Bioavailability of copper\textsuperscript{1,2}

Bo Lönnerdal

ABSTRACT Copper intakes of infants and adults are often much lower than current recommendations. Copper status, however, appears adequate in most populations. This suggests that copper requirements may be lower than believed earlier, except those for premature infants, who have high requirements as a result of low prenatal stores. Infants, in general, constitute a risk group because milk is low in copper. Bioavailability of copper from human milk is high, whereas it is lower from cow milk and infant formula. Protein source, amino acids, carbohydrates, and ascorbic acid can affect copper availability, whereas phytate, zinc, and iron appear to have little influence on copper absorption, at least at physiologic intakes. Am J Clin Nutr 1996;63:821S–9S.

KEY WORDS Copper, copper absorption, copper bioavailability, copper status, infants

DIETARY SUPPLY OF COPPER

Copper intake

Similar to several other trace elements, copper in the diet is affected by the concentration of copper in the soil (1). Concentrations of copper in plants and animals are influenced by local conditions. Thus, food tables in which copper concentrations are given need to be reviewed with some caution; local conditions may make published values irrelevant. Some foods are always high in copper, such as organ meats (liver and kidney) and, to some extent, cereals, some fruit, and nuts, whereas other foods are always low in copper (2). Among the poor sources of copper are milk and dairy products. Representative samples of foods and their copper contents are given in Table 1, but it should be kept in mind that these values will vary with location.

Another source of copper that must be considered is drinking water (3). Well water has a highly variable copper content that is dependent on the soil and the underlying water table. Concentrations can occasionally be high, although concentrations $>5$ mg/L usually lead to discoloration of the water and a bitter taste. However, even if very high values are unusual and represent a range that is at risk of precipitating acute toxicity, the contribution of copper from water to the dietary supply must be included. Because copper pipes are now common in many areas, the additional amount of copper provided from the pipes must also be considered. This is of particular concern when the water is slightly acidic or very soft. The World Health Organization (WHO) has a provisional limit of 2 mg Cu/L for tap water (4), but the foundation for setting a limit for copper toxicity is limited (5).

In some early studies, daily intake of copper in adults was estimated to be $2–5$ mg (2). More recent dietary intake studies showed mean values of $1.24$ mg/d in men and $0.93$ mg/d in women (1). Gibson (6) compiled several studies and found that copper intakes in adults were $\approx 1.0–1.5$ mg/d from omnivore diets, whereas vegetarian diets provided larger quantities of copper, $2.1–3.9$ mg/d. Copper intakes of children were $\approx 0.8–1.9$ mg/d, with most of the higher intakes from vegetarian diets. These intakes should be contrasted with the estimated safe and adequate daily dietary intake (ESADDI) of the Food and Nutrition Board, which is $1.5–3.0$ mg/d (7). Further amplifying the discrepancy between common copper intakes and ESADDI values is the youngest age group, infants aged 0–0.5 y, who often have very low intakes of copper ($0.08–0.16$ mg/d) due to the low concentrations of copper in breast and cow milk. For this group the ESADDI is $0.4–0.6$ mg/d. A study by Salim et al (8) also emphasized the large discrepancy between the copper intake of breast-fed and formula-fed infants and the WHO recommended minimum intake of $80 \mu g \cdot kg^{-1} \cdot d^{-1}$ ($1.3 \mu mol \cdot kg^{-1} \cdot d^{-1}$) for this age group. Sorenson and Butrum (9) also found low copper intakes from infant foods but no signs of deficiency.

Infant diets

The copper concentration of human milk is $\approx 0.4–0.6$ mg/L in early lactation (10, 11) and $\approx 0.2–0.3$ mg/L in mature milk (12). Milk from women giving birth prematurely appears to be higher in copper than milk from women with term infants (13). Most of the copper in milk is associated with serum albumin, whereas a minor fraction is associated with casein (14). In contrast with zinc, little copper in human milk is associated with low-molecular-weight ligands such as citrate (15, 16). Despite the declining concentration of copper in breast milk during lactation, serum copper and ceruloplasmin concentrations in exclusively breast-fed infants increase, suggesting that the copper requirements of these infants are met (17).

Because cow milk is even lower in copper concentration than human milk, most formulas are fortified with copper. However, a large survey of infant formulas from various countries showed that there is little consensus and that these products exhibit a wide range of copper concentrations (18). In some cultures, copper may be leached out from cooking utensils into milk that is heated or stored in such containers (19). Contamination of milk with copper from brass vessels is believed to cause a rare but severe copper toxicity called Indian childhood cirrhosis (19, 20). Studies have shown that copper may be,

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released from the brass alloy by low-molecular-weight ligands (such as citrate) and then associate with casein in the milk (21).

