New Aspects on the Role of Copper in Human Nutrition: a Toxicological Approach

I. Delimar1, I. Iakovidis 2
1 External Postdoctoral Scientific Coordinator, Section of Morphological and Clinical-Laboratory Sciences, Medical School of Ioannina, Greece, 2 Lecturer, Department of Physics Chemistry & Materials Technology, Technological Educational Institute of Athens, Greece.

ABSTRACT
Although it is widely accepted that copper plays a key role in human nutrition the clinical picture of its deficiency is not always so straightforward. Moreover, serious concerns about the overuse of copper dietary supplements arouse when investigations revealed that copper toxicity in humans has probably been underestimated. This paper reviews the present knowledge on the deficiency and toxicity of dietary copper in humans.

Keywords: Copper deficiency, copper toxicity, chalcosis, nutritional supplements.

INTRODUCTION
Copper (Cu) is a key element in cellular biochemistry, and its involvement in critical enzyme systems within the human organism is wide. It is a transition metal with three oxidation states: Cu0, Cu1+ (cuprous), and Cu2+ (cupric). In biological systems, including water, copper tends to be in the cupric state, although it is also found as Cu(I). Easy release and absorption of one electron is part of the basic chemistry of copper. At physiologic pH, there is little or no free copper in solution, especially if chelating agents are available. Most copper in foods is bound to specific proteins [1]. Natural copper intake in Europe is about 1-2 mg Cu/person/day [2]. In the United States, the median intake of copper from food is 0.93–1.3 mg/day for adults (0.013–0.019 mg Cu/kg body weight/day using a 70-kg reference body weight). A recommended dietary allowance (RDA) of 0.9 mg/day (0.013 mg/kg/day) has recently been established [3]. The tolerable upper levels (UL) of intake from food, water, and supplements for trace elements and metals according to the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the International Atomic Energy Agency and the National Academy of Science and the Food and Nutrition Board Dietary Reference Intakes (DRIs) are 9 and 10 mg copper/d respectively (Table 1).

Based on the values established by the WHO for copper intake (1996) the meeting set an upper limit for copper intake of 0.15 mg Cu/kg bw/day [2].

The only way copper normally enters human body is through the alimentary tract. In general enteral absorption may occur throughout the whole length of the gastrointestinal tract but three areas are of special importance, depending on the formation constants of the particular Cu-ligand complexes [4, 5]. These are the mouth (pH - 7.4) the stomach (pH - 1.6) and the small intestine (pH - 6-6.5 in the duodenum and - 6.5-7 in the jejunum) [5]. Generally, copper does not enter human body through the skin, unless, for example, it is applied in high concentrations in the form of specific ointments or if copper bracelets are worn [1]. According to its content in copper food can be classified as rich (more than 5 mg/Kg), adequate (0.1 to 4 mg/Kg) and poor (less than 0.1 mg/Kg). Most of the foods are of adequate content. Organ meats and shellfish are the richest food sources of copper. The more abundant plant sources include nuts, seeds, legumes, whole grains, some fruits, potatoes, and chocolate [6]. Drinking water is the primary source of excess copper. Copper concentrations in drinking water vary widely as a result of variations in pH and hardness of the water supply; the levels range from a few ppbs to 10 ppm.
The mean concentration of copper in soil in the United States ranges from 5 to 70 mg/kg [3].

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<tr>
<th>Advisory Board</th>
<th>Reference Value</th>
<th>Copper</th>
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<tbody>
<tr>
<td><strong>Dietary copper requirements</strong></td>
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<tr>
<td>WHO International Programme on Chemical Safety, 1998</td>
<td>Acceptable Range of Oral Intake (AROI)</td>
<td>1.2 to 2 or 3 mg/d</td>
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<tr>
<td>Department of Health, UK, 1991</td>
<td>Reference Nutrient Intake (RNI)</td>
<td>1.2 mg/d</td>
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<tr>
<td>Food and Nutrition Board, US, 2001</td>
<td>Recommended Daily Allowance (RDA)</td>
<td>0.9 mg/d</td>
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<td>Food and Nutrition Board, US, 2001</td>
<td>Estimated Average Requirements (EAR)</td>
<td>0.7 mg/d</td>
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### Recommended limits of copper intake in drinking water

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<th>Advisory Board</th>
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<tr>
<td>WHO Guidelines for Drinking Water Quality, 1993</td>
<td>WHO standard</td>
<td>2.0 mg/L</td>
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<tr>
<td>EU Directive 98/83 L330</td>
<td>EU standard</td>
<td>2.0 mg/L</td>
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<tr>
<td>Water Quality Regulations, 1989</td>
<td>UK standard</td>
<td>3.0 mg/L</td>
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<tr>
<td>EPA Drinking Water Regulations, 1988</td>
<td>US maximum contaminant level</td>
<td>1.3 mg/L</td>
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### Upper limit (UL) for total copper intake

