variable. Organ meats, oysters, and chocolate are the richest sources of copper in the diet (8–10). Human and cow milk are poor sources of copper (11); however, breast milk has a higher copper content than does cow milk. On the other hand, the copper concentration in breast milk declines with the time of lactation: colostrum and transitory milk have the highest values (12, 13).

Most infant formulas are supplemented with copper. The copper content in infant formulas varies depending on the need of the infant (full-term or preterm) (14, 15). The US Food and Drug Administration, the Codex Alimentarius (1976), and the American Academy of Pediatrics (1985) recommend a minimum specification for infant formulas of 0.2 μmol Cu/kJ (0.6 μg Cu/kcal) (16–18). The current recommendation of the European Society of Paediatric Gastroenterology and Nutrition Committee on Nutrition (1987) is 0.3 μmol Cu/kJ (0.9 μg Cu/kcal) (19). Recommendations for premature formulas are 0.3–0.7 μmol Cu/kJ (0.9–2 μg Cu/kcal) (11, 18, 19).

The copper content of drinking water is also highly variable and is influenced by the natural mineral content and pH of the water and by the plumbing system (20). Soft, acidic water, especially if it is conducted through a copper pipeline, has a higher copper concentration (20). In addition, copper salts are added in some countries to control the growth of algae. Therefore, the copper in drinking water may constitute an important source of copper for the adult population (20, 21). Assuming that the copper content of drinking water is between 1.6 and 7.9 μmol/L (0.1 and 0.5 mg/L), an adult who ingests 2 L water daily will obtain 13–50% of the estimated safe and adequate daily dietary intake (ESADDI) proposed by the US National Academy of Sciences (22). This may explain why there is little evidence of copper deficiency in adult populations even when the consumption of foods rich in copper is low. In infants, the contribution of water to copper intake may be higher because infants consume proportionally more water than do adults.

DIETARY INTAKE

The Total Diet Study (1982–1986), in which the copper content of US diets was measured by chemical analyses (23), indicated that the daily mean intake of copper was below the

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ESADDI (22). The average copper intake of eight sex-and-age groups is shown in **Figure 1**. Additional studies conducted in the United States, the United Kingdom, France, Finland, Germany, Sweden, New Zealand, and Belgium showed daily copper intakes under or in the low range of the ESADDI (24–30). Despite these inadequate copper intakes, there was a low prevalence of copper deficiency in these countries. This discrepancy questions the validity of the ESADDI. The assessment of the adequacy of the diet to fulfill copper requirements must take into account the issue of bioavailability. Depending on the copper content of the diet and other diet-related factors, only 25–60% of ingested copper is absorbed (31).

DEFICIENCY OF COPPER IN HUMANS

Acquired copper deficiency

An acquired deficiency of copper is mainly a pathology of infants; however, cases have also been described in children and adults. The liver stores copper prenatally, which provides a sufficient amount of copper to sustain normal copper nutrition in the body during the first 2 and 5 mo of life in preterm and full-term infants, respectively (15). A deficiency of this mineral could be the result of decreased copper deposits at birth, inadequate dietary copper intake, malabsorption, increased requirements, or enlargement of the copper loss (**Table 1**). Many of these etiologies are found together in copper-deficient subjects.

Premature infants are much more prone to develop copper deficiency because of their reduced storage of liver copper at birth and their higher requirements due to their high growth rate (32–36). Inadequate copper intake could occur in infants fed cow milk exclusively because of the minor content of copper and low absorption of this mineral in cow milk compared with breast milk (37, 38). Other causes of insufficient copper intake occur in subjects receiving total parenteral or enteral nutrition when the nutritional formula is not supplemented with copper (39–42). High oral intakes of zinc, iron, or ascorbic acid decrease copper absorption and might predispose to copper deficiency (43–46). Yet, copper deficiency has been described in subjects who received penicillamine or high doses of oral alkali therapy (46). Some cases of copper deficiency occurred in subjects with malabsorption syndromes such as