The contribution of copper from copper piping to tap water (which is originally low in copper) was recently examined in a study of formula-fed infants (22). Parents were instructed to allow the tap water to be standing in the pipes by not running water for 12 h and then to make up formulas based on powder with this water. Copper was analyzed in formula made of tap water or deionized water. The additional amount of copper provided by tap water was only 0.03 mg/L compared with the US formula copper concentration of 0.4–0.6 mg/L. This may represent a low end of the spectrum of copper contamination from copper piping but could also indicate that such contamination is lower than believed earlier.

## COPPER STATUS

The indicators most commonly used to assess copper status include serum (or plasma) copper, serum ceruloplasmin, and erythrocyte superoxide dismutase (SOD). These indexes are all decreased in copper deficiency, but their value in detecting marginal copper deficiency is questionable (23). Serum copper and ceruloplasmin concentrations are usually closely correlated with each other because ceruloplasmin is the major copper-binding protein in serum. However, serum copper and ceruloplasmin concentrations may be affected by factors other than copper status. Infection (inflammation), pregnancy, use of oral contraceptives, and cancer result in higher-than-normal concentrations of ceruloplasmin and serum copper. Erythrocyte SOD activity is believed to better reflect long-term copper status but has not been used in many studies to date (24). Turnlund et al (25) found no change in erythrocyte SOD in young men consuming three different levels of dietary copper (0.78, 1.68, or 7.53 mg/d for 90 d). There were no significant differences in serum copper, ceruloplasmin, or urinary and salivary copper either, suggesting that these indexes may respond only to suboptimal copper intakes. A large interindividual variation was observed in this study, however, possibly reducing the diagnostic value of these indicators. Hair copper concentrations have also been used as an indicator of copper status, particularly in infants (26, 27). However, several factors other than copper status (such as growth and concentrations of copper in water) may also affect hair copper concentrations (28), making this a less reliable indicator.

### Infants and children

Although copper concentrations in human and cow milk are low, 1.5–5 μmol/L (0.1–0.3 mg/L), copper deficiency is rare in term infants (29). Formulas that are not fortified with copper have also been shown to result in satisfactory copper status (30, 31). However, most infant formulas are supplemented with copper to a total concentration of 6–9 μmol/L (0.4–0.6 mg/L), most likely because these formulas may also be fed to prematurely born infants. Copper deficiency in premature infants fed cow milk has been shown in many studies (32–34). Copper stores, particularly in liver, accumulate dramatically during the third trimester (35), and any shortening of the fetal accumulation of copper results in lower copper stores than normal. It is believed that these copper stores are utilized during early infancy when copper intake is low. Serum copper and cerulo-

