Estimating risk from copper excess in human populations

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ABSTRACT
Risk assessment for nutrients assumes a single population with a normal distribution of indexes of requirements and excess. Toxic levels are by definition intakes above the upper level; for copper, however, because we lack noninvasive, sensitive biomarkers of storage or early damage from excess, excess is based on the infrequent occurrence of clinical disease, such as unexplained liver cirrhosis. We examine the limitations of this approach for copper given the very low prevalence of clinical and subclinical disease and suggest that the population risk for copper excess be based on hepatic copper loading as a potentially quantifiable measurement. The challenge ahead is to develop biomarkers that predict the population risk of elevated hepatic copper stores and thus the possibility of disease in a population. Am J Clin Nutr 2008;88(suppl):867S–71S.

INTRODUCTION
Traditional risk assessment of nutrient deficit and excess assumes a single population with requirements that follow a normal distribution; on the basis of this, a mean requirement and a recommended intake to cover a fixed (97.5) percentage of the group are established. An upper level defines the upper end of the range that is considered safe. Toxic levels are by definition intakes above the upper level. We examine the limitations of applying this approach to copper given known and potentially susceptible populations and propose a potential method for quantifying population risk from copper excess on the basis of available data.

Proof of the nutritional essentiality of copper in humans is established on the basis of the presence of copper in the body, the demonstration of adverse effects if it is removed from the diet, and finally the beneficial effect of restoring it. Whole-body copper content in adults is ∼80 mg (range: 50–120 mg), with the highest concentrations found in liver and brain (5.1 and 6.3 μg/g wet weight, respectively; 1, 2). Liver copper represents the main form of storage, whereas the high content in the basal ganglia of the brain is explained by copper’s role in neurotransmitter metabolism. Patients receiving intravenous nutrition without the addition of minerals develop deficiency, exhibiting anemia and neutropenia within a few weeks of copper deprivation, which are reversed by providing copper (2). Copper requirements and their basis and the potential for deficiency and excess are presented in other articles in this supplement.

Humans can adapt to excessive copper exposure from food, water, or supplements by decreasing the absorbed fraction as exposure increases (3, 4). Some metals and other dietary components interfere with copper absorption, whereas protein and histidine enhance uptake. As pointed out in the section of this supplement on intestinal copper metabolism, the gut is not only responsible for regulated copper uptake but also serves as a place for storage, thus preventing excess copper from entering the portal blood. Under normal conditions, the transfer of copper to the liver is complexed with proteins via the portal vein. Copper in the enterocyte is mainly bound to metallothionein. If for any reason metallothionein storage binding capacity is exceeded, metallothionein is denatured, thus losing its capacity to bind copper, and cell death ensues. This in fact is a further potential protective mechanism in the gut representing a first line of defense against copper excess (4, 5).

Copper uptake from the portal circulation occurs in the fenestrated capillaries; thus, hepatocytes and Kupfer cells are exposed to copper. Once copper enters the hepatocyte via specific transporters (CTR1, ATP7A), it is taken up by specific chaperones that define the final destination of the metal in terms of functional proteins, of which cytochrome c oxidase, superoxide dismutase, and ceruloplasmin are the most important. Excess copper is bound to metallothionein, whereas the copper necessary for ferroxidase activity is transferred to the Golgi with the corresponding apo-ceruloplasmin for export to other organs via the systemic circulation. Copper is thus secreted from the liver into the systemic circulation predominantly bound to ceruloplasmin or bound to small peptides. Cellular uptake in some tissues is mediated by specific ceruloplasmin cell surface receptors. If there is excess copper in the hepatocytes, ATP7B translocates from the Golgi to a vesicular transport system responsible for the transport and is secreted into the biliary canaliculi. Copper may also be excreted to the canaliculi bound to glutathione or stored in lysosomes bound to metallothionein or to lysosomal proteins (6, 7). The exact distribution of copper into these alternate transport systems is not known, but apparently the lysosomal pathway for excretion is induced only in the presence of excess liver copper.

