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Review

Dietary copper and human health: Current evidence and unresolved issues

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ABSTRACT

Although copper (Cu) is recognized as an essential trace element, uncertainties remain regarding Cu reference values for humans, as illustrated by discrepancies between recommendations issued by different national authorities. This review examines human studies published since 1990 on relationships between Cu intake, Cu balance, biomarkers of Cu status, and health. It points out several gaps and unresolved issues which make it difficult to assess Cu requirements. Results from balance studies suggest that daily intakes below 0.8 mg/day lead to net Cu losses, while net gains are consistently observed above 2.4 mg/day. However, because of an incomplete collection of losses in all studies, a precise estimation of Cu requirements cannot be derived from available data. Data regarding the relationship between Cu intake and potential biomarkers are either too preliminary or inconclusive because of low specificity or low sensitivity to change in dietary Cu over a wide range of intakes. Results from observation and intervention studies do not support a link between Cu and a risk of cardiovascular disease, cognitive decline, arthritis or cancer for intakes ranging from 0.6 to 3 mg/day, and limited evidence exists for impaired immune function in healthy subjects with a very low (0.38 mg/day) Cu intake. However, data from observation studies should be regarded with caution because of uncertainties regarding Cu concentration in various foods and water. Further studies that accurately evaluate Cu exposure based on reliable biomarkers of Cu status are needed.

Contents

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1. Introduction

Copper (Cu) is an essential trace element in both humans and animals. Needed only in trace amounts, the human body contains approximately 100 mg Cu. As a transition metal, it is a cofactor of many redox enzymes, Ceruloplasmin being the most abundant Cu-dependent ferroxidase enzyme with a Cu-dependent oxidation activity. Beyond its role in iron metabolism, the need for Cu also derives from its involvement in a myriad of biological processes, including antioxidant defense, neuropeptide synthesis and immune function [1,2]. As a consequence, the wide range of clinical features resulting from perturbations in the activities of cuproenzymes [3] means that although severe Cu deficiency is relatively straightforward to diagnose, identifying marginal deficiency is somewhat problematic. Dietary Cu deficiency can result in adverse consequences throughout the life span. *In utero*, Cu deficiency may result in impaired development of the cardiovascular system, bone malformation and ongoing neurologic and immunologic abnormalities into infancy and beyond [4,5]. In adulthood, prolonged marginal Cu deficiency has been associated with alterations in cholesterol metabolism [6,7].

Though an essential micronutrient for man, Cu is toxic at high levels. An overload of this metal easily leads to Fenton-type redox reactions, resulting in oxidative cell damage and cell death. However, Cu toxicity as a result of dietary excess is generally not considered a widespread health concern, probably as a result of the homeostatic mechanisms controlling Cu absorption and excretion [8].

Dietary reference values for Cu were established for the French population more than a decade ago. Because of the lack of data on Cu metabolism, dietary reference values have been based on data from dietary surveys and balance studies.

The French population reference intakes (ANC—*apports nutritionnels conseillés*) for Cu are presented in Table 1, along with those established in other countries.

Discrepancies between current recommendations highlight the difficulties in establishing a requirement for dietary Cu. The aim of this literature review is to present the current knowledge regarding Cu intake and metabolism, biomarkers of Cu intake, and the relationship between dietary Cu and health.

2. Copper metabolism and balance

A description of the mechanism involved in Cu transport across the GI tract is beyond the scope of this review and readers interested in this topic are invited to refer to recent reviews [9–11].

Cu absorption occurs mainly in the proximal part of the small intestine, where it is transported into the liver via the portal vein. It is complicated to measure true fractional absorption of Cu because of important fecal Cu excretion and the existence of only two stable isotopes, ^{63}Cu (69.2% natural abundance) and ^{65}Cu (30.8% natural abundance), which makes it impossible to use a dual labeling technique to correct apparent absorption for biliary and gastrointestinal re-excretion. In most studies, fractional Cu absorption has been measured using extrinsic meal labeling with ^{65}Cu and fecal Cu monitoring for several days [12–22]. Recently, the addition of the non-absorbable rare earth element Holmium to ^{65}Cu-labeled meal has been used to accurately quantify the amount of absorbed Cu re-excreted in fecal matter [13,14]. Using this approach, it has been concluded that true fractional absorption of Cu was close to 50%, and remained constant for Cu intake ranging from 0.7 mg/day to 6.0 mg/day. This estimation has recently been confirmed by the analysis of fecal Cu excretion and plasma Cu appearance data after the oral or intravenous administration of ^{65}Cu, using a simple compartmental model [12].