### TABLE 1

**Copper content of foods**

<table>
<thead>
<tr>
<th>Food</th>
<th>Copper content mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td>Human colostrum</td>
<td>0.57 (0.24–0.76)</td>
</tr>
<tr>
<td>Human</td>
<td>0.2–0.76</td>
</tr>
<tr>
<td>Cow</td>
<td>0.1–0.88</td>
</tr>
<tr>
<td>Nonfat dry</td>
<td>0.7</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>157</td>
</tr>
<tr>
<td>Lamb</td>
<td>56</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>2.1–4.3</td>
</tr>
<tr>
<td>Muscle meat</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>0.1–1.8</td>
</tr>
<tr>
<td>Pork</td>
<td>0.1–9.1</td>
</tr>
<tr>
<td>Cereals</td>
<td></td>
</tr>
<tr>
<td>Corn (maize products)</td>
<td>0.6–16.6</td>
</tr>
<tr>
<td>Wheat products</td>
<td>3.3–36.0</td>
</tr>
<tr>
<td>Rice products</td>
<td>0.6–3.1</td>
</tr>
<tr>
<td>Wheat bread</td>
<td>2.9</td>
</tr>
<tr>
<td>Whole-wheat bread</td>
<td>3.4</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>0.48–16.0</td>
</tr>
<tr>
<td>Potato chips</td>
<td>2.2–3.6</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>0.15</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.37–0.62</td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.68–0.87</td>
</tr>
<tr>
<td>Peas</td>
<td>1.9–2.4</td>
</tr>
<tr>
<td>Lettuce</td>
<td>0.1–2.9</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.1–3.4</td>
</tr>
<tr>
<td>Sweet corn</td>
<td>0.02–0.15</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0.1–1.7</td>
</tr>
<tr>
<td>Seafoods</td>
<td></td>
</tr>
<tr>
<td>Oysters</td>
<td>0.3–16.0</td>
</tr>
<tr>
<td>Tuna</td>
<td>0.1–1.2</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.5–0.8</td>
</tr>
<tr>
<td>Shrimp</td>
<td>2.0–2.9</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>0.1–3.3</td>
</tr>
<tr>
<td>Flounder</td>
<td>0.1–2.5</td>
</tr>
<tr>
<td>Catfish</td>
<td>1.4–2.5</td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>0.1–2.3</td>
</tr>
<tr>
<td>Bananas</td>
<td>0.7–3.0</td>
</tr>
<tr>
<td>Grapes</td>
<td>0.74–1.5</td>
</tr>
<tr>
<td>Peaches</td>
<td>1.1–1.4</td>
</tr>
<tr>
<td>Pineapple</td>
<td>0.86–0.96</td>
</tr>
<tr>
<td>Prunes</td>
<td>3.7–5.0</td>
</tr>
<tr>
<td>Raisins</td>
<td>2.7–4.1</td>
</tr>
<tr>
<td>Oranges</td>
<td>0.8–0.9</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>0.3–0.6</td>
</tr>
<tr>
<td>Ice cream</td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td>0.3–3.4</td>
</tr>
<tr>
<td>Vanilla</td>
<td>0.1–0.9</td>
</tr>
<tr>
<td>Strawberry</td>
<td>0.1–1.4</td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
</tr>
<tr>
<td>Peanuts</td>
<td>2.7–9.6</td>
</tr>
<tr>
<td>Pecans</td>
<td>9.7–13.6</td>
</tr>
<tr>
<td>Walnuts</td>
<td>2.0–13.9</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>14.3–19.0</td>
</tr>
</tbody>
</table>

*From Davis and Mertz (2).*
plasmin concentrations increase rapidly after birth, making this a likely scenario. When copper stores are low, low-copper diets like cow milk cannot provide enough copper for incorporation into tissues and ceruloplasmin synthesis; thus, the infant becomes copper deficient. Although breast milk is also low in copper, copper deficiency is rare in premature infants fed breast milk—perhaps because of a higher bioavailability of copper from human than from cow milk.

When cow milk is fed to term infants for prolonged periods of time, copper deficiency may be induced. Levy et al (36) showed in a case report on two 6-mo-old infants, born at term and otherwise healthy and well nourished, that copper deficiency was manifested by low serum copper and ceruloplasmin, microcytic anemia, neutropenia, and X-ray abnormalities. These infants were not given copper supplements; provision of chicken, meat, and vegetables restored their copper status to normal and the anemia resolved. This indicates that most other foods have higher concentrations of copper than does milk, and that amounts of copper in these foods are sufficient to compensate for a prior deficiency. After infants are weaned, cereals and other foods will provide more copper than milk, and copper intake increases rapidly. Studies on copper status in older infants and children indicate that copper intakes at this age meet requirements for growth and maintenance (9).

**Preterm infants**

Both term and preterm infants are born with low serum copper concentrations (37–39). Values of 0.29 and 0.33 mg/L, respectively, have been reported at birth. The values then increase rapidly, more rapidly in term infants than in healthy preterm infants (40) or, particularly, in sick preterm infants (41). Whereas serum copper values have reached adult concentrations at 1 mo in term infants (37), such concentrations are not attained in preterm infants until 4–6 mo of age (40). A slower increase in serum copper continues up to 12 mo of age (39). Another important factor is the growth of the infant. Preterm infants with normal growth were found to have lower serum copper than those with poor growth (41), thereby increasing the risk for copper deficiency. Hair copper concentrations of preterm infants are lower than those of term infants at this age, suggesting somewhat lower copper status (42). Supplementation with copper in the formula by up to four times the normal amount fails to increase serum copper significantly during early life (43). L’Abbé and Friel (40) also noted that serum copper was similar in very-low-birth-weight (VLBW) infants regardless of copper intake; however, erythrocyte SOD and hair copper were higher in infants receiving copper supplements. It should be cautioned, though, that erythrocyte SOD is affected by transfusion and that a large proportion of VLBW infants are transfused (44).