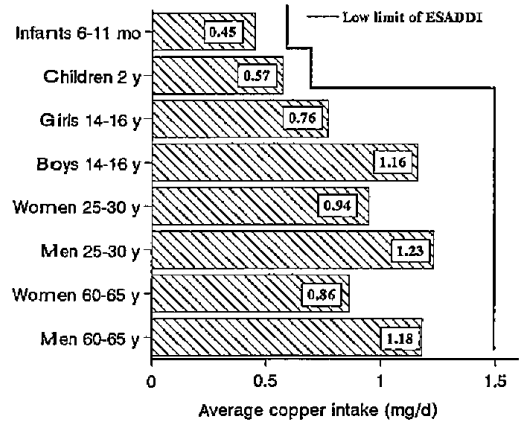

FIGURE 1. Average daily copper intakes in different sex-and-age groups in the Total Diet Study (23).

TABLE 1
Etiology of acquired copper deficiency

Decreased copper stores at birth
Low birth weight
Inadequate copper supply
Low dietary copper
Low bioavailability of dietary copper
Total parenteral nutrition with inadequate copper supplement
Inadequate copper absorption
Malabsorption syndrome
Increased requirements
High growth rate
Increased losses
Repeated or prolonged episodes of diarrhea
Abnormal bile loss
Intestinal loss from small intestinal ostomies

celiac disease, tropical and nontropical sprue, cystic fibrosis, or short bowel syndrome (46, 47). Copper deficiency due to an increase of nutrient losses could also occur in infants with prolonged or recurrent diarrheal episodes, abnormal bile loss, or intestinal loss from small intestinal ostomies (44, 46).

Most cases of copper deficiency have been described in malnourished children (15). In these subjects, several factors are frequently associated with deficiency, such as low birth weight, short breast-feeding time, cow milk–based feeding, and increased losses of nutrients as a result of diarrheal disease. An increase in copper requirements, imposed by a high rate of growth during the rehabilitation period, can be an additional predisposing cause of copper deficiency in these subjects.

In a recent review, 51 copper-deficient children were analyzed (15). Of the 51 subjects, 48 were < 19 mo of age at the time of diagnosis. Forty percent of the patients had a birth weight < 2.5 kg. Fifty-four percent of the infants were fed exclusively or predominantly cow milk. Twenty-three percent received total parenteral nutrition and 25% had an antecedent of malnutrition. The average age of presentation of the copper deficiency was 8.3 mo (range: 5–18 mo) for full-term infants and 3.0 mo (range: 2.2–15 mo) for low-birth-weight infants.

The most constant clinical manifestations of acquired copper deficiency are anemia, neutropenia, and bone abnormalities (2, 6, 15, 39, 46). The frequencies of anemia and neutropenia observed in infants were 92% and 84%, respectively (15). Hematologic changes are characterized by the existence of hypochromic, normocytic, or macrocytic anemia, which is also accompanied by a reduced reticulocyte count, hypoferremia, neutropenia, and thrombocytopenia (2, 6, 15, 46–48). In a few cases microcytic anemia is present (46). In bone marrow, megaloblastic changes, vacuolization of the erythroid, and myeloid progenitors may be found, in addition to a maturation arrest of myeloid precursors and the existence of ringed sideroblasts (15, 46, 48). All of these alterations are reversed through copper supplementation and are unresponsive to iron therapy (49, 50).

It has been hypothesized that the anemia associated with copper deficiency is due to defective iron mobilization resulting from reduced ceruloplasmin activity (6, 15, 51). This enzyme, by its ferroxidase action, is fundamental for the transformation of Fe^{2+} to Fe^{3+} (52), a step indispensable for the incorporation of iron into circulating transferrin. The reduction of ceruloplasmin may determine that the iron remains trapped

in the reticuloendothelial system and is therefore not available for erythropoiesis. However, in Menkes syndrome and Wilson disease there is a reduction in the concentration of ceruloplasmin that is not accompanied by anemia (7, 46). On the other hand, there is some evidence of a reduction in the synthesis of heme because of decreased ferrochelatase or cytochrome *c* activity (53). These enzymes are fundamental for the reduction of Fe^{3+} to Fe^{2+} in mitochondria and the subsequent incorporation of iron into protoporphyrin IX (15). A reduction of erythropoietin has been described in rats (54).