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<th>Advisory Board</th>
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<td>European Food Safety Authority, EU, 2008 [2]</td>
<td>UL</td>
<td>0.15 mg Cu/kg bw/d</td>
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Many of the well established biological functions of copper in the body arise directly from its role in a number of copper-containing metalloenzymes (e.g. cytochrome oxidase, lysyl oxidase, caeruloplasmin, superoxide dismutase, monophenol mono-oxygenase (tyrosinase), dopamine B-monooxygenase) [7], and possibly also indirectly from the effect that a change in copper status may have on other enzyme systems which do not contain copper [7]. This wide ranging involvement of copper in various enzyme systems is used as an argument by some individuals to justify their belief that copper supplementation in healthy people could be beneficial as it might prevent various diseases [4]. On the other hand, several scientists claim that the overuse of copper supplements could cause damage to human health [4]. The aim of this review study is to unravel the role of copper in human nutrition with emphasis in Cu deficiency and toxicity. Original articles were searched via Google Scholar and PubMed published between 1960 and 2012. Search terms were “copper”, “copper and nutrition”, “copper and diet”, “copper deficiency”, and “copper toxicity”. All papers identified were English language full text papers. Studies that did not involve human subjects in vivo were excluded. The reference lists of identified articles for other relevant papers or textbooks were also searched.

### Cu compounds in the human body

Copper enters the human body mainly through the alimentary track. After digestion of food an amount up to 75% of the copper contain is absorbed in the small intestine and is subsequently transferred to the interstitial fluid and blood plasma where it binds to albumin, mainly in the form of Cu(II) [8]. The main features of copper chemistry of significant biological interest include: (a) the soft Lewis acid character of the Cu(I) state which prefers to coordinate to S sites of biomolecules, while the hard Lewis acid Cu(II) coordinates to O and N binding sites and (b) the low reduction potential of the Cu(II)/Cu(I) E0= 0.153 V which facilitates the interconversion between the two states [9]. It has been estimated that the adult human body contains 80 mg of copper, with a range of 50-120 mg. Tissue copper levels range from < 1 pg/g (dry weight) in many organs to > 10 pg/g (dry weight) in the liver and brain [10]. Copper in human blood is principally distributed between the erythrocytes and the plasma. In erythrocytes, most copper (60%) occurs as the copper-zinc metalloenzyme superoxide dismutase, the remaining 40% being loosely bound to other proteins and amino acids. Total erythrocyte copper in normal humans is around 0.9-1.0 pg/ml of
packed red cells [10]. In plasma, about 93% of copper is firmly bound to the enzyme caeruloplasmin, while the remaining plasma copper (7%) is bound less firmly to albumin and amino acids, and constitutes transport copper capable of reacting with receptor proteins. Plasma or serum copper in normal humans is in the range 0.8-1.2 pg/ml [11].

Cu deficiency in humans with emphasis on dietary aetiology
There is no single specific index of copper deficiency. Biomarkers which, despite major limitations, are currently considered to be of value in establishing a range for normal copper status include serum copper (normal range 0.64-1.56 pg/ml), caeruloplasmin (0.18-0.40 mg/ml), urinary copper (32-64 pg/24 h) and hair copper (10-20 pg/g), all of which are lower in frankly copper-deficient subjects but are less sensitive to a marginal copper status [12]. Hypocupraemia is defined as a serum copper level of 0.8 pg/ml or less, and since about 93% of serum copper is normally bound to caeruloplasmin, is usually accompanied by hypo-caeruloplasminaemia [4]. At the clinical level copper deficiency has been recognized infrequently. Symptoms associated with copper deficiency in humans include normocytic, hypochromic anemia, leukopenia, and osteoporosis [8]. Well-documented reports are generally limited to: a) infants with Menkes' disease (an X-linked recessive disorder caused by defects in a gene that encodes a copper-transporting ATPase) [8], b) patients given inadequate copper in parenteral alimentation fluids [13], c) an insufficient copper supply during the nutritional recovery of malnourished children or preterm infants. Several factors are frequently associated with copper deficiency in these cases: low birth weight, short duration of breast-feeding or cow’s milk consumption (due to the lower copper content of cow’s milk), increased losses of nutrients as a result of diarrheal disease, and frequent infections d) in subjects with malabsorption syndromes—such as celiac disease, sprue, cystic fibrosis, and short-bowel syndrome—resulting from intestinal resection [14]. However, despite the significant progress in nutritional research the evidence-based knowledge of factors affecting the bioavailability of dietary copper is limited. Intestinal absorption of copper appears to be facilitated by L-amino acids. Persons who consume diets high in zinc and low in protein are at risk of copper deficiency. High intakes of sources of dietary fiber apparently increase the dietary requirements for copper [13].