Intravenous copper supplementation of preterm infants showed a marginal increase in serum copper concentrations when 40 μg·kg⁻¹·d⁻¹ was used compared with 20 μg·kg⁻¹·d⁻¹ (45), but ceruloplasmin values were not affected. This supports the earlier hypothesis that liver ceruloplasmin synthesis is immature in preterm infants. Friel et al (27) found higher serum copper concentrations in preterm infants receiving parenteral nutrition; however, these infants had received blood transfusions providing both copper and ceruloplasmin. Graham and Cordano (46) showed that a combination of these is more efficient in raising serum copper than a similar amount of copper injected as a salt. Because copper deficiency can cause bone abnormalities in preterm infants, Koo et al (47) studied a group of preterm infants with rickets and bone fractures. No differences in serum copper or ceruloplasmin concentrations were found between this group and a group without radiographic indications of bone problems.

Not only gestational age but also maternal nutrition during pregnancy may affect infant copper status. Krishnamachari and Rao (48) found that infants born to undernourished mothers had significantly lower serum copper and ceruloplasmin concentrations at birth than did infants of well-nourished women. Because albumin concentrations were also lower in infants of undernourished women, it was suggested that the liver’s capacity to synthesize protein was impaired in general in these infants.

In copper-deficient infants, it is mainly the ceruloplasmin-bound fraction of serum copper that is decreased (49). The nonceruloplasmin fraction of serum copper is much less affected and is restored more rapidly when copper supplementation is started. Apoceruloplasmin cannot be detected in human serum during copper deficiency, suggesting that even if the apo form can accumulate in the liver (49), ceruloplasmin is not released until the holo form can be formed. However, even if apoceruloplasmin cannot be detected in its completely unsaturated form, low ceruloplasmin enzyme activity concomitant with normal immunoreactive ceruloplasmin concentrations have been observed in copper-deficient human adults. In fact, it has been suggested that the ratio between ceruloplasmin activity and its concentration determined by immunologic methods may be used as an indicator of copper status (50). To date, this ratio has not been used for infants.

The importance of normal bile flow for copper excretion is illustrated by infants with hepatobiliary disease (51). These infants have elevated liver copper concentrations. After surgery, biliary output of copper increases and liver copper concentrations decrease. The authors suggest that it is not a simple bile duct obstruction and that the metabolic pathway for copper excretion from the liver is different from that for bilirubin and bile acids. No correlations were found between biliary copper, biliary lipid, or bile pigment excretion.

**COPPER ABSORPTION**

Radioisotope studies in experimental animals suggest that copper is absorbed from the stomach to some extent, but that the major site of absorption is the duodenum (52). Some absorption of copper also occurs at the distal part of the small intestine, making it possible that some copper excreted via bile may undergo enterohepatic circulation. It has been shown that copper absorption in rats is high during the neonatal period (53) but that it decreases by the time of weaning. A large proportion is retained by the small intestinal mucosa 6 h after gastric intubation of a radiolabeled meal and by 24 h postdosing much of this absorbed copper remains in the perfused intestine (53). With increasing postnatal age, more copper is transported to the liver and a smaller fraction is bound to the intestine. By the time of weaning (∼21 d), neither the mucosa nor the liver retains much of the ⁶⁴Cu from a labeled meal; the major fraction is now found in the cecum-colon and most likely corresponds to unabsorbed copper. Thus, age has a profound effect on the absorption of copper.
Varada et al (54) also studied the development of copper absorption in rats. Through use of kinetic studies, they found that copper absorption was saturable only in adolescent animals; in suckling and weaning rats, copper absorption was linear and nonsaturable. This may also explain why earlier we found a similar efficiency of copper absorption when the copper content of human milk was increased 10 times by the addition of a copper salt (53). Varada et al (54) found intestinal copper retention to be concentration-dependent, and that suckling rats had much higher tissue concentrations of copper than did weaning or adolescent rats. These authors found that induction of intestinal metallothionein that can bind copper was significantly higher in adolescent rats than in younger rats, strongly suggesting that metallothionein induction is not responsible for the high copper retention during the early neonatal period.

Absorption data from human infants are limited. Balance studies in preterm infants by Cavell and Widdowson (55) and Dauncey et al (56) showed negative balances of copper for several months after birth. Most of the copper was found in the stool, suggesting ineffective absorption mechanisms or that the capacity of the preterm infant to retain copper is poor and that copper is re-excreted in the bile. Negative copper balance was also found in a study of preterm infants by Tyrala (57); not even a formula with a copper concentration of 2.1 mg/L resulted in consistently positive copper balance. Copper absorption from formula was estimated to be 11–13% at 34 wk postconceptual age. Dörner et al (58) also performed balance studies in preterm infants and found a slightly negative balance (2–5 μg/kg) when infants were fed formula that was not supplemented with copper (0.12 mg/L), whereas they found a slightly positive balance (5 μg/kg) when infants were fed a copper-supplemented formula (0.62 mg/L). Mean retention of copper in breast-fed, term infants was considerably higher, 88 μg/kg, with a mean daily intake of 114 μg/kg. Copper retention decreased with age, though: at 2 wk of age, 130 μg/kg was retained whereas 64 μg/kg was retained at 16 wk. Urinary copper was low, ~6.4% of intake. When fed supplemented formula, term infants had a mean retention of 55 μg/kg, whereas 5 μg/kg was retained from the unsupplemented formula. When mean relative retention was calculated, 75% of copper was retained from breast milk, 52% from copper-supplemented formula, and 23% from unsupplemented formula. A linear relation between copper intake and copper retention was found, supporting the suggestion from studies in rats that copper absorption during infancy is nonsaturable (54). Thus, within a normal range of copper intake, copper retention increases with dietary copper.

