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Biomarkers of copper status: a brief update

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The essentiality of copper (Cu) in humans is demonstrated by various clinical features associated with deficiency, such as anaemia, hypercholesterolaemia and bone malformations. Despite significant effort over several decades a sensitive and specific Cu status biomarker has yet to be identified. The present article updates a comprehensive review recently published by the authors which assesses the reliability and robustness of current biomarkers and outlines the on-going search for novel indicators of status[1]. The essential features of this earlier review are reiterated whilst considering whether there are other approaches, not yet tested, which may provide valuable information in the quest for an appropriate measure of copper status. Current biomarkers include a range of cuproenzymes such as the acute phase protein caeruloplasmin and Cu-Zn-superoxide dismutase all of which are influenced by a range of other dietary and environmental factors. A recent development is the identification of the Cu chaperone, CCS as a potential biomarker; although its reliability has yet to be established. This appears to be the most promising potential biomarker, responding to both Cu deficiency and excess. The potential for identifying a 'suite' of biomarkers using high-throughput technologies such as transcriptomics and proteomics is only now being examined. A combination of these technologies in conjunction with a range of innovative metal detection techniques is essential if the search for robust copper biomarkers is to be successful.

Copper status: Biomarkers: Copper deficiency: Copper Excess

Copper is an essential micronutrient. As with iron (Fe), it can undergo valency changes, from Cu (II) to Cu (I), and this ability to either accept or donate electrons makes it an important part of many catalytic processes. Some enzymes and biological processes where it plays a central role are given in Table 1. Perturbations in cuproenzyme activities are largely responsible for the clinical features of Cu deficiency, whereas overt signs of Cu overload stem from intracellular oxidative damage, particularly in the liver. Not surprisingly, Cu deficiency has a wide spectrum of consequences. In different species, these are manifest in a different order, with cardiac effects being seen first in ruminants[2,3], for example, while changes in glucose and cholesterol metabolism are observed first in humans[4–6].

The importance of Cu means that it has been the subject of intensive investigation over several decades, but despite these efforts, the ideal biomarker remains elusive. A comprehensive review covering the search for Cu biomarkers has recently been published[1], and this paper summarises some of that material, whilst also considering whether there are other methodological approaches, not yet tested, which may generate data suggesting potential new status indicators. Despite the unmistakeable importance of Cu in maintaining health, there remains on-going difficulty with setting dietary recommendations due to the lack of sensitive and specific Cu biomarkers. Whilst severe deficiency and toxicity are relatively easy to recognize due to the obvious clinical signs, it is virtually impossible to identify marginal deficiency.

Cu deficiency can result from both primary and secondary causes. Primary causes usually relate to diet, though there are inherited disorders of Cu metabolism, such as Menkes' and Wilson's diseases, that result in systemic deficiency and overload respectively[7]. Despite the adverse health consequences of these rare diseases, both have provided fundamental information for understanding the molecular basis of human Cu metabolism and status. Dietary Cu bioavailability undoubtedly influences Cu status, and whilst factors that affect the former are not fully characterized, nutrient-Cu interactions play a significant role. In infants the interaction of Cu with Fe is potentially the most important, with a reduction in Cu absorption demonstrated in formula-fed infants given high dietary levels of Fe (10·8 mg/L) compared with lower levels (1·8 mg/L)[8]. Use of zinc (Zn) supplements also increases the risk of Cu deficiency, since Zn blocks Cu absorption by up-regulating metallothionein transcription in enterocytes[9]. Several case studies have been reported with Cu deficiency occurring as a result of taking high levels of over-the-counter Zn supplements[10]. This interaction is exploited in the treatment of Wilson's disease patients who are given pharmacological doses of zinc to avoid the accumulation of copper in the tissues[11,12].