Several parameters affect the absorption rate of dietary Cu, including age, gender, food type, amount of dietary Cu and oral contraceptives. These parameters can cause the absorption rate to vary

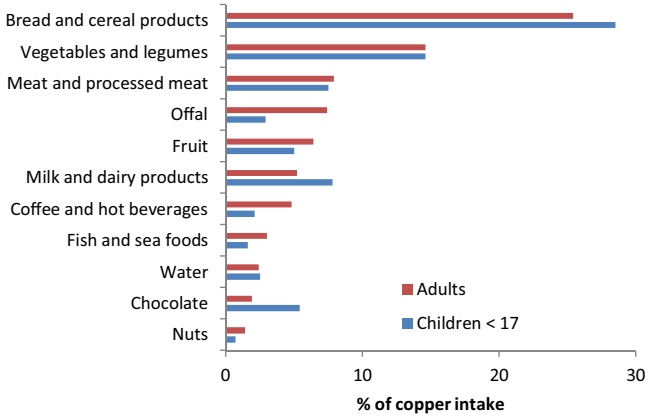

Fig. 1. Contribution of the main food groups to Cu intake in the French population (INCA-2).

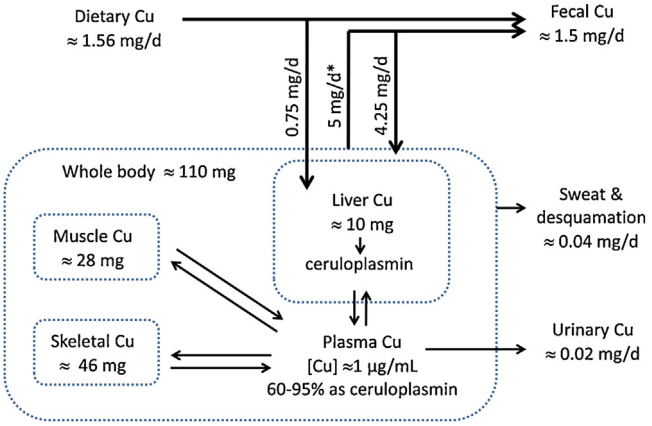

Fig. 2. Whole-body Cu pools and fluxes.

Table 1
Population reference Cu intake (mg/day) in different countries.

	0–6 months	6–12 months	1–3 years	4–6 years	7–10 years	11–20 years	20–50 years	>50 years	Pregnancy	Breastfeeding
France (2001)	0.4	0.6	0.75	1	1.2	1.5	2 (m) 1.5 (w)	1.5	2	2
USA (2001)	0.2	0.22	0.34	1	1–1.3 (m) 1.1 (w)	1.3–1.5 (m) 1.1 (w)	1.7 (m) 1.2 (w)	1.7 (m) 1.2 (w)	1.2–1.3	1.4–1.5
Australia (2004)	0.2	0.22	0.7	1	1–1.3 (m) 1.1 (w)	1.3–1.5 (m) 1.1 (w)	1.7 (m) 1.2 (w)	1.7 (m) 1.2 (w)	1.2–1.3	1.4–1.5
Nordic countries (2010)	–	0.3	0.3–0.4	0.4	0.5	0.7–0.9	0.9	0.9	1	1.3
UK (1991)	0.3	0.3	0.4	0.6	0.7	0.8–1	1.2	1.2	1.2	1.5

USA (2001): Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, Cu, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Institute of Medicine, National Academy Press, Washington DC.
Australia (2004): Nutrient reference values for Australia and New Zealand. Melbourne, Ministry of health, Government of Australia.
Nordic countries (2010): Nordic Nutrition recommendation 2012, 5th edition, Nordic Council of Ministers, Copenhagen.
UK (1991): Dietary reference values for food, energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference values of the Committee on Medical Aspects of Food Policy, HSMO.

between 12 and 71% [11]. The effect of age and gender on Cu absorption in adults has been reported in only one study [23]. Because this study relies on whole-body counting after the oral administration of the radioisotope ^{67}Cu to estimate dietary Cu absorption, the fractional absorption values cannot be compared to later estimations from Harvey et al. However, within this study, Cu absorption was consistently found to be 10% higher in women than men, and was unaffected by hormone use in women. According to this study, the fractional absorption of Cu was similar in young adults and elderly subjects. In contrast, limited evidence suggests that the intestinal absorption of Cu is higher in infants than adults. By monitoring fecal ^{65}Cu elimination after an oral dose, Olivares et al. have reported an apparent fractional absorption of Cu close to 80% in fully or partially breast-fed infants aged 13 months [24]. Because this estimation was not corrected for intestinal Cu re-excretion, it can be assumed that Cu uptake from breast milk is almost total in infants.

