Copper absorption and bioavailability[1,2]

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ABSTRACT The human gastrointestinal system can absorb 30–40% of ingested copper from the typical diets consumed in industrialized countries. Experimental data support the existence of a carrier-mediated transport mechanism with an affinity constant in the micromolar range. Aging probably decreases the efficiency of copper homeostasis, resulting in higher plasma copper concentrations in the elderly. Physiologic differences may account for the higher cupremia of females. Supplements of minerals with similar chemical characteristics could reduce copper absorption. This property has pharmacologic applications in Wilson disease. Manipulation of the fiber content of the diet may have an indirect effect on copper bioavailability by altering the bioavailability of mineral antagonists. Proteins and soluble carbohydrates tend to improve copper absorption and bioavailability by enhancing its solubility and intestinal bulk flow. Organic acids, other than ascorbic acid, or agents that form low-molecular-weight chelates, are likely to have a positive effect on overall copper absorption. Conditions associated with malabsorption of macronutrients and gastrointestinal disease can impair copper uptake and contribute to suboptimal copper status. *Am J Clin Nutr* 1998(suppl);67:1054S–60S.

KEY WORDS Copper bioavailability, copper absorption, intestinal absorption, milk, food processing

GENERAL ASPECTS OF COPPER ABSORPTION

Overall view of intestinal copper absorption

In mammals, copper can be absorbed from the stomach to the distal small intestine. A critical component of copper gastrointestinal balance involves enterohepatic circulation. At least one-half of the amount of copper reaching the small intestine reappears in the bile as strongly bound compounds, and is lost in the stool. The distribution of copper throughout the body is mediated by ceruloplasmin, albumin, and other quantitatively less important copper binders. In humans it has been convincingly demonstrated in a series of studies by Turnlund et al (1, 2) that the rate of copper absorption varies inversely with copper intake and can be as low as 12% with very high copper intakes. A theoretical maximum absorptive capacity of 63–67% has been estimated (**Figure 1**). However, with typical diets in developed societies, the average true copper absorption is in the 30–40% range. These findings are consistent with the view that intestinal absorption is a key regulatory step in copper assimilation.

Site and mechanisms of absorption

The capacity for copper absorption is about equally distributed along the small intestine of rodents and, presumably, higher mammals. However, only a small fraction of dietary copper is sufficiently solubilized in the stomach, so its absorption at that site is not considered to be nutritionally significant. Data on copper absorption were obtained by the use of plasma concentration slopes after oral and intravenous boluses in humans, in addition to information gained through the use of stable isotopes ^{65}Cu and ^{63}Cu in balance studies. In experiments with laboratory animals, more traditional techniques such as intestinal perfusion and creation of everted sacs have been applied, as well as the overall determination of copper sufficiency by measuring tissue content, hematocrit, and ceruloplasmin. The radioactive short–half-life isotopes ^{64}Cu and ^{67}Cu have been largely used in experimental protocols with animals. Evaluations of markers of copper sufficiency are discussed elsewhere in this supplement. A rate-limiting, active-transport mechanism and a diffusion component have been reported in the small intestine of rodents (3). There are no comparable studies in humans that provide evidence on the kinetics of the intestinal absorption of copper. The decrease in the proportion of copper absorbed at high dietary intakes is compatible with a carrier-mediated transport process. Because dietary copper is incorporated into solid foods, it appears less likely that bulk flow across the intestinal mucosa is of quantitative significance.

Ontogeny of copper absorption: age and sex differences

Mediated intestinal transport of copper appears not to be congenital. It has been shown in rats that during lactation and through the weaning period the intestinal absorption of copper occurs largely by diffusion or solvent drag. A saturable copper transport system becomes evident in early maturity, both in rats (4) and in mice. Mediated mucosal transport of copper in mouse duodenum has a Michaelis constant (K_m) of 4.3 ± 0.7 μmol (5). In young adult rat jejunum, the K_m in the presence of sodium was 21 μmol (6). Transport from the enterocyte to the circulation