Biliary excretion is the main form of copper elimination in humans and is the critical preventive factor in response to high exposure (7, 8). The operation of this system requires not only the

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presence of the respective copper-related proteins and Golgi-derived vesicular system but also needs the cytoskeletal structural proteins necessary for secretion into the canaliculi (7, 8). This intricate, redundant system explains the difficulty in inducing liver copper excess; mathematical modeling of the biological responses of the enterocyte regulatory responses and the liver uptake-storage and excretion system based on actual data suggests that the capacity of healthy humans to adapt for copper excess is at least 10-fold daily copper needs (5–9). The homeostatic data predict that liver stores will increase; however, if excretory pathways are intact, biliary excretion will increase linearly with exposures up to 20–30 times the mean requirement without evidence of adverse effects.

**RISK OF COPPER EXCESS**

It is >80 y since the London neurologist SAK Wilson defined a familial syndrome of progressive lenticular degeneration associated with cirrhosis of the liver. Wilson disease is now recognized as an autosomal recessive genetic disorder characterized by a defect in copper biliary excretion; copper accumulates in liver and brain causing altered structure and function of these organs (10). Considerable advances have been achieved in elucidating the clinical, biochemical, genetic, and histologic features as well as the management of patients with this disease. The cloning of the Wilson disease gene accelerated our understanding of the disease; however, wide scale application of genetic screening has yet to be resolved. The Wilson disease gene is distributed worldwide and has been shown in most races. The current prevalence estimate is 1:30 000 live births; gene frequency varies between 0.3% and 0.7% corresponding to a heterozygote carrier rate of 1:100.

Other examples of chronic copper toxicity include Indian childhood cirrhosis and Tyrolean infantile cirrhosis (11, 12); these cases are related to high exposure to copper due to animal milk stored or heated in copper or copper alloy containers. Intakes associated with cirrhosis are commonly 50–100 times what a breastfed infant receives; genetic susceptibility may contribute to explaining individual risk. Copper may act in synergy with environmental toxins. These unusual occurrences involve either extremely high exposure to copper (11, 12) or unconventional dietary practices, such as taking supplements without medical approval. Copper-associated infantile cirrhosis is an extremely rare condition; for idiopathic copper toxicosis the estimated incidence based on prospective data from Germany is 1:500 000 to 1:1 000 000 (13). The data for Indian childhood cirrhosis from India reveal a dramatic decline of this condition resulting from the avoidance of copper vessels for storing and heating milk. More recent observations from the Pune district based on hospital admissions reveal major drops in prevalence; no cases have been diagnosed since 1974 (11). In conclusion, Indian childhood cirrhosis/idiopathic copper toxicosis is a disease of unknown etiology. The most likely explanation for this condition appears to be a combination of a genetically determined defect in copper metabolism and a high copper intake. The relative contribution of each factor remains to be determined; risk management in this case includes practical advice and dissemination of existing guidelines.

The long-term toxicity of copper at moderately high exposures has been less studied. A study performed in 7 towns in Massachusetts with a total of 64 124 child-years of exposure of children under the age of 6 y, with copper concentrations in drinking water ranging from 8.4 to 8.8 mg/L, did not show a higher prevalence of deaths due to liver disease (14) than in towns with low copper in drinking water. A systematic evaluation of the association of infantile liver disease and the copper content of drinking water in the United Kingdom showed no association between these variables (15). The range of acceptable intake to prevent copper deficiency and toxicity should be based on the protection of healthy populations and should not be expected to meet requirements or prevent excess of individuals with special susceptibility. Disease conditions or genetic alterations in copper metabolism that determine special sensitivity for excess or deficit deserve the attention of public health authorities on the basis of the relevance of these conditions within a given ecological setting. The upper and lower cutoffs for the range of acceptable oral intakes are defined by using a population-based model for the assessment of health risks associated with deficiency or excess. The lower cutoff should be sufficient to meet the requirements of most individuals in the population. Similarly, the upper endpoint should protect most individuals from the risk of toxicity. This homeostatic risk assessment model as applicable to essential elements has been proposed by the World Health Organization International Program for Chemical Safety as the basis for risk assessment of essential elements (16).