Abbreviations: ATOX1, copper chaperone for ATP7A (Menkes protein) and ATP7B (Wilson protein); ATP7A, human copper-transporting P-type adenosine triphosphatase; ATP7B, human copper-transporting P-type adenosine triphosphatase; CCO, cytochrome *c* oxidase; CCS, copper chaperone for SOD1; CTR1, copper transporter 1; Cox17, copper chaperone for CCO; CU, copper; Fe, iron; SOD1, Cu, Zn superoxide dismutase; ZN, zinc.

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Table 1. Biological processes involving Cu-binding enzymes or proteins

Function	Enzyme/Protein
Iron mobilization	Caeruloplasmin (ferroxidase I), hephaestin
Antioxidant defence	Cu,Zn-superoxide dismutase (SOD1), caeruloplasmin, metallothionein
Cu transport	Caeruloplasmin, albumin, transcuprein, ATP7A, ATP7B, CTR1
Formation of connective tisssue	Lysyl oxidase, cartilage matrix glycoprotein
Electron transport	Cytochrome C oxidase (CCO)
Blood clotting	Blood clotting factors V and VIII
Deamination of primary amines	Amine oxidases
α-amidation of neuropeptides	Peptidylglycine monooxygenase
Pigment production e.g. melanin	Tyrosinase
Catecholamine metabolism	Dopamine β-monooxygenase
Oxidation of phenylalanine to tyrosine	Phenylalanine hydroxylase
Metal detoxification	Glutathione
Cu Chaperones	ATOX1: delivery of Cu to ATP7A and ATP7B CCS: delivery of Cu to SOD1 Cox17: delivery of Cu to CCO in mitochondria

Growing children and pregnant women are particularly vulnerable to mild/moderate Cu deficiency. The developing fetus accumulates significant Cu stores during the third trimester to provide for the first 3–4 months of life when dietary Cu intake is minimal[13]. In order to meet this demand, maternal Cu absorption is up-regulated as demonstrated by a stable isotope study conducted during pregnancy[14]. Studies in children have shown that malnutrition commonly induces Cu deficiency, though of course the symptoms are confounded by other nutritional problems[15,16]. Many foods high in Cu, especially offal such as liver, are less commonly consumed now, and others, such as chocolate are high in fat, and hence are not considered beneficial for a healthy lifestyle. These factors also contribute to the risk of deficiency, especially in young women.

Cu deficiency can also arise as a consequence of other disorders and treatments. For example, coeliac disease[17], Crohn's disease[18] and other gut absorption problems all increase the risk of Cu deficiency, as do diseases of the immune system, such as AIDS and autoimmune diseases[19]. The long-term consumption of high doses of antacids and other cation chelating agents reduce absorption, whilst excessive losses of caeruloplasmin–bound Cu may be experienced by patients undergoing ambulatory peritoneal dialysis[20].

Cu overload is less frequent but it also carries risks. Brewer and colleagues have campaigned for some time about the dangers of high Cu intake, and suggested that it may be associated with an increased risk of diseases such as Alzheimer's disease[21]. It has also been implicated in the development of prion diseases such as Creutzfeld-Jacob disease and kuru[22]. The data are equivocal, but provide further support for the need for sensitive and specific biomarkers of Cu status.

Current biomarkers

Most current approaches use cuproenzymes of one form or another. Many studies have used caeruloplasmin (Cp), for example, which is an acute phase protein, affected by the age and hormonal status of the individual. Cu homeostasis is tightly maintained by changes in both the absorptive efficiency and biliary excretion in the gut. At low and high intakes the efficiency of absorption is up- and down-regulated, respectively[23], but is predominantly controlled via endogenous excretion[24]; however this control mechanism is imperfect at extremes of intake. Consequently, intervention studies have shown little or no effect of marginal or short-term Cu deficiency on either plasma Cp concentrations or activity[25,26]. Cp also does not respond to high levels of dietary copper at the level of either mRNA transcription or protein translation. However, its activity is reported to decrease in response to severe Cu deficiency, so that it has value for indicating moderate/severe Cu deficiency[27].

Other cupro-enzymes that have been tested, with greater or lesser success, include (SOD1), platelet CCO, lysyl oxidase and peptidylglycine α-amidating monooxygenase (refer to recent review for further information[1]).