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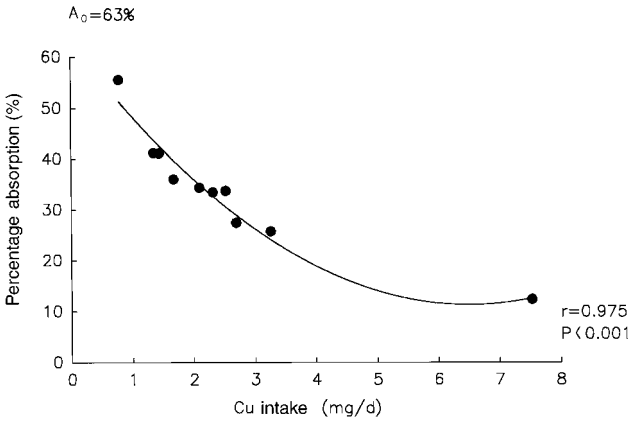

FIGURE 1. Aggregate results of human copper absorption studies at various copper daily intakes. The data conform to a quadratic equation ($P < 0.001$). A_0 = extrapolation of copper absorption rate at zero copper intake (adapted from references 1 and 2).

also exhibits progressive development. Suckling rats retain a greater proportion of copper in the small intestinal mucosa after perfusion with copper than do weanling and adolescent rats, a finding consistent with an immature distribution mechanism.

In humans, changes in the absorption of copper at different ages have only been assessed indirectly. Plasma concentrations of copper increase steadily from childhood to old age (7). This stands in contrast with stable zinc concentrations over the human life span. The changes in circulating copper have been attributed to a decline of biliary secretion, as a component of the regulation of copper absorption, rather than to an increasingly efficient gastrointestinal absorption capacity throughout life.

Sex differences in plasma copper in humans are well documented for all ages. Females have consistently higher plasma copper concentrations than males. This difference is exacerbated in women taking estrogens. It is yet undetermined whether gastrointestinal factors play a role in this difference, although it has been speculated that copper requirements may differ between men and women (8).

FACTORS INVOLVED IN THE INTESTINAL ABSORPTION OF COPPER AND ITS BIOAVAILABILITY

Assessment of bioavailability

Mineral bioavailability has been defined as the efficiency with which a natural or manufactured source of an element delivers the element to storage or supplies it to metabolically active tissue or to a protein. In general, the following criteria are applied for the assessment of bioavailability: *1*) whether the level of intake is below or above the physiologic requirement, *2*) which tissue mineral contents should be examined as dependent variables to assess bioavailability, *3*) the range at which there is linearity between dose and response, and *4*) the results of a slope ratio analysis to determine relative bioavailability.

With the introduction of the stable isotope ^{65}Cu, present as 30.8% of the natural element, it has become possible to undertake meaningful balance studies in humans without the hazards of ionizing radiation (9). However, because the proportion of the stable isotope in native copper is high, the amount of the material required for balance studies in humans demands that a substantial fraction of the total dietary copper intake be in a chemical form different from the one present in common foods, which makes this technique costly. A novel approach, restricted to experimental studies, is based on the use of a diet enriched in ^{63}Cu, the isotope that has a concentration of 69.1% in native copper. In rats, this design has allowed balance studies to be extended for up to 100 d (10).

Identity of copper sources

Chemical speciation and bioavailability from various food sources

The ingestion of copper as a mineral salt is relevant in humans only when this element is taken as a nutritional supplement. In this case the potential of interactions with other mineral elements (discussed below) may have nutritional significance. Organomineral complexes of copper with amino acids or organic acids are used extensively in animal nutrition. Copper-lysine has been shown to be as effective as sulfate for chicks, but has only two-thirds of the bioavailability of the mineral salts in lambs (11). Proprietary combinations of proteins with minerals, called *proteinates*, are effective as a feed for growing calves in areas with high molybdenum contents in forages (12).

Information on the chemical speciation of copper from vegetable and animal foodstuffs is limited. In humans, vegetables are the major sources of copper nutriture, although they require a more extensive digestive enzymatic attack than do copper-containing animal proteins, including milk. The latter are, in general, more easily solubilized. The metal-matrix binding of copper in vegetables and herbage is not well defined and may be present in a variety of forms. A substantial fraction of copper in grains is thought to be associated with lectins and glycoproteins (13, 14). Although solubility in water or physiologic fluids is not an overriding factor in determining bioavailability, this property may still be a good indicator of digestibility (15, 16). Macronutrients and bulk material also play a role in determining copper bioavailability (*see* below).