**EXPOSURE TO EXCESS COPPER**

Population exposure to copper is mainly through drinking water and depends on several factors: water composition, the stagnant contact time between water and the pipe, the age of the pipe, the installation procedures of copper pipes, the use of copper pipes in water distribution networks, and the drinking habits of the population. The concentration of copper after water transport and distribution in a copper pipe depends on the chemical composition of the water, mainly its pH and alkalinity. Other compositional variables such as the dissolved inorganic carbon, organic substances, and other compounds also play a role in copper byproduct release (17, 18). Illustrated in Figure 1 is the cumulative distribution of copper exposure from food and water (broken lines) relative to population distributions of physiologic needs or of toxic amounts represented by the bell shaped curves.

Risk of copper excess in apparently healthy human populations can be hypothesized under relatively rare conditions of exposure. These include the following. 1) Populations exposed to high copper intake, ie, subjects that consume water containing >5 mg Cu/L or those in the general population with high intakes of copper from food or nutritional supplements high in copper, may be at risk of copper excess. Food may account for >90% of copper intake in adults if water has low copper content (<0.1 mg/L). If water copper content is higher (1–2 mg/L), it may account for up to 50% of total intake. In infants consuming copper-supplemented artificial formula, the contribution of water may be <10%, whereas if the formula is not fortified with copper, water may contribute >50% of total copper intake, especially when water copper content is 1–2 mg/L (19). The situation of persons taking copper as a supplement differs; there is one case report in the literature described by O’Donohue et al (20) of a man who after taking a supplement of 30 mg/d for 2 y and then 60 mg/d for the next year, an estimated intake of 600–900 μg · kg⁻¹ · d⁻¹, developed severe liver failure and required a liver transplant to survive.
2) Formula-fed infants consuming powdered formula containing copper and tap water containing >2 mg/L may also exceed the World Health Organization upper limit of 150 μg · kg⁻¹ · d⁻¹. However, a controlled study in a group of 100 healthy infants consuming close to double that amount for 1 y from water with 2 mg Cu/L presented no biochemical or clinical evidence of health problems (19). Population-based studies in Germany also failed to demonstrate a link between copper exposure from water with concentrations of ≈0.8 mg/L and clinical or laboratory evidence of liver abnormalities (21).

3) Finally, population groups that may have greater susceptibility to copper excess as the result of genetic defects or gene-nutrient interactions, for example, persons heterozygous for the Wilson disease gene, may be at risk of copper excess (see B in Figure 1). This group may represent up to 1% of the general population considering the prevalence of Wilson disease across different geographic regions. For now, it remains unknown whether these subjects have increased susceptibility to accumulate copper under exposures considered safe for the general population.

Long-term safety data of normal populations are limited, and prospective studies controlling exposure over time are extremely difficult to conduct. Controlled studies over 3–6 mo have been conducted assessing responses to exposure to 8–10 mg Cu/d (22, 23). The present upper levels for copper are 8–10 mg/d; this constitutes the limit on what can be ethically assessed in normal humans. Thus, it may not be possible to demonstrate evidence of classical disease unless genetic factors condition enhanced susceptibility. Markers to assess early effects of excess accumulation of copper before any pathological changes occur might permit assessing risk in an ethical and valid form. If such a marker was available, effective risk management targeting susceptible individuals or population groups could be implemented. Unfortunately, the problem is not resolved by markers of exposure; the need is for specific biomarkers of liver copper load that would predict storage at levels compatible with liver cirrhosis. Even markers of subclinical disease would be inadequate, because once the inflammatory process leading to fibrosis is triggered, it might too late for preventive actions.

FIGURE 1. Population risk of copper excess on the basis of distribution curves of requirements (bell shaped curves) and exposure (broken lines represent various cumulative distributions of population exposure from food and water). The open curve on the far left indicates normal subjects under a safe range of copper exposure (A–B), and the solid curve on the extreme right indicate normal subjects under excessive exposure. Genetically distinct population groups are depicted as solid curves (Wilson disease, heterozygous Wilson disease, and idiopathic copper toxicosis). These groups may be toxic at intakes that are below normal requirements (A), at the recommended intake (B), or close to the upper level (C) for normal subjects. All subjects, including the normal population, will be toxic at extremely high exposure (D).