The presence of bone abnormalities is common with copper deficiency in infants and young children (6, 15, 39). These abnormalities 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 (6, 15). Other less frequent manifestations of copper deficiency are hypopigmentation of the hair, hypotonia, impaired growth, increased incidence of infections, and alterations of phagocytic capacity of the neutrophils (6, 15, 55–57). In addition, less well-established manifestations of this deficiency are abnormalities of cholesterol and glucose metabolism and cardiovascular alterations (58–61). The hypopigmentation of the hair described in Peruvian children with copper deficiency is difficult to interpret because these children were also severely malnourished (5). It is well known that hypopigmentation of the hair occurs frequently with protein malnutrition.

Castillo-Durán and Uauy (55) showed that copper deficiency impaired weight gain of infants recovering from malnutrition. Additionally, these infants had an increased frequency of lower respiratory tract infections (56). In animals, a deficiency of copper is associated with pronounced alterations of immunity (62). However, there is little information concerning nutritional copper deficiency in humans. Heresi et al (57) reported an impaired phagocytic activity in copper-deficient infants. On the other hand, Kelley et al (63) described a decrease in the proliferation of peripheral blood mononuclear cells cultured with different mitogens in 11 men consuming a low-copper diet.

An increased concentration of total cholesterol and low-density-lipoprotein (LDL) cholesterol and a reduced concentration of high-density-lipoprotein (HDL) cholesterol have been observed in subjects fed an experimental diet that was low in copper (58, 59). Other observations are a diminished tolerance to glucose (60), abnormal electrocardiograms, and a hypertensive response to a hand-grip test (61). Furthermore, these changes can be reversed with copper supplementation (58–61). All these alterations are well-known risk factors for the development of atherosclerosis (64). However, other experiments have not reproduced these changes in cholesterol and glucose metabolism (63).

Several effects of copper on atherogenesis can be postulated. Although copper intake and a high ratio of copper to zinc have been traditionally considered to be protective factors (65), more recently the opposite has been proposed (66, 67). Some epidemiologic studies showed an association between cardiovascular mortality and copper intake or serum copper concentrations. An increase in risk with both high and low copper intakes has been described, as has an increase with high and low serum copper concentrations (65–67). In U-shaped relations, it is possible that the different mechanisms act in both extremes. In copper deficiency, alterations of glucose and cholesterol metabolism, increased blood pressure, endothelial cell peroxidation due to a decrease in superoxide dismutase (SOD) activity, and arterial prostacyclin production may contribute to atherogenesis (68). In copper overload the atherogenesis can be attributed to a direct effect on LDL-cholesterol oxidation by copper. This is clearly demonstrated in vitro yet it has not been proven in vivo (69). Copper is a prooxidant; thus, it will act on cysteine residues of the LDL apolipoprotein B component, modifying the structure and binding properties of LDL to cell receptors affecting cholesterol uptake by cells (70). The atherogenicity of oxidized LDL particles has been evaluated and is considered to be greater than that of the nonoxidized forms (70, 71). In addition, oxidation of LDL could be potentiated by a reduction of selenium, vitamin E, and ascorbic acid concentrations (72).

Genetic copper deficiency

Menkes syndrome is an X-linked recessive disorder that is characterized by an altered absorption and transport of copper, which causes an abnormal distribution of the mineral between organs as well as within cells (7). Symptoms of Menkes syndrome appear before 3 mo of age and the illness usually ends the life of the child before 5 or 6 y of age. The disease is characterized by growth retardation, hypothermia, skin and hair depigmentation and abnormal spiral twisting of the hair (pili torti), lax skin and articulations, tortuosity and dilatation of major arteries, varicosities of veins, osteoporosis, flaring of metaphyses, bone fractures, excessive wormian bone formation, retinal dystrophy, and profound central nervous system (CNS) damage (7). CNS alterations include severe mental retardation, seizures, and ataxia. Pathologic studies show intense degenerative changes of the brain and the cerebellum, with a pronounced alteration of the Purkinje cells (73). The existence of a prenatal critical phase in CNS development has been suggested, during which copper deficiency can cause CNS damage (6). This explains why nutritional copper deficiency is not accompanied by neurologic abnormalities. Menkes syndrome is not accompanied by anemia or neutropenia (7).