Following intestinal absorption, 75% of portal Cu is taken up by the liver [12] and the remainder flows into the peripheral circulation, mainly bound to albumin. In the liver, 20% of the Cu taken up is re-excreted back into the gastrointestinal tract and 80% is exported to the periphery bound to ceruloplasmin [12]. Based on Cu concentration in the different gastrointestinal fluids, it has been estimated that approximately 2.5 mg of Cu are excreted daily in the biliary flow, and that an equivalent amount is excreted through other gastrointestinal secretions, including saliva, gastric juice, pancreatic secretion and intestinal fluid secretion [25]. Because balance studies indicate that daily fecal losses of Cu are of the same order of magnitude as dietary Cu intake [8,13,19,20], it can be concluded that most of the endogenous Cu secreted into the gastrointestinal lumen is reabsorbed across the intestinal epithelium. Moreover, using Holmium to distinguish unabsorbed dietary Cu from endogenous Cu secretion in the gastrointestinal tract, it has been shown that the gastrointestinal secretion of Cu plays a major role in the control of Cu homeostasis, with endogenous losses increasing from 0.45 to 2.46 mg/day for Cu intake increasing from 0.7 to 6 mg/day [13].

Compared to fecal excretion, urinary Cu excretion is low (10–25 μg/day) [8,26,27]. The effect of dietary Cu level on urinary Cu excretion is inconsistent, with some studies reporting a small but significant positive relationship [8,27] and others showing no effect [26]. While tubular Cu re-absorption involving the ATP7A protein has been reported [28], there is no evidence that urinary excretion plays a role in the control of Cu homeostasis in response to changes in Cu intake.

Little is known regarding the respective importance of all the other routes of Cu excretion. While an early report from Jacob et al. suggests that surface losses of Cu in adult men were close to 300 μg/day [29], a more careful estimation from the same team later concluded that sweat and integumentary losses averaged 42 μg/day [30]. Small amounts of Cu have also been reported to be lost in normal menstrual flow in women, but there is no evidence that women's Cu status could be compromised by menstruation. Information regarding Cu absorption, losses and organ pools are summarized in Fig. 2.

The results of balance studies have been used to set nutritional requirements in adults for several minerals, including calcium, magnesium and zinc. Four balance studies with a different level of dietary Cu have been published since 1990 [8,13,16,20]. Because balance calculation requires a thorough assessment of nutrient losses, and because none of these four balance studies meet this criterion, they must be considered likely to be biased, although dietary intake was usually carefully controlled. Fecal, urinary, sweat and integumentary losses were only measured in some subjects of one study [16]. In this study, however, sweat and integumentary losses were not taken into account in the final balance calculation. Urinary and fecal losses were taken into account in two studies [8,31] while only fecal losses were considered in the remaining study [13]. At Cu intakes above 2.4 mg/day, Cu balance was consistently reported to be positive [8,13,20] and Cu balance values were always negative for Cu intakes below 0.8 mg/day [13,20]. The minimal amount of dietary Cu required to achieve a null balance lies somewhere between 0.8 and 2.4 mg/day, but the quality of the studies—and more specifically the fact that urinary and/or surface losses were not measured—preclude the precise estimation of Cu requirement.

3. Dietary sources and copper intake

Cu content in foodstuff varies according to local conditions. Soil Cu concentration, slurry/manure spreading, use of Cu compounds as bactericides or fungicides on many crops and Cu emissions from smelting and casting industries may affect the Cu content in cereals, fruit and vegetables and, to a lesser extent, meat and animal products [32–35]. Moreover, the Cu concentration in drinking water may also vary depending on groundwater Table composition and household plumbing systems. Soft acidic water causes corrosion in Cu pipes and increases tap water Cu concentration. For instance, tap water Cu concentration has been shown to vary largely in dwellings, with 0.02–3.5 mg/L in Berlin [36] and 0.1–5 mg/L in Uppsala and Malmö [37]. Altogether, these variations represent serious hindrances in the assessment of Cu intake both at individual and population levels, and Cu content in food composition databases must be considered with caution. However, despite those variations, food groups such as offal and nuts, and to a lesser extent cereals and fruit, can be regarded as good sources of Cu, while milk and dairy products contain low amounts (Table 2).

The Cu intake of representative samples of healthy populations have been assessed in several national dietary surveys. In France, the second National Individual Survey on Food

Table 2
Cu content of selected foods.

Foods	Cu (mg/kg)
• Milk and dairy products	
Milk, plain	0.1–0.88
Fresh cheese	0.03
Processed cheese	0.025
• Meat and offal	
Liver, beef	157
Kidney, beef	2.1–4.3
Muscle meat, beef	0.1–1.8
Muscle meat, pork	0.1–9.1
• Cereal products	
Maize products	0.6–16.6
Wheat bread	2.9
Whole grain wheat bread	3.4
Whole grain pasta	0.08–0.52
• Vegetables	
Potato	0.48–16.0
Carrot	0.37–0.62
Broccoli	0.68–0.87
Peas	1.9–2.4
Lettuce	0.1–2.9
Tomato	0.1–3.4
Cabbage	0.1–7.7
• Seafood	
Oysters	0.3–16.0
Tuna	0.1–1.2
Salmon	0.5–0.8
Shrimp	2.0–2.9
Flounder	0.1–2.5
• Fruits	
Apples	0.1–2.3
Bananas	0.7–3.0
Oranges	0.8–0.9