Turnlund et al (23) used stable-isotope methodology to study copper absorption in adults. Diets were labeled extrinsically with 65Cu and mass ratios were analyzed in the diets and stools by thermal-ionization mass spectrometry. Copper absorption was dependent on the amount of copper in the diet; when a low-copper diet (0.78 mg/d) was given, absorption was 55.6%, whereas it was 36.3% from the same diet with copper added to an “adequate” level (1.68 mg/d), and 12.4% from the same diet but with a high copper content (7.53 mg/d). Thus, it appears that copper absorption at this age is saturable and that the percentage absorbed decreases with the level of dietary copper. However, total retention of copper increased with the level of dietary copper. Balances were positive even at the lower copper intake studied, suggesting that copper intakes of ~0.8 mg/d are adequate and that current recommendations (ESADDs) may be higher than necessary. The authors concluded from data obtained early and late in each balance period that there is more rapid adaptation of absorption to a low intake of copper than to a high intake of copper. Thus, sudden intakes of high concentrations of copper increase the risk of copper toxicity.

Early studies of copper absorption in human adults with radioactive copper isotopes showed a mean absorption of 28% in young men, but the amount of copper in the diet was not analyzed (59). Sternlieb (60) studied normal subjects and found a mean copper absorption of 40% when 2 mg Cu was given. Other studies in which whole-body counting (61) or monitoring of fecal radiocopper (62) was used resulted in highly variable absorption values (15–97% and 40–70%, respectively).

The length of the balance period is critical in all copper-absorption studies, regardless of whether radioisotopes or stable isotopes are used. If the balance period is too short, all the unabsorbed copper has not yet left the body. Studies in which fecal markers were used showed that some individuals excrete the markers within 6 d after administration, but that it is more often 12 d or even longer (23). This may explain the high value of 61% obtained through use of fecal monitoring in a pilot study of four subjects (63), because only five stool samples were collected. However, as pointed out by Turnlund et al (23), when longer collection periods are used, some of the initially absorbed copper will be re-excreted during the balance period. This fraction was estimated to be ~12–15% in 12 d on the basis of excretion of an intravenously injected isotope (59, 61, 64). This value can then be used to correct absorption data, making the corrected values somewhat larger than initially calculated. On the other hand, some correction should also be made for urinary losses that are often not taken into account in methods that make use of fecal monitoring. This amount was determined to be 10–20 μg/d in a study of young men with dietary copper intakes of 2.7 mg/d (64).

**DIETARY FACTORS AFFECTING COPPER BIOAVAILABILITY**

**Infant diets**

Balance studies in term infants who were breast-fed or fed cow-milk formula (discussed above) suggested that copper was absorbed to a higher extent from human milk than from cow-milk formula (58). Studies with a stable isotope of copper also indicated this (65). However, the extent of copper absorption was difficult to evaluate because the copper concentration varied between diets. In our studies in suckling rats, we found slightly higher copper bioavailability (estimated from liver by 64Cu uptake 6 h postdosing) from human milk than from cow-milk formula (53). Less copper was absorbed from cow-milk, cereal-milk, and soy formulas. Detailed studies of the distribution of the radiolabel and native (cold) copper in the diet were used to validate the extrinsic tag method. A more recent study in the same rat pup model evaluated several varieties of infant formula (66). In general, copper absorption was relatively high from milk formulas but lower from soy formulas. One milk-based product for premature infants resulted in lower copper absorption than that from the other products. The lower copper bioavailability from cow milk combined with its low copper content most likely explains the
copper deficiency found in some premature infants fed cow milk for long periods. Soy formula, which contains phytate, has been shown to result in low plasma zinc concentrations in infants (67). Plasma copper concentrations, however, were similar for infants fed soy formula and milk-based formula.