Cu toxicity in humans with emphasis on dietary aetiology
Hypercupraemia occurs naturally during pregnancy and is associated with the so-called "acute phase" reactions of a number of diseased states. It is almost always accompanied by hypercaeruloplasminaemia [4,15]. Regardless of this, exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, and developmental toxicity [8]. Copper "supplements" taken in a dose of 30-60 mg/day during 3 years caused severe liver cirrhosis necessitating liver transplantation [16]. Moreover, fatalities from acute copper sulphate poisoning have been reported. An 11 year old female died within hours of accidentally ingesting a solution of copper sulphate [17]. In India, copper sulphate poisoning has been used as a method of suicide. Of 48 cases of copper poisoning examined, 12 were fatal and ingested doses ranged from 1 g to 100 g copper dissolved in water [18]. WHO have concluded that the fatal oral dose of copper salts is about 200 mg/kg body weight [19]. A particularly interesting clinical case refers to Indian childhood cirrhosis (ICC). ICC is a fatal disease of infants in India associated with massive levels of copper accumulation in the liver [20]. Occurrence of ICC has been attributed to the practice of boiling and storing milk in copper and brass vessels [21]. Idiopathic copper toxicosis has been attributed to high levels of copper (up to 6.8 mg/L) in drinking water [22]. Another 138 cases termed Tyrolean infantile cirrhosis (TIC) have been identified in the Tyrol (western Austria) and have been associated with high dietary copper concentrations [22]. There exists a spectrum of pathomorphological alterations in exogenous infantile copper disease correlating with the clinical outcome and it has been proposed that copper intoxication of the liver should be of diagnostic concern in any unclear case of micronodular cirrhosis in early infancy [23]. Furthermore, it has been reported an unusual case of acute copper intoxication in a patient who died after swallowing more than 700 coins. At autopsy the liver showed fibrosis. The histological analysis demonstrated the presence of copper in the hepatic tissue as well as extensive copper deposition in the histological sections [24]. In a similar case two hundred seventy-five United States coins were discovered in the stomach of a mentally disturbed individual at autopsy. Many coins containing copper were
corroded by prolonged contact with gastric juice, with subsequent absorption and deposition of copper in the liver and kidneys. The patient died from complications related to the acute toxic phase of chronic copper poisoning [25].

Acute copper toxicity is infrequent in humans and is usually a consequence of contamination of foodstuffs or beverages from copper containing vessels or dispensers [26]. In cases of suspected copper poisoning, the chelating agent penicillamine ((2S)-2-amino-3-methyl-3-sulfanyl-butanoic acid) is the drug of choice. Regarding the pathophysiological mechanisms explaining the damage to various organs due to copper excess it has been suggested that Cu facilitates oxidative tissue injury through a free-radical-mediated pathway analogous to the Fenton reaction [8]. It could generate reactive oxygen species which damage proteins, lipids and DNA [27]. Persons at special risk include those with impaired pulmonary function, especially those with obstructive airway diseases, since the breathing of copper fume might cause exacerbation of symptoms due to its irritant properties [28]. Relatively rare conditions of excessive copper exposure in apparently healthy human populations could include the following [29]: a) 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 rich in copper, b) formula-fed infants consuming powdered formulas containing copper, and c) individuals that may have greater susceptibility to copper overload as a result of genetic conditions or gene-nutrient interactions, for example, persons heterozygous for the Wilson’s disease (an autosomal recessive disease of copper metabolism) gene.

Copper is not classifiable as to human carcinogenicity. There are no human data, inadequate animal data from assays of copper compounds, and equivocal mutagenicity data [30]. It is generally agreed that copper itself is less toxic than its salts [31]. It has been shown that administration of high doses of copper to monkeys induces transcriptional activation of hepatic proliferative responses [32]. Regarding the pathology of the liver in copper overload it is considered that copper accumulation in the liver is associated with cellular and apoptotic injury [33]. Copper and copper-associated protein accumulation may also be seen in chronic biliary obstructive processes [33]. Moreover, in a large community study, high copper intake was associated with a significantly faster rate of cognitive decline among persons who also consumed a diet rich in saturated and trans fats [6]. Currently, the role of dietary copper in neurodegenerative diseases (Alzheimer’s disease, Parkinson’s and prion diseases) is under evaluation [34, 35]. Several reports of acute hemolytic anemia in patients undergoing hemodialysis have been attributed to excess copper in the dialysis fluid [36]. Copper allergies have been reported, either related to skin contact with copper salts or to copper containing intrauterine devices [37, 38]. Also, copper itself has been known to cause keratinization of the palms of hands and soles of feet [31].