Absorption from inorganic sources

Copper salts, including chloride, acetate, sulfate, and carbonate, are highly bioavailable in animal nutrition when added to feed; the exception is copper oxide, which has < 40% of copper chloride bioavailability in sheep (17) and has negligible value in chicks (18). In cattle, cupric carbonate is better absorbed than the nitrate, followed by the sulfate, the chloride, and cuprous and cupric oxides (19). The slope-ratio technique, in which the concentration of liver copper as related to daily copper intake, was used to compare the bioavailability of this element in chicks. When copper bioavailability of acetate was set at 100%, those of the sulfate, carbonate, and oxide were found to be 88.5%, 54.3%, and 0%, respectively (18).

Effects of food processing on bioavailability

Physical and chemical treatments of foodstuffs often reduce their initial mineral content. Milling of grains for the removal of bran and germ can reduce the copper content by up to 45%. Canning of vegetables can result in the leaching of minerals to the surrounding water. Salt addition for food preservation may alter the solubility of protein and organometallic natural compounds that are subsequently lost during processing or become less available (20). Chemical treatments on foods can decrease copper bioavailability, especially when oxidation or reduction treatments are applied or take place as a result of air exposure or the presence of reducing or oxidizing agents.

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The best evidence regarding the influence of the type of dietary copper source and the role of food preparation on the bioavailability of this element comes from animal studies. Copper added to cooked foods with high protein contents, such as chicken liver or chick peas, was more poorly absorbed by rats than copper supplied from other vegetable and animal sources (21). Some of the reduction in mineral bioavailability has been associated with the formation of sugar–amino acid condensation products (Maillard reaction) during heat treatments. This results in a reduction of free amino acids and available sites for metal–nitrogen bond formation, with a subsequent decrease in organometallic compounds with greater bioavailability (22).

Milk as a special copper source

The bioavailability of trace elements in milk is extremely high for the young of any given species, regardless of the elements' absolute concentrations. Human breast milk has the highest concentration of copper among mammalian milks consumed by humans, which generally range from 0.25 to 0.60 mg/L (3.9 to 9.5 μmol/L). There is a progressive decline in concentration as the duration of lactation is prolonged (23). Copper concentrations in cow milk are four to six times lower than in human milk. In addition, the distribution of copper among protein fractions of different molecular weights and among lipids are widely different. Human milk has, in comparison with cow milk, a greater proportion of copper bound to lipids (15% compared with 2%, relatively) and to whey (56% compared with 8%), but much less associated with casein (8% compared with 44%) (24). The overall bioavailability of copper in human breast milk has been estimated to be 24%; that of cow milk is 18% (25). Nevertheless, cow milk can exert a positive effect on the absorption and retention of trace elements later in life, above and beyond that achieved by vegetable protein supplementation (26).

MODIFIERS OF COPPER ABSORPTION

Physiologic modifiers

Effect of pH and digestion

The acid environment in the stomach contributes to the freeing of copper bound to foodstuffs, and to setting the conditions for peptic digestion, which releases copper from natural organic complexes (27). Once gastric contents empty into the duodenum, the increase in lumenal pH affects copper absorbability, presumably because of a diminished concentration of free copper and the consequent predominance of cupric hydroxide and basic copper salts with low dissociation constants. This hypothesis is supported by the finding in rats that in the ileum, and to a lesser extent in the jejunum, copper absorption is lower at a pH of 6.3–7.3 than at a pH of either 5.8 or 7.8 (6).

An assessment of the digestibility of a mineral source is the proportion of an element released from a high-molecular-mass matrix into fragments no larger than 12–14 kDa. This process is substantially affected by pancreatic digestion, and can be experimentally measured and monitored (28). There is evidence that the fraction of copper tightly bound to bile remains unabsorbable during its passage through the gastrointestinal tract (29).

Role of sodium in copper absorption

There is experimental evidence that sodium may be involved in copper uptake by the small intestinal mucosa. A significant reduction in rates of removal of copper from the jejunum and ileum of rats was observed in vivo when sodium was excluded from the perfusate (6). Similarly, when amiloride, an inhibitor of the sodium-proton antiporter and the sodium-calcium exchanger, was added to a perfusing solution, copper transport was decreased (30). The nutritional significance of the sodium-copper connection is still uncertain, although these findings suggest there may be a link between mineralocorticoid and copper metabolism.