Proteins

The effect of animal protein compared with that of plant protein on copper absorption was evaluated by stable-isotope methodology by Turnlund et al (68). Copper absorption was higher from the animal-protein diet (41%) than from the plant-protein diet (34%) in nonpregnant women. However, the copper content of the plant-protein diet was higher, resulting in more copper being absorbed from it than from the animal-protein diet. Gregor and Mulvaney (69) compared tissue copper in rats fed either a lactalbumin (whey protein)-based diet or five different types of soy protein–based diets. Copper retention was significantly lower from the lactalbumin diet than from the soy diets. It is not clear why less copper was retained from the lactalbumin diet, but the authors suggested that the higher zinc absorption from the lactalbumin diet had a negative effect on copper absorption. In a study on infant rhesus monkeys, we found lower plasma copper concentrations in infants with elevated zinc absorption due to marginal zinc deficiency (70), giving some support to this hypothesis. Zinc and copper are known to interact at the level of absorption. It is also possible that the whey protein had a negative effect on copper absorption. Different milk-protein sources have been shown to have various effects on copper status in rats (71). Some studies have evaluated the effect of soy protein on copper bioavailability (69, 72); however, because soy protein isolates also contain phytate, the isolates need to be evaluated in this context and are discussed below.

Phytate and fiber

Several studies in experimental animals have shown a negative effect of phytate on copper bioavailability (73, 74). However, Lo et al (72) did not observe such an effect of phytate in soy protein in a rat bioassay. The authors suggested that the phytate content of the soy protein isolate used (0.05–0.27%) was considerably lower than that of isolates used in some earlier studies, eg, 1% in the study of Davis et al (73).

Turnlund et al (75) used stable isotopes to study copper absorption from phytate and α-cellulose in young men. They found no effect of either component in these human subjects and suggested that high amounts of phytate or fiber do not decrease copper absorption, even if zinc absorption is markedly reduced (76). The authors suggested that the fact that zinc–phytate complexes precipitate at the pH of the gastrointestinal tract, whereas complexes of copper do not (77, 78), could explain the lack of an effect on copper absorption. Because phytate in the soluble copper–phytate complex can be easily replaced by other chelators such as amino acids (78), there may be no inhibitory effect of phytate on copper absorption. A study on cereal products supports this notion (79); zinc solubilized from cereal by the addition of acid precipitated completely when the pH was raised to 7, whereas copper stayed in solution.

Amino acids

Some amino acids are known to form complexes with divalent cations such as copper. Histidine can chelate copper with an affinity about three orders of magnitude greater than for zinc (80). In duodenal-jejunal, single-pass perfusion studies, it was found that copper accumulation in the mucosal tissue was higher when an excess of histidine to copper and zinc was used (81). However, the author of this report cautioned that the time period used for the study was too short to allow induction of metallothionein. It is possible that a copper–histidine complex may be an effective way to provide available copper when needed. Such a complex has successfully been used to administer copper subcutaneously to infants with low concentrations of circulating copper (82). In contrast, high concentrations of cysteine have an inhibitory effect on copper utilization in chickens (83, 84). This effect on copper absorption is evident at both copper-deficient and excess copper concentrations (85). It is possible that the negative effect of cysteine on copper absorption is mediated by a positive effect on zinc absorption (85) because these two elements compete at the level of absorption.

Novel complexes of copper and amino acids such as copper–methionine and copper–lysine appear to have no significant effect on copper absorption (86, 87), although provision of copper as these complexes may be advantageous in the presence of an inhibitor of copper absorption such as cysteine or ascorbate (85). This may be explained by the fact that copper may be absorbed by an amino acid transport pathway rather than through the conventional zinc-copper transport mechanism (85). Note that these studies to date have been performed in chicks and with high concentrations of inhibitors (cysteine, ascorbate). Further studies in other species and at other concentrations are needed to evaluate these effects.

Ascorbic acid

It is well known that ascorbic acid has a negative effect on copper absorption in laboratory animals (88, 89). There is now considerable evidence that this effect is achieved by reduction of cuprous (Cu⁺) ions to cupric ions (Cu²⁺) and that the latter form is less well absorbed. This is supported by the fact that other reducing agents have similar effects (88). Early studies in which radiocopper was administered in the small intestine or intraarterially showed that the effect was exerted at the absorptive stage in the intestine and that increased copper excretion was not the cause of lower copper status in ascorbate-supplemented animals (89). An interactive effect between ascorbic acid and carbohydrate on copper metabolism has been described and is discussed below (90).