Consumption (INCA2) includes data on dietary Cu intake, gathered over seven days from a representative sample of the metropolitan population, consisting of 2624 adults aged 18–79 years old and 1455 children aged 3–17 years old [38]. After excluding subjects with incomplete dietary records and under- or over-reporters, dietary Cu intake was calculated for 1863 adults and 1382 children aged 3–79 years old (Table 3). In adults, daily intake (mean ± SE) was significantly higher in men than women (1.53 ± 0.03 *vs* 1.30 ± 0.02, respectively). In both sexes, Cu intake was lower in subjects aged 18–35 years old than in subjects over 35 years old, in accordance with a lower consumption of fruit, vegetables, nuts and offal in young French adults [38]. In children, daily Cu intake (mean ± SE) was 1.06 (±0.02) and 0.94 (±0.01) in boys and girls, and increased with age. Intakes of Cu in the 95th percentile are 2.4 g/day in men, 2.1 g/day in women and 1.6/day in children. Similar levels have been reported in

Table 3
Daily Cu intake in the French population, by gender and age group.

Age (years)	Male	Female
3–10	1.0 ± 0.5	0.9 ± 0.3
11–14	1.2 ± 0.5	1.0 ± 0.3
15–17	1.2 ± 0.5	1.0 ± 0.3
18–34	1.5 ± 0.7	1.1 ± 0.4
35–54	1.6 ± 0.7	1.4 ± 0.6
55–79	1.6 ± 0.9	1.4 ± 0.7

Data are from the INCA2 study [38].

other European countries for adults and the elderly (Table 4), according to two recent reviews of representative national dietary surveys and total diet studies [35,39] and in the US adult population (1.5 ± 0.02 and 1.2 ± 0.02 for dietary Cu in adults over 20 years old) [40].

Food products are seldom fortified with Cu in the European Union. The main contributors to Cu intake in the French population are bread and cereal products (≈25–30% of dietary Cu), meat and offal (15–20%) and legumes and vegetables (≈15%), while dairy products, water and beverages provide less than 10% of dietary Cu (Fig. 1) [38]. The respective contributions of these food groups to total intake were similar in adults and children (3–17 years old) and close to those reported in other European countries [35]. Few data are available regarding the contribution of dietary supplements to daily Cu intake in the European Union [41]. In France, Cu intake from food supplements is very low in adults and children and does not contribute to excessive intakes (ANSES data, working paper). In contrast, dietary supplements significantly contribute to daily Cu intake in the US population. In adults, results from the NHANES 2003–2006 survey indicate that male dietary supplement users obtain 50% of their daily Cu intake from supplements and females, more than 60% [42]. For both men and women, the use of dietary supplements resulted in a small but significant reduction in the prevalence of Cu inadequacy. However, with less than 10% of adults having an intake below the estimated average requirements, Cu inadequacy is far from being a public health issue in the US population, and the prevalence of inadequate intake is already lower in supplement users than non-users when considering only Cu from food. Dietary supplements are thus of little interest for Cu nutrition. On the contrary, the use of dietary supplements was associated with a large increase in the prevalence of two- to eight-year-old children with a total intake above the upper limit (7.7 *vs* 39% for non-users and users respectively) [43]. Although little information is available regarding the long term effects of high Cu intake in children, these results suggest that the risk-benefit ratio of dietary supplement usage inclines toward risk as far as Cu nutrition is concerned.

Little information is available regarding Cu intake and adequacy in populations with specific diets such as vegetarians, vegans or those practicing macrobiotics. An analysis of duplicated meals has shown that the Cu density of a vegan diet was more than twice that

Table 4
Mean Cu intake in adults and the elderly in different European countries according to representative national dietary surveys.

Country	Study—years	Survey method	Men		Women	
			N	Mean ± SD	*N*	Mean ± SD
Adults (19–64 years)						
FI	FINDIET 2007	adj 48H DR	730	1.6 ± 0.7	846	1.3 ± 0.5
IRL	SLAN 2007	FFQ	662	1.5 ± 0.8	717	1.2 ± 0.7
IT	INN-CA 1994-1996	7 day record	660	1.6 ± 0.7	801	1.3 ± 0.5
UK	Health Survey for England 2000-2001	7 day record	219	1.4 ± 0.7	210	1.0 ± 0.4
Elderly (>64 years)						
FI	FINDIET 2007	adj 48H DR	229	1.4 ± 0.7	234	1.2 ± 0.5
IRL	SLAN 2007	FFQ	580	1.4 ± 0.9	742	1.3 ± 0.7

Data from Ref. [39].

of an omnivorous diet (0.7 ± 0.29 *vs* 2.0 ± 0.34 mg/1000 kcal for the vegan and omnivorous diet respectively) [44]. In the same way, daily Cu intake was 27% higher in vegetarians than in omnivorous adolescent females [45]. The higher Cu content of a plant-based diet has been consistently shown to compensate for the slightly reduced bioavailability of Cu resulting from the presence of phytates and fibers, suggesting that diets low in or devoid of animal products provide an adequate amount of Cu [17,21,46].