Dietary modifiers

Macronutrients and natural and pharmacologic bulk materials, as well as other mineral elements, can significantly alter the bioavailability and absorptive mechanisms of copper. These are summarized in **Table 1**.

Binding of copper by natural vegetable fibers

The effect of vegetable fibers on copper absorption is varied. The negative effect of phytate on copper absorption is not as severe as that of other divalent cations such as zinc and calcium. In one study, the addition of sodium phytate or α-cellulose did not reduce copper absorption (1); hence, the frequently used dephytinization process, important for increasing the bioavailability of other trace elements, does not have a direct effect on copper nutriture (31). Dephytinization can indirectly alter copper bioavailability. If iron, zinc, and other divalent cations that may interact with copper become more bioavailable, intestinal absorption of copper can be partially inhibited. Although there is evidence that copper may be coprecipitated by phytate in the presence of an excess of calcium, it has been shown that the amino

TABLE 1
Potential modifiers of copper intestinal absorption[1]

	Effect on test subjects	
	Humans	Laboratory animals
Fiber		
Phytate	—	±
Hemicellulose	↓	NA
Vegetable gums	—	NA
Carbohydrates		
Fructose	↓[2]	↓[2]
Glucose polymers	NA	↑
Fats		
Triaglycerols	—	—
Long-chain fatty acids	NA	↓
Medium-chain fatty acids	NA	—
Protein		
High-protein diet	↑	↑
Excess amino acids	±	↓
Organic acids		
Ascorbic acid	±	↓[3]
Natural polybasic amino acids	↑	NA
Divalent cations (Zinc, Iron, Tin, and Molybdenum)	↓	↓

[1] —, No effect; ±, uncertain or variable effect; ↑, increased absorption; ↓, decreased absorption; NA, data not available.
[2] Effect may be only systemic.
[3] In laboratory animals, systemic administration of ascorbic acid may have stimulatory effects on absorption; administered locally, it is inhibitory.

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nitrogen content of the intestinal lumen can easily solubilize copper from copper, zinc, and calcium phytate complexes (32).

Crude soybean proteins can decrease copper bioavailability and induce copper deficiency in chicks (33). Purified soybean protein, one of the products used in infant formulas, can reduce copper bioavailability by 90% (25). Hemicellulose has also been shown to induce a negative copper balance in adolescent males, though pectin and intact cellulose were inactive (34). Other refined fibers and gums, such as locust bean and karaya gums, as well as carboxymethylcellulose, have been shown to be either without effect or beneficial to trace element balance, including copper (35).

Effect of carbohydrates

Dietary fructose can exacerbate the effects of insufficient copper intake in male rats (36, 37). Similarly, copper requirements are increased if the diets contain fructose. However, this carbohydrate effect seems to be species- and sex-dependent. The fructose-copper deficiency interaction appears to be associated with changes in lipid and energy metabolism (38, 39). Copper status in humans can deteriorate when the predominant carbohydrate in the diet is fructose (40). The extensive use of fructose-containing sweeteners in convenience foods and beverages has made this finding relevant. Copper sufficiency has been linked to cholesterol metabolism; the risk of developing cardiovascular disease has been putatively related to copper nutritional status (41).

The effect of carbohydrates on copper absorption is not exclusively systemic. During the lumenal phase of absorption, glucose polymers, such as those present in corn syrup solids, can enhance copper uptake, presumably through the stimulation of water transport across the intestinal mucosa. In the absence of other factors, water fluxes correlate with copper disappearance from the intestinal lumen (42).

Effect of fats

In addition to alterations in copper nutritional status experimentally produced by a high-fat diet, as mentioned above, there is limited knowledge on what effect dietary fats may have on copper absorption. In humans, preliminary data indicated that dietary polyunsaturated fatty acids had no effect on copper retention, but that they did reduce iron and zinc uptake (43). In laboratory animals, long- or medium-chain triacylglycerols presented to the small intestinal mucosa simultaneously with copper had no effect on its absorption. However, free palmitate (16:0) and stearate (18:0) reduced considerably the rate of copper removal from the jejunal lumen, whereas free caprylate (8:0) and caproate (6:0) had no effect (44). In a comparison among diets with relatively high fat contents (9.5%) in rats, beef tallow, introduced as a source of saturated fatty acids, interacted with fructose to increase plasma triacylglycerol, cholesterol, and liver iron, but hepatic iron remained unchanged (45).