QUANTIFYING RISK OF COPPER EXCESS

Currently, the only marker of hepatic copper overload is the serial measurement of copper content performed in liver biopsies. Normal liver copper content ranges from 15 to 55 μg/g dry liver. Virtually all untreated patients with Wilson disease have elevated hepatic copper concentrations, ranging from 250 to 3000 μg/g dry liver. The finding of a normal hepatic copper concentration effectively excludes the diagnosis of untreated Wilson disease. However, an elevated liver copper concentration alone is insufficient to establish a diagnosis of Wilson disease, because concentrations >250 μg/g may be found in other chronic hepatic disorders, including primary biliary cirrhosis, primary sclerosing cholangitis, extrahepatic biliary obstruction or atresia, chronic active hepatitis, intrahepatic cholestasis of childhood, and Indian childhood cirrhosis. An alternative marker could be the concentration and level of saturation of ceruloplasmin; unfortunately, this indicator is responsive to low copper intake but fails to increase in the presence of excess copper. The normal serum concentration of ceruloplasmin is 200 to 400 mg/L; although concentrations are low in human newborns, they gradually rise during the first 2 y of life, coinciding with the postnatal decline in hepatic copper concentration. Concentrations are below the normal range in ≈90% of all patients with Wilson disease. Difficulty may arise with regard to the 10% of heterozygous carriers of the gene for Wilson disease who manifest diminished serum concentrations of ceruloplasmin yet never develop clinical symptoms or signs of the disease. These individuals, who represent 1:2000 persons in the general population, may present a difficult diagnostic dilemma if they fortuitously develop hepatitis or cirrhosis secondary to other etiologies, thereby mimicking the clinical, biochemical, and histological features of Wilson disease.
TABLE 1

<table>
<thead>
<tr>
<th>Specific risk</th>
<th>A (0.5 mg/d)</th>
<th>B (3 mg/d)</th>
<th>C (10 mg/d)</th>
<th>D (60 mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical disease</td>
<td>0.00005</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.1</td>
</tr>
<tr>
<td>Subclinical disease</td>
<td>0.00001</td>
<td>0.0010</td>
<td>0.0301</td>
<td>0.5</td>
</tr>
<tr>
<td>Liver copper loading</td>
<td>0.00010</td>
<td>0.0033</td>
<td>0.1111</td>
<td>1.0</td>
</tr>
<tr>
<td>Risk of any effect†</td>
<td>0.00016</td>
<td>0.0044</td>
<td>0.1412</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1 For details on types of specific risk, see the text. Risk proportions are based on the assumption that all subjects were exposed to the corresponding level (A–D). See Figure 1 for population risk assessment of a normal population and susceptible groups at given levels of exposures.

2 Sum of each specific risk at a given exposure level.

Systematic studies in humans suggest that the consumption of beverages or drinking water with copper in excess of 5–6 mg/L results in nausea, vomiting, and diarrhea in >5% of those exposed (24). Thus, the present World Health Organization guideline for drinking water has been set at 2 mg/L. This figure was considered to be safe for chronic population exposure, because for now there is no evidence to the contrary, except for patients with Wilson disease (24). However, a US National Academy of Science expert group asked to examine whether the US limit of 1.3 mg Cu/L in water could be raised to 2 mg/L kept the existing level because only half the experts accepted the proposed change (25, 26). The remaining half were concerned about potential chronic health effects due to uncertain frequency of genetic susceptibility. They considered that up to 1% of the population was heterozygous for the Wilson disease gene and potentially another 1% could have other susceptibility genes, thus the need to define the long-term effect of this level of exposure in genetically susceptible individuals.

Even if we had the perfect marker for Wilson disease, with a 100% predictive power, it would require a large sample size to demonstrate the presence or absence of risk with sufficient power to guide preventive action at the population level. Shown in Table 1 is the specific risk for various potential clinical and subclinical endpoints and the overall population risk of any measurable effect considering different population exposure levels based on the model presented in Figure 1. The endpoints are based on the known frequencies of genetic defects that define increased susceptibility at lower exposure levels. It is apparent that at usual exposures it would be extremely rare to find clinical signs or even subclinical signs; thus, the only sensitive measure for copper excess would be indexes that predict liver copper intake on copper absorption in young men at three levels of dietary copper by use of the stable isotope 64Cu. Am J Clin Nutr 1989;49:870–8.


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