4. Biomarkers of copper exposure

The assessment of dietary adequacy of Cu is constrained by the absence of recognized biomarkers of Cu status. Despite the widely held view that there is a lack of sensitive and specific biomarkers of Cu status, several putative indexes—including plasma Cu, ceruloplasmin and Cu/Zn superoxide dismutase (Cu/Zn SOD)—are routinely assayed in human studies. However, it has been pointed that a minimum of four weeks is needed to observe a variation in these biomarkers. This suggests that Cu metabolism adapts only slowly to changes in dietary Cu intake [47]. Regardless of the possible futility of these analyses [3,48], their widespread use continues mainly due to the absence of anything better.

4.1. Plasma and serum Cu

We have identified in our review eight recent controlled trials reporting the effect of dietary Cu intake on plasma or serum Cu (Table 5).

In one of these trials, judged as at high risk of bias, a small but significant decrease in plasma Cu was observed in young healthy men at the end of a Cu depletion period (dietary Cu = 0.38 mg/day), compared to the preceding equilibration period (dietary Cu = 0.66 mg/day) or the subsequent repletion period (dietary Cu = 2.49 mg/day) [27].

In the other trials [13,16,26,49–51], the range of dietary Cu intake was 0.57–6.9 mg/day. In none of these studies did the authors report any difference in plasma Cu between the different dietary Cu periods. Therefore, it seems that plasma Cu does not vary for daily intakes between 0.57 mg and the upper level. A very low Cu intake (0.38 mg/day) may result in a decreased plasma Cu but this remains questionable as such a low intake was imposed in only one trial.

In a review on the methods for assessing Cu status, including studies among unhealthy populations, serum Cu appeared to reflect changes in Cu status in both Cu-depleted and Cu-replete individuals, with smaller changes in Cu-replete individuals [47].

Plasma Cu concentrations are consistently reported to be lower than serum values, which possibly contributed to a lack of response with the biomarker in Cu-replete individuals.

4.2. Ceruloplasmin (Cp)

It is well-accepted that the responsiveness and concentration of Cp can be affected by a range of non-dietary factors. Concentrations tend to increase with age and are generally higher in females than males throughout the life span. The latter is exacerbated in premenopausal women as a result of estrogen-dependent Cp synthesis and secretion by the liver, and oral contraceptive use [52]. Cp is also an acute-phase protein regulated by inflammatory hormones and, consequently, levels are raised by chronic inflammatory conditions, such as rheumatoid arthritis. Therefore, Cp concentrations should be viewed with extreme caution. As for plasma Cu, plasma Cp and Cp activity significantly declined when dietary Cu intake shifted from marginal (0.66 mg/day for 24 days) to low (0.38 mg/day for 42 days) in one controlled trial involving healthy male subjects [27,53]. However, Cp concentration and activity did not increase after a repletion period providing 2.49 mg Cu/day for 24 days.

In the other trials, the range of dietary Cu intake was 0.57–6.9 mg/day. No effect of dietary Cu on plasma Cp was reported [13,16,49–51,54–58].

The Cp values are consistent with the serum Cu levels. However, in this case, supplementation of healthy, replete individuals showed a significant increase in serum Cu concentration.

In a review on the methods of assessing Cu status, also including studies among unhealthy populations, Harvey identified two studies suggesting that Cp total protein may be affected by Cu supplementation, thus reflecting Cu exposure. However, these results were only observed in unhealthy, highly depleted individuals [47].

4.3. Cu-enzymes

4.3.1. Erythrocyte superoxide dismutase (SOD)

The effect of dietary Cu on the activity of Cu/Zn erythrocyte SOD has been assessed in five controlled trials or balance studies. None reported any change in erythrocyte SOD in response to dietary Cu within a range of 0.38 mg Cu/day to 6 mg Cu/day [13,27,50,55,56].

We can conclude, in agreement with the conclusions of Harvey et al. [47], that erythrocyte SOD is not a suitable marker of Cu status.

4.3.2. Diamine oxidase (DAO)

Serum DAO was increased (compared to a placebo) after 3 mg Cu supplementation for six weeks in two RCTs, each with 24 participants [55,59]. However, the studies were both considered at high risk of bias due to an incomplete report of outcomes: in the study of Kehoe et al. [55], results were reported for 19, 14, 20 and 17 subjects respectively (out of 24) in the case of SOD, platelet cytochrome c oxidase, ceruloplasmin oxidase and serum diamine oxidase. In the study of O'Connor, the effects of Cu supplementation as $CuSO_4$ on DNA damage and liver function enzymes were reported for 17 and 21 subjects respectively.

We can conclude that DAO is not a suitable marker of Cu status as reported by Harvey et al. [47].