Effect of dietary protein and protein-derived products

Natural proteins. It is generally accepted that the greater the amount of protein in the diet, the lesser the likelihood of essential mineral deficiency (46, 47). However, the nature of the protein source and the amount of phosphorus consumed may be important. Young women consuming diets containing between 25 and 46 g protein/d had similar copper retention rates (48). However, when the two extreme values were either 50 or 150 g/d and phosphorus content was either 1.0 or 2.5 g/d, the apparent retention of copper was highest with a diet low in phosphorus and high in protein. Fecal copper losses inversely correlated with retention (49). In a study on the effect of varying concentrations of zinc in the diet on copper balance, it was concurrently shown that protein intake inversely correlated with the copper requirement (50).

Changes in the size and quaternary structure of proteins may affect copper assimilation. This can be inferred from experiments in which rats were fed raw meat, which produced copper deficiency—presumably because of incomplete digestion of proteins. In contrast, when the rats were fed cooked meat, a process consistent with the partial breakdown of proteins, copper replenishment was possible (51). Other effects of heat and cooking on copper bioavailability were discussed earlier (*see* Effects of food processing on bioavailability).

Amino acids and small peptides. Amino acids are suggested to be mandatory ligands for the uptake of copper by the brush border membrane (52). A preliminary report stated that when a supplement of methionine was introduced in the diet of human volunteers, the net absorption of copper was doubled (53). However, an excess of dietary amino acids may result in copper malabsorption and deficiency. This has been corroborated in rats, because an excess of dietary histidine produced increased copper losses in the urine concurrently with a decline of serum and tissue copper (54). Similarly, a 10:1 molar excess of histidine or proline produced a reduction of copper uptake by a perfused segment of a rat upper jejunum, possibly because of competition between the amino acid complex and binding proteins of the intestinal mucosa (55).

Cysteine is also an effective chelating agent for copper, but, in addition, it produces a reduction in bioavailability similar to that of ascorbic acid (*see* below), probably due to the reduction of copper from the divalent to the monovalent state (56). However, when chicks were fed other highly bioavailable copper complexes, such as copper-methionine (relative bioavailability 96% compared with copper sulfate) or copper-lysine (relative bioavailability 120% compared with copper sulfate), the inhibition produced by cysteine was far less marked (57). Other sulfur amino acids, methionine and cystine, become regulating factors in determining copper status. This interaction is also associated with the sex-dependent sensitivity to copper deficiency exhibited by rats (58).

During in situ perfusions, a large excess of a mixture of amino acids and small peptides with a molecular masses < 200 Da had a deleterious effect on copper uptake, similar to that observed with amino acids with high copper affinities (59). As in the case of individual amino acids, the proportion of ligand to metal determines whether there is a positive or negative effect of copper chelators on absorption.

A biologically important tripeptide, glutathione, probably has significant postabsorptive importance in copper transport. It has been proposed that glutathione may form an intermediary complex with copper in the enterocyte before the transfer of the cation to other proteins, namely superoxide dismutase and metallothionein (60, 61).

Effect of organic acids

The most significant organic acid–copper interaction during absorption takes place between ascorbic acid and copper, most likely because of the reduction of cupric to cuprous copper and a consequent lowering of the bioavailability of this element (62). In humans,

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although a diet with 605 mg/d (3.4 mmol/d) ascorbic acid reduced serum ceruloplasmin by 21%, this amount did not alter intestinal copper absorption or other markers of copper status (63). In rats, addition of ascorbic acid to a diet containing 5 mg Cu/kg (79 μmol/kg) reduced copper's apparent absorption from 42% to 23%. This effect was much less marked in copper deficient diets (64). Administration of ascorbate during the postabsorptive period, in contrast, greatly enhanced copper tissue utilization (65). Ascorbate in tissues reacts with ceruloplasmin, facilitating the transfer of the element across certain membranes (61). It has also been proposed that monovalent copper resulting from ascorbate electron transfer is the form that is absorbed from ceruloplasmin by select types of cells (66).