Recent developments in copper biomarkers

More recently, several groups have examined the expression of CCS, a Cu 'chaperone'. When Cu is taken up by cells, it binds to one of a series of proteins (termed chaperones) which transport the metal to its target protein (Table 1). One of these, CCS, has been shown to change expression in response to Cu levels in a variety of models. Initial experiments carried out in rat models demonstrated that CCS protein levels were inversely proportional to Cu status and, that regulation appeared to act through degradation by the 28S proteosome[28]. Subsequently it was shown that Cu deficiency induced by feeding rats increased Zn in the diet could also de detected by erythrocyte CCS[29]. Interestingly, at a high level of Zn intake, Cu deficiency was actually improved, and this was correlated with a decrease in CCS expression. These data have been confirmed in mice on Cu deficient diets, supporting the idea that CCS or possibly the CCS:SOD1 ratio is a good indicator of Cu deficiency. Whether it will act as a good indicator of Cu excess remains yet to be tested, although data obtained in *Drosophila melanogaster* S2 cells suggest it may not be[30].

There is a body of evidence relating Cu deficiency to bone metabolism at all life stages. Skeletal defects such as osteopenia and spontaneous rib fractures are common features of Menkes disease in young children[31–33], bone defects in pre-term infants respond to Cu supplementation[34], and Cu deficiency is reportedly a factor in age-related osteoporosis[35]. Urinary pyridinoline and deoxypyrodinoline (biomarkers of

British Journal of Nutrition

bone resorption) may be useful functional indicators of Cu status. Studies have demonstrated increased bone resorption associated with Cu depletion in adult males[36], and a reduced rate of bone loss at the lumbar spine in Cu-supplemented middle-aged women[37]. However, the complex nature of bone metabolism suggests that these biomarkers are non-specific for Cu status and will be influenced by a variety of nutritional and environmental factors. Other suggested Cu biomarkers include immune and blood lipoprotein biomarkers (further information can be found in the recent review[1]).

The potential for multiple markers, using high throughput methods such as proteomics, transcriptomics and other methods is being investigated. The current state-of-the-art for these techniques in relation to human nutrition is reviewed elsewhere in this supplement. Whilst several papers have reported the identification of suites of potential biomarkers in experimental models, application to the human situation is only just beginning to be investigated. Proteomics technology offers significant potential for the identification of novel Cu biomarkers particularly in relation to the analysis of Cu-transporting or Cu-binding proteins in both healthy individuals and those with Cu-related conditions such as Menkes' or Wilson's disease. There are specific technological problems associated with the investigation of metalloproteins, including analysis at low concentrations and the inherent instability in response to environmental changes. Consequently, isolation of Cu-containing proteins in physiological conformations is particularly challenging. A comprehensive summary of the proteomics of metal transport can be found in the review by Kulkarni and colleagues[38]. The ability of these techniques to screen the entire proteome of a cell may ultimately facilitate the identification of biomarker(s) with no obvious role in Cu metabolism. Potentially, a protein-product substantially down-stream from processes clearly related to Cu metabolism may provide an unexpected component of the 'suite' of Cu biomarkers. Ultimately, a combination of 'standard' proteomics and transcriptomics technologies in conjunction with a range of innovative metal detection techniques will be required to drive the search for robust copper biomarkers.

Conclusions

In the absence of robust sensitive and specific biomarkers, it is difficult to know whether Cu status, either in relation to deficiency or excess, is a significant public health problem. Nonetheless, given the intake data that suggest levels may be lower than optimal and given the serious consequences of deficiency, there is a strong argument for developing such markers of status. After many years of searching, we believe that success is not too far away. CCS and the other chaperones, high throughput methods and identification of mechanisms of regulation all add to our knowledge and will hopefully contribute, so that one day we will be able to accurately assess an individual's Cu status and determine whether he or she is at risk of deficiency or overload.

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British Journal of Nutrition

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