Ascorbic acid can also affect copper metabolism at a stage later than absorption in the small intestine (91). DiSilvestro and Harris (92) showed in copper-deficient chicks that ascorbate given during or before (75 min) provision of copper intraperitoneally significantly impaired copper utilization. However, when ascorbate was given after (75 min) CuSO₄, activity of copper-dependent enzymes was increased. Thus, ascorbate may have an important role in the metabolism of copper. This effect, though, appears to be specific to l-ascorbic acid because D-isoascorbic acid, which is also a reducing agent, had no effect. It was proposed that l-ascorbate may be one of the reducing agents necessary for the reduction of ceruloplasmin-bound copper to make it available intracellularly. It is also
possible that l-ascorbate is needed in the transport of copper from mucosal cells to other tissues.

Van den Berg and Beynen (93) attempted to evaluate the direct effects of ascorbic acid on copper absorption and the postabsorptive effects on copper metabolism. They found that the primary effect of high dietary ascorbic acid was reduced intestinal absorption of copper, but that it also increased hepatic uptake as well as biliary excretion of $^{64}$Cu. They also found that the effect of ascorbic acid on copper metabolism was more pronounced in copper-deficient than in copper-adequate animals. The authors suggested that the increased biliary excretion of copper was not specific, but rather due to increased uptake of copper into the liver. They also emphasized the importance of evaluating the effects of ascorbic acid and altered copper status separately.

The effect of ascorbic acid on copper metabolism in humans may be less pronounced than in experimental animals. Finley and Cerklewski (94) found decreased ceruloplasmin oxidase activity and a trend toward lower serum copper after 64 d in young adult men after they consumed 1500 mg ascorbic acid/d. However, this effect may not have been the result of lower copper absorption because Jacob et al (95) found no difference in copper absorption in young men given different amounts of ascorbic acid. These authors suggested that ascorbic acid promotes the dissociation of copper from ceruloplasmin and thus lowers its oxidase activity. This was supported by the finding that immunologic quantitation of ceruloplasmin showed no change in protein concentrations. A clinical study on low-birthweight infants fed formula supplemented with ascorbic acid (50 mg/d) did not show any negative effects on copper balance (96). However, the low-birthweight infants were largely in negative mineral balance and it is possible that ascorbic acid under these conditions may not exert any effects.

**Zinc**

Copper and zinc have the same electron configuration and form similar coordination complexes in water. Hill and Matrone (97) hypothesized that elements that share these properties will interact at the level of absorption. They subsequently showed that this, in fact, occurs in vivo. Thus, high amounts of dietary zinc can have a negative effect on copper absorption. Because supplemental zinc often is used in infants, children, and pregnant women to avoid possible zinc deficiency, the possibility of this practice interfering with copper status should be considered.

A direct interference of zinc with intestinal absorption of copper was shown by van Campen and Scaife (98), who administered $^{64}$Cu in duodenal segments and gave zinc intraluminally or intravenously. Intravenous zinc or elevated zinc status did not affect copper absorption; the effect was localized to the intestinal mucosa. The mechanism behind the zinc-copper interaction in the small intestine was studied by Hall et al (99). They found that high dietary zinc induced intestinal metallothionein and proposed that this newly synthesized metallothionein may act as a “trap” for absorbed copper. This theory was supported by findings of Fischer et al (100), who found a correlation between the decrease in copper absorption and the appearance of intestinal metallothionein. They suggested that newly absorbed copper could displace zinc from metallothionein because of its higher affinity toward this protein. Oestreich and Cousins (101) examined this hypothesis in detail using isolated vascularity perfused intestinal segments in rats, which allow direct measurements of mineral ion fluxes across the intestine as well as intracellular events. When zinc and copper ranges were varied within a relatively normal range, there was no inhibitory effect on copper absorption. However, when very high concentrations of zinc were used to perfuse the intestine, a negative effect on copper absorption was observed. This was in agreement with the findings of Hall et al (99), who found no effect on copper absorption after a dietary zinc intake of 450 mg/kg, but a 40% decrease when 900 mg/kg was fed. However, Oestreich and Cousins (101) also found that intestinal metallothionein concentrations increased 10-fold when dietary zinc was increased from 5 to 180 mg/kg, there was no change in the amount of copper (or zinc) transported to the portal circulation. Thus, the metallothionein gene is sensitive to dietary zinc, but at the concentrations of zinc studied, these changes were insufficient to alter copper absorption.