4.3.3. Skin lysyl oxidase (SLO)

We identified only one CT carried out in 12 men [53] which addressed the effect of Cu intake on skin lysyl oxidase activity. SLO activity decreased when switching from a diet providing 0.66 mg Cu/day to a diet providing 0.38 mg Cu/day, and increased after a repletion period (2.48 mg Cu/day). However, based on this sole study it is not possible to conclude that SLO can serve as a useful indicator of Cu status.

4.3.4. Cu in hair

Compared to serum, Cu content of scalp hair is 10–100 time higher and much more variable, with values ranging from 7 to 95 μg/g [60,61]. Very few studies have investigated the relationship between Cu intake and Cu content in hair. In one long term study in healthy young men fed controlled diet, Cu content in scalp hair increased from 9.2 ± 3.1 to 21.1 ± 5.9 μg/g when intake shifted from 1.6 to 7.8 mg/day. In contrast, no correlation was observed between Cu content in scalp hair and Cu intake assessed with three 24 h recall in a cohort of 70 menstruating women [62]. Beyond Cu intake, factors affecting the Cu content of scalp hair are still largely unknown and may include sex, use of hormonal contraception, cancer and other pathological situations associated with change in Cu distribution between plasma and tissues as well as environmental pollution [63]. Hair matrice presents advantages, with easy collection and conservation, with retrospective characteristics in addition. However, these advantages are still hindered by the problems of external contamination, the analytical difficulties of this matrice for metal determination with the lack of internal

Table 5
Plasma or serum Cu in healthy subjects with different levels of Cu intake.

Study	Population	Cu intake per period (mg/day)	Plasma or serum Cu ($\mu mol\ L^{-1}$)
Turnlund et al. [27]	Healthy young men (26 ± 4 years)	Equilibration: 0.66 Depletion: 0.38 Repletion:2.49	Plasma Cu: 13.8 ± 0.3 Plasma Cu: 12.6 ± 0.3 Plasma Cu: 13.6 ± 0.3
Milne et al. [16]	Men (18–36 years)	Equilibration: 1.22–1.57 Depletion: 0.73–0.99 Repletion: 4.34–6.42	Plasma Cu: 12.9 ± 1.1 Plasma Cu: 11.8 ± 1.9 Plasma Cu: 12.2 ± 2.0
Milne et al. [51]	Postmenopausal women (63.1 ± 8.8 years)	Equilibration: 1.37 Depletion: 0.57 Repletion: 2.57	Plasma Cu: 17.3 ± 2.2 Plasma Cu: 18.1 ± 2.4 Plasma Cu: 17.0 ± 3.1
Baker et al. [49]	Young adults (20–50 years)	Medium: 1.6 Low: 0.7 High: 6.0	Serum Cu: 14.4 ± 2.3 Serum Cu: 14.0 ± 1.8 Serum Cu: 15.2 ± 2.0
Harvey et al. [13]	Men (20–59 years)	Medium: 1.6 Low: 0.7 High: 6.0	Serum Cu: 15.9 ± 1.7 Serum Cu: 14.4 ± 2.1 Serum Cu: 15.5 ± 2.2
Araya et al. [54]	Healthy adults	Dietary Cu: 14.2 ± 12.6 μmol/day Four levels of drinking water Cu: 0 (Q1) to 177.4 ± 40.9 (Q4) μmol/day	No relationship between total (dietary + drinking water) Cu intake and serum Cu

and external quality controls. For all these reasons, hair copper is not regarded as a suitable marker of Cu status.

All the biomarkers identified in our review show very little or no variation to Cu exposure in the dietary intake range. More informative biomarkers are needed to assess Cu status in the general population. Currently, other biomarkers—such as exchangeable Cu [64]—are used in pathological conditions (Wilson Disease), and should be explored in further studies.

5. Dietary copper and health

There are few reports of Cu excess or deficiency in the general population except for formerly obese patients after gastric bypass surgery, in whom Cu deficiency has been reported in 8 to 18.8% [65,66].

However, we have identified several studies addressing the relationship between dietary Cu and health issues for intakes in the nutritional range.

5.1. Cardiovascular risk

5.1.1. Cardiovascular disease

High serum Cu has been reported as an independent risk factor for cardiovascular disease in both case-control [67] and large prospective population studies [68,69]. The mechanisms underlying these relationships are mainly unclear. A synergistic effect between the pro-oxidation of Cu and low status of selenium (an antioxidant, leading to atherogenesis via an imbalance of defense systems against free radicals, has been suggested) [69].

In a cohort study, in 1,054 subjects aged 65 years old and over (mean age 76.6 ± 7.4 years old, 49% females) [70], dietary Cu intake was not predictive of cardiovascular mortality over 14 years.

5.1.2. Lipoproteins

A cross-sectional study at high risk of bias by Bo et al. [71] carried out on 1197 apparently healthy subjects (no use of prescription medicine and without diabetes, cardiovascular disease or dyslipidemia) of both sexes observed a negative relationship between dietary or serum Cu and total and LDL-cholesterol, suggesting that a high Cu intake and status is associated with a better metabolic profile. A second cross-sectional study with 189 participants (without diabetes, cardiovascular disease or hypertension) considered at high risk of bias showed that serum Cu was positively associated with cholesterol HDL [72].