ASSESSMENT OF COPPER STATUS

Measurements of serum copper and ceruloplasmin concentrations are currently used to evaluate copper status (74, 75). These indexes are less sensitive to marginal copper deficiency, especially if the deficiency only recently appeared (76–78). However, concentrations of these laboratory indexes are diminished in severe to moderate copper deficiency. The normal ranges for these indexes are 10.1–24.6 μmol/L (64–156 μg/dL) for serum copper and 180–400 mg/L for ceruloplasmin (3). Serum concentrations of copper and ceruloplasmin change in relation to age and sex (76). During the first months of life, concentrations of copper and ceruloplasmin are low; adult values are attained at 4–6 mo of age (79). In low-birth-weight infants these concentrations rise more slowly (80). On the other hand, it is well known that adult women have higher concentrations of serum copper than do men (76, 81). During pregnancy there is a progressive rise in the concentrations of serum copper and ceruloplasmin (76, 81).

Other conditions also modify these laboratory indexes. The concentration of copper has a diurnal variation: it is slightly higher in the morning than at other times during the day (82). In the inflammatory or infectious processes, with neoplasm, and with therapy with anticonvulsants or estrogens, copper and ceruloplasmin concentrations are increased (75, 76, 81, 83–85). The effect of estrogens can partly explain the increase in copper and ceruloplasmin observed during pregnancy (86). On the contrary, corticosteroids and adrenocorticotropic hormone reduce copper concentrations (75). Copper or ceruloplasmin concentrations, or both, are also decreased in other conditions such as Menkes syndrome, Wilson disease, and nephrosis (87, 88).

Studies by one group in which the enzymatic activity and concentration of ceruloplasmin were measured showed that in copper deficiency enzymatic activity of ceruloplasmin is reduced and the ceruloplasmin concentration is conserved (76, 89). Therefore, the ratio of enzymatic activity to concentration of ceruloplasmin may be a better indicator of copper status, with the additional advantage that such an index is not influenced by factors such as hormones and sex (76).

Measurements of copper in hair have not proven to be very useful because copper is reduced only in prolonged copper deficiency and can vary as the result of the action of external agents, including environmental contamination with copper (6, 75).

SOD is an enzyme found in the cytosol of many cells, including the erythrocyte. Reduced SOD activity has been shown in animal models of copper deficiency; the decrease is proportional to the magnitude of the deficiency (90). Studies in humans have shown decreased activity of erythrocyte SOD in copper-deficient patients or in subjects with low copper intakes (91, 92). SOD activity was restored to normal when the subjects were supplemented with copper or when this mineral was added to the diet (58, 76, 92). Erythrocytic SOD activity does not seem to vary with age, sex, or hormonal therapy (74, 81, 93). However, a higher SOD activity can be found in demented patients with Alzheimer disease and conditions that produce oxidative stress (94–96).

Studies carried out in humans by one group have shown that cytochrome *c* activity of leukocytes and platelets is reduced in copper deficiency (89, 97). Furthermore, this decrease occurs before the appearance of reduced SOD activity (89). These findings suggest that the measurement of cytochrome *c* activity in leukocytes or platelets is a sensitive indicator of copper status (76). However, these results are not confirmed by others and therefore deserve further investigation.

Metallothionein is a protein that selectively binds heavy metals and participates in the metabolism of zinc and copper (98). The tissue metallothionein concentration is modified by zinc and copper status (98). A reduction of erythrocyte metallothionein has been shown in moderate zinc deficiency (99). Therefore, it is conceivable that erythrocyte metallothionein measurement may be another useful laboratory indicator of copper status. However, it has been shown that stress and inflammatory processes increase tissue concentrations of metallothionein (100, 101). Further investigations are needed to develop simple laboratory indicators that are sensitive to marginal copper deficiency and that are not influenced by factors other than copper status.

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