Industrial Cu exposures as metal fume could lead to atrophic changes in the nasal mucous membranes [37]. The acute inhalation of copper fume during refining or welding processes may cause typical metal fume fever with upper respiratory irritation, chills, and aching muscles [39]. Other signs and symptoms of metal fume fever include nausea, fever, dry throat cough, weakness, and lassitude. There is usually leucocytosis, which may amount to 12,000 to 16,000/ml, and in some instances discoloration of the skin and hair [28]. In an interesting case-report it was shown that two children developed green hair with copper absorbed from swimming pools to be considered as responsible (the source being either copper piping or a copper-containing algicide) [40]. In rare instances open angle glaucoma has developed in eyes with disseminated chalcosis from copper foreign bodies, but not in endogenous chalcosis of Wilson’s disease [41]. Verdigris, formed by atmospheric corrosion of the surface of metallic copper presumably composed of copper carbonates and oxides, causes immediate irritation and inflammation when accidentally dropped on the eyes of patients [41]. It has to be mentioned that in an extensive investigation of 1,910 workers exposed to the dust of metallic copper and its oxides the clinical symptoms were acute gastrointestinal disturbance, pain in chest, metallic taste in mouth, nausea, vomiting, as well as some respiratory irritation, and dyspnea. The digestive disturbance was attributed to the conversion of the swallowed metallic copper to its irritating salts [31]. Moreover, copper fume is considered to be associated with increased risk of pancreatic cancer [42], while incubation of human spermatozoa with metallic copper is found to bring about a significant fall in the percentage of motile sperm [43].

CONCLUSIONS
Copper present health beneficial effects at physiological doses versus potential deleterious effects at high doses in humans. It is both essential and toxic, depending on the dose ingested. Acute toxicity is not usual and is often an after-effect of contamination of food stuffs or beverages from copper containing vessels or dispensers [26]. However, long term use of copper supplements has been implicated in liver cirrhosis [15,16,29]. Copper supplements (multivitamin or mineral products) are most likely to cause side effects or even harm: a) a child b) when people take them instead of prescribed medicines c) when people take many supplements in combination d) due to interaction with certain prescription drugs e) when women take them in pregnancy or nursing f) when copper is added to foods. It is evident that there were always good ideas in nutritional research (sometimes based on reasonable biochemical mechanisms), which, however, were not accompanied by corresponding good results in the “arena” of randomized clinical trials [44]. Guidelines for diet should adhere closely to what has been clinically proved [45], and by this standard there is currently no basis to recommend copper supplements for disease prevention to healthy people. We must realize that their use is not an alternative to regular consumption of food sources rich in copper (as organ meats, shellfish, nuts, seeds, legumes, whole grains, potatoes, and chocolate). In future studies closer attention should be given initially to the definition and later to the monitoring of the many biological processes that are directly or indirectly responsive to alterations in copper homeostasis [8]. Studies could include the monitoring of copper-responsive biochemical changes that may suggest appropriate indices for the early detection of pathologically relevant changes in subsequent studies with human subjects.

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ΑΝΑΣΚΟΠΗΣΗ

Νεότερα δεδομένα για το ρόλο του χαλκού στη διατροφή του ανθρώπου: μια τοξικολογική προσέγγιση

Ι. Δελμαρίς1, Ι. Ιακωβίδης2

1 Εξωτερικός Μεταδιδακτορικός Επιστημονικός Συνεργάτης, Μορφολογικός-Κλινικοαρσενημικός Τομέας Ιατρικής Σχολής Πανεπιστημίου Ιωαννίνων, 2 Λέκτορας, Τμήμα Φυσικής Χημείας & Τεχνολογίας Υλικών, Τεχνολογικό Εκπαιδευτικό Ίδρυμα Αθήνας, Αιγάλεω

(Επιστημονικά Χρονικά 2013;18(1):7-12)

ΠΕΡΙΛΗΨΗ

Μολονότι είναι ευρέως αποδεκτό ότι ο χαλκός παίζει ένα βασικό ρόλο στην ανθρώπινη διατροφή, η κλινική εικόνα της έλλειψης του στον οργανισμό δεν είναι πάντα έξοχες. Επιπλέον, σοβαρές αντισυνθέσεις εγκαίνιαται σχετικά με την υπερβολική χρήση διατροφικών συμπληρωμάτων χαλκού, εξαιτίας της δημιουργίας ερευνών που υποστήριζαν ότι η τοξικότητα του στον άνθρωπο πιθανός έχει υποτιμηθεί. Η παρούσα εργασία εξετάζει την τρέχουσα γνώση αναφορικά με την ανεπάρκεια και την τοξικότητα του χαλκού στον άνθρωπο σε σχέση με τη διατροφή.

Λέξεις ευρετηρίου: Ανεπάρκεια χαλκού, τοξικότητα χαλκού, χάλκωση, συμπληρώματα διατροφής