In the RCT by Davis et al. [50], for which risk of bias was unclear, total and LDL cholesterol did not differ between the low (0.59 mg Cu/day) and adequate Cu (2.59 mg Cu/day) periods (6 weeks each). In the RCT of Medeiros et al. [73] considered at moderate risk of bias, supplementation with 2 mg Cu/day for 6 weeks triggered an increase in total serum LDL cholesterol at 4 weeks while VLDL cholesterol declined (in comparison with the placebo group). The percentage of cholesterol as LDL increased at 6 weeks of supplementation compared to the initial baseline value, while the percentage of cholesterol as VLDL decreased compared to the baseline value in the supplemented group.

Therefore, limited results from cross-sectional studies tend to suggest that dietary Cu is associated with a better lipoprotein profile. This observation is not fully supported by the results of RCTs, but the short duration of these studies may have precluded the occurrence of an effect.

5.1.3. Cardiac arrhythmia

Limited evidence from RCTs also suggests that marginal intake of Cu may lead to cardiac arrhythmia.

In one trial, a significant increase in the number of ventricular premature discharges (VPDs) was observed in three women out of 13 after 3 to 12 weeks on a low Cu diet (0.57 mg/day for 105 days) [51].

In another study, three women out of 12 on a low Cu diet (1 mg/day during a 90-day controlled period) exhibited abnormal electrocardiographic recording (premature ventricular discharge) requiring Cu supplementation before the end of the study. However, because two of these women still exhibited an increased number of abnormal ventricular discharges after Cu supplementation, no clear conclusion can be drawn regarding the relationship between dietary Cu and cardiac arrhythmia [56].

5.2. Cognitive decline

The hypothesis that Cu intake might be linked to cognitive decline is based on the long recognized age-related accumulation of metal-transporting proteins and compounds in key sites of the attentional circuits [74].

Lam et al. [75] found an inverse association between serum Cu concentrations and cognitive performance in a large cohort of elderly healthy women. Long-term and short-term recall scores were significantly lower in women with serum Cu over 2.15 mg/L than women with serum Cu below 0.9 mg/L. This relationship was not observed in men.

More recently, Salustri et al. [76] have reported that MMSE scores were correlated with serum levels of free Cu, but not of bound Cu, in healthy women. They also revealed an inverse

correlation between free Cu and an attention-related neuropsychological test, the digit span (verbal and spatial short-term memory test; attention), both in its forward and backward forms. These observations have prompted some authors to suggest that Cu accumulation, resulting from drinking water piped through Cu plumbing, may promote mental decline [76,77].

However, this hypothesis has been challenged by Klevay [78], who suggests that free serum Cu may increase even when total body Cu decreases, and questions the relevance of free serum Cu as a marker of Cu exposure. This controversy points out the need for studies evaluating the direct relationship between Cu intake and cognition. Our literature review has identified only one study addressing this point.

A cohort study of 3718 males and females by Morris et al. [79] identified a potentially adverse effect of Cu-containing supplements on cognitive functions in subjects with high saturated and trans fatty acid intake, but not in subjects with a diet low in saturated and trans fatty acids. Among such individuals, an average Cu intake of 2.75 mg/day resulted in a rate of mental decline almost 50% higher than that of individuals whose average Cu intake was 0.88 mg/day. However, because factors other than Cu intake are involved, this cohort study does not enable clear conclusions to be drawn regarding the influence of Cu intake on cognitive decline.

5.3. Cancers

Two cohort studies examined the link between Cu intake and lung cancer and lymphoma.

In a large cohort study (482,875 subjects), Mahabir et al. [80] showed no relationship between total Cu intake and lung cancer risk. In the Iowa Women's Health Study, Thompson et al. [81] did not identify any link between total or dietary Cu and the risk of Non-Hodgkin's lymphoma, diffuse large B-cell lymphoma or follicular lymphoma in a cohort of 35,159 women. For other types of cancer, no cohort studies have assessed Cu intake. In a third cohort study in 1,054 subjects aged 65 years old and over (mean age 76.6 ± 7.4 years old, 49% females) [70], dietary Cu intake was not predictive of cancer mortality over 14 years.

The link between Cu intake and breast cancer has not been evidenced by the cross-sectional and case-control studies identified in our review. The case-control study [82] did not show any relationship in 522 women (261 cases and 261 controls) aged between 25 and 65 years old. In the cross-sectional study [83], pre-menopausal women with breast cancer ($n = 23$) had significantly higher plasma Cu levels than the pre-menopausal controls ($n = 48$). However, levels of Cu intake were similar in both groups.

For other cancers, our review did not identify any relevant studies assessing Cu intake.

Thus, according to our review, no conclusion can be drawn regarding Cu intake and cancers.