The balance between chelating amino acids (such as histidine), other trace elements (like zinc), and copper may be of critical importance. Wapnir and Lee (102) showed that the inhibitory effect of zinc on copper absorption is enhanced in the presence of excess histidine. Similarly, the effects of iron and tin on copper absorption were also exacerbated by the addition of histidine (103). It is possible that histidine either enhances the removal of copper from the intestinal lumen or increases the retention of copper by the mucosa.

Studies on human subjects fed diets with different ratios of zinc to copper also failed to show a significant effect on copper absorption. August et al (104) used a stable isotope of copper to study copper absorption in young adults and elderly subjects. They used ratios of zinc to copper of 2:1, 5:1, and 15:1, but found limited effects of these ratios on copper absorption. Feeding a low-copper diet was found to increase copper absorption compared to a normal level of dietary copper.

Very high doses of zinc have been used successfully to treat patients with Wilson disease, an inborn error in copper metabolism that results in copper toxicity (105, 106). High concentrations of zinc can be used to significantly limit the amount of copper absorbed and, therefore, slow the progression of the disease. However, high intakes of zinc should be viewed with some concern. We recently found that a patient with acrodermatitis enteropathica who was being treated with zinc was copper deficient (107). Acrodermatitis enteropathica is a genetic disorder of zinc metabolism and patients need high doses of zinc to prevent zinc deficiency. Thus, the dosing of zinc becomes critical; too much zinc may cause copper deficiency and too little may result in zinc deficiency. This is illustrated by a recent case report of an infant who was given daily doses of zinc (16–24 mg) and who developed copper deficiency (108). In addition, Salim et al (8) showed lower plasma zinc concentrations in infants fed copper-supplemented formulas, although these zinc concentrations were within the normal range. In this context, it should also be realized that even if copper supplementation may be beneficial in some situations, supplementation may have a negative effect on zinc status. Castillo-Durán et al (109) found in patients hospitalized for diarrhea that copper supplementation caused a reduction in zinc absorption.

**Iron**

Copper absorption may also be affected by high levels of dietary iron. Haschke et al (110) studied the effect of two levels of iron fortification of infant formula on copper balance in
term infants. They found that the higher amount of iron (10.8 mg/L) resulted in lower copper balance than the lower amount of iron (1.8 mg/L). Barclay et al (111) showed reduced SOD concentrations in premature infants given iron supplements. Earlier studies in experimental animals showed a reduction in liver copper concentrations when dietary iron was increased 10-fold (112). However, modest supplements of iron did not appear to affect serum copper concentrations in older infants (113). On the other hand, it is possible that in iron deficiency, enhanced iron absorption may interfere with copper absorption. Morais et al (114) found that serum copper and ceruloplasmin concentrations decreased in iron-deficient children given 5 mg iron/kg body wt for 2 wk. Although these decreases were significant, they were still within the normal range. As noted earlier, copper status may be affected adversely without significant effects on serum copper. Several studies suggest that high dietary iron affects copper absorption only when copper status is low or marginal, possibly explaining some of the discrepancies noted between studies (90, 115, 116). It should be cautioned that high intakes of iron and ascorbate may act together to adversely affect copper status. Johnson and Murphy (90) found that high intakes of iron with ascorbic acid caused severe anemia in copper-deficient rats and decreased plasma ceruloplasmin by 44% in copper-adequate rats. Because iron and ascorbate are commonly used supplements in humans, the possibility of a negative effect on copper metabolism should be explored.

Carbohydrates

The effects of different carbohydrates on copper absorption have received considerable attention. In particular, the role of fructose in copper metabolism has been explored in both experimental animals and humans. In rats, dietary fructose appears to worsen the effects of copper deficiency in that fecal and urinary excretion of copper are elevated when the rats are fed fructose compared with starch (117, 118). The results of the experiments are more difficult to interpret; copper balance appears to be increased when subjects are fed low-copper diets with fructose compared with starch (117), whereas copper-zinc SOD concentrations in red blood cells were reduced. Copper-zinc SOD is believed to be a good indicator of long-term copper status and the reduced concentrations were restored when copper was added back to the diet. The effect of fructose in humans does not appear to be specific for copper: when a low-copper diet was fed, balances of calcium, magnesium, iron, and zinc were increased compared with when starch was fed (119).

The mechanism or mechanisms behind the interaction between fructose and copper is not yet known. Fructose forms complexes with cations and is absorbed via a different pathway than other carbohydrates (120). Also, fructose accumulates in the liver and may cause some redistribution of copper between different body pools. Wapnir and Balkman (121) studied water flux in perfused rat intestine and found enhanced bulk flow by fructose. It is evident that the direct effects of fructose on copper absorption and the postabsorptive effects need to be evaluated further.

REFERENCES


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