The presence in foods of other organic acids such as citric, lactic, acetic, and malic acids, can contribute to the solubilization of copper and increase the bioavailability of this element. Citric acid in particular forms stable complexes with copper. Therefore, it is not surprising that fruits, generally rich in organic acids, have been shown to improve copper retention (67, 68).

Interaction of copper with other mineral elements.

Overview. In general, because of its electron configuration, its lower proportion in human food sources, and its susceptibility to being tightly bound to certain macromolecules and to some low-molecular-weight ligands, copper tends to become displaced, its intestinal absorption easily inhibited, and its bioavailability decreased. In these elemental confrontations, copper appears to be a "victim" nutrient. The most relevant cases involve divalent cations.

Zinc. The antagonism between zinc and copper has been known for decades (69, 70). The physiologic effects of dietary zinc follow classical pharmacologic responses: serum and liver copper decrease linearly as the logarithm of zinc concentration in the diet increases (71).

A ternary relation between zinc, copper, and dietary protein can result in marginal copper deficiency, especially when zinc intake is high and protein consumption low (50). If dietary zinc is increased from 5 to 20 mg/d (76 to 306 μmol/d), the intake of copper needs to be 60% higher to maintain balance. Further indication of the delicate interaction between the two elements came from a study by Greger and Snedeker (49) that showed that only 3 mg/d (46 μmol/d) more of zinc sufficed to increase copper losses and diminish copper retention.

Availability of large doses of zinc as over-the-counter supplements has lead to cases of iatrogenic, microcytic, and hypochromic anemia and leucopenia, which are all unresponsive to iron. Intravenous cupric salts were needed to normalize the condition of one such patient (72). This is a good example of the potential dangers of inappropriately manipulating the diet. However, as described elsewhere, substantial doses of zinc salts are the therapy of choice for the long-term maintenance of patients with Wilson disease (73).

Other cations. Divalent cations may also act competitively, as zinc does, on the intestinal absorptive process. This has been clearly shown for ferrous iron (74, 75) and stannous tin (75, 76). Both elements can enter the diet in abnormally high amounts: iron as a therapeutic hematopoietic precursor, or from exposure to cooking utensils, and tin from the use of superficially tinned iron pots and pans, a common craftsmanship practice in certain Asian societies.

Molybdenum, which when present in high concentrations in soil produced copper deficiency symptoms in sheep, was recently introduced as the thiomolybdate complex in the initial treatment of Wilson disease. When ingested with foodstuffs, thiomolybdate effectively scavenges dietary copper. The inhibition of copper intestinal uptake by this unabsorbable form of molybdenum has been clearly shown (77).

Pathologic factors in the absorption of copper

Malnutrition and diarrhea

Nutritional status and integrity of the small intestinal mucosa are important determinants of copper absorbability. Malnourished infants are also at risk of recurrent diarrheal disease. When fed animal (cow or goat) milk they become susceptible to copper malabsorption (78, 79). The link between gastrointestinal disease and copper deficiency may stem from the preponderance of low-molecular-weight ligands in ruminant milk, which can inhibit copper absorption, as mentioned earlier in regard to protein-breakdown products. Animal models have not yet been tested to assess whether intestinal alterations due to malnutrition or mucosal changes identified in viral or bacterial diarrhea induce copper malabsorption and lead to copper deficiency.

Copper deficiency and copper malabsorption

Demonstrable copper deficiency need not to be accompanied by copper malabsorption. It is still unknown how copper deficiency interacts with the intestinal absorption of this element, although there is evidence of an up-regulation of absorption as copper sufficiency falls (80). Because in many cases, particularly in infancy, low serum copper and ceruloplasmin concentrations are not necessarily associated with other hallmarks of malnutrition (81), it is difficult to discriminate the effect of protein and protein-energy malnutrition, including frank kwashiorkor and marasmus, with respect to the specific absorptive ability for copper. Moreover, because fecal excretion is a key aspect of copper homeostasis, alterations of the enterohepatic circulation and bile secretion may play a role as important as that of the intestinal mucosa uptake of copper in the maintenance of copper nutritional status (2).

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