5.4. Arthritis

One cohort study carried out in 29,368 female subjects (mean age 61.4 years old) [84] found no link between total (diet and supplements) or dietary Cu intake and risk of rheumatoid arthritis. There was a weak but significant inverse relationship between the use of Cu supplements and the risk of rheumatoid arthritis, which did not persist after further adjustment for confounders (age and energy intake, other risk factors).

Thus, no conclusion can be drawn regarding Cu intake and rheumatoid arthritis.

5.5. Copper and immune function

It has been suggested that Cu influences both humoral and cellular factors of the immune system. Cu-deficient animals have a lighter thymus and enlarged spleen compared with controls. Animals with severe Cu deficiency have reduced populations of neutrophils and T cells, impaired proliferation of T lymphocytes in response to mitogens and decreased activity of phagocytes, B-lymphocytes and natural killer cells. Antibody production by splenocyte T cells is reduced. In humans, the relationship between Cu intake and immune function is less well documented.

Recently, there have been several reports of Cu deficiency in formerly obese patients following gastric bypass surgery [65,66]. In these patients, features of Cu deficiency included leucopenia and neutropenia. In these subjects, Cu deficiency is likely to result from malabsorption consecutive to stomach and duodenum bypass [85]. Kelley et al. [86] have examined the effect of three levels of Cu intake on several markers of immune function in 11 healthy humans successively fed diets providing 0.66, 0.38 and 2.49 mg/day Cu. At the end of the 0.38 mg period, peripheral blood mononuclear cell proliferation and secretion of the IL-2 receptor in the culture medium were reduced. Neither parameter returned to baseline levels at the end of the 24-day repletion period. In this study, the peripheral blood number of leucocytes, monocytes, neutrophils, lymphocytes or natural killer cells were unaffected by the diet.

More recently, Turnlund et al. [58] have investigated the effect of increasing Cu intake from 1.6 (normal) to 7.8 (high) mg/day on several immune-related parameters and humoral response to influenza vaccination in healthy humans. However, because influenza vaccination took place between the normal and high Cu periods, the results of this study cannot be taken into account because of its high risk of bias.

A significant impact of Cu on the immune function can only be observed in specific situations where Cu malabsorption may be combined with low Cu intakes, such as post-bariatric surgery patients.

In healthy subjects, marginal Cu intake such as observed in the 5th percentile of dietary Cu in the French population (EAT) is unlikely to result in modifications to immune parameters.

6. Conclusions

This review points out several gaps and unresolved issues which make it difficult to assess links between Cu intake and health and Cu requirements.

First, although several putative biomarkers of Cu status including plasma or serum Cu, ceruloplasmin, Cu/Zn SOD, diamine oxidase or skin lysyl oxidase have been explored to date, none of them have yet unequivocally proved to reflect changes in Cu intake within the range observed in Western populations (0.5–2.5 mg/day). Even though serum and plasma Cu were recently proposed as markers of Cu status at the population level [47], neither has been shown to vary for Cu intake ranging from 0.57 to 6 mg/day in well controlled studies, and plasma Cu barely decreased when Cu intake fell well below the 5th percentile of dietary Cu observed in Western populations. For other putative biomarkers, either their specificity and sensitivity is too low or the quality and number of studies examining their link with Cu intake is insufficient to support their use in the evaluation of Cu exposure. At present, the lack of a suitable biomarker for Cu intake greatly limits our capacity to assess the relationship between dietary Cu exposure and health, and highlights the urgent need for high quality randomized controlled trials in healthy subjects evaluating the responsiveness of putative new biomarkers of Cu status over a wide Cu intake range.

Second, although Cu intake is almost consistently assessed in most representative national dietary surveys and in many prospective studies, major uncertainties remain regarding the actual distribution of total Cu intake within the population. Within the same city/region, considerable variations in the Cu concentration in drinking water have been observed as a consequence of the nature of household plumbing systems and water softness. The Cu content of vegetables has also been shown to vary depending on soil composition and agricultural practices. The use of national food composition databases with low Cu water concentration in cohort studies and national representative dietary surveys may thus lead to an underestimation of Cu exposure in a significant number of individuals, although the actual percentage is unknown.

Last, the number of large scale high quality studies examining the relationship between Cu intake or putative biomarkers of Cu status and health outcomes is very limited. The failure to observe a relationship between Cu exposure or status and negative health outcomes in most of these studies might suggest that Cu nutrition is not an issue in Western countries and that for most people, Cu intake meets nutritional requirements. Alternatively (i) uncertainties regarding Cu concentration in foods and water and (ii) the lack of biomarkers of Cu status make it impossible to simply rule out the risk of subject misclassification in term of Cu exposure and may confuse the link between Cu intake and health.

It is clear regarding Cu and health that the absence of proof is not proof of absence, but simply reflects the number of unresolved questions and uncertainties, and calls for studies with a more complete and precise evaluation of Cu exposure based on reliable biomarkers of Cu status.

Conflict of interest

The authors declare no conflict of interest.

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