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Copper exposure and potential biomarkers of copper metabolism

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Abstract

Relevant biological effects associated with mild to moderate copper deficiency and copper excess are unknown. It is difficult to identify markers of these early changes because limits of the homeostatic range are still undefined and early changes may represent adaptive responses that do not imply necessarily risk of damage. We report here a series of studies carried out to shed light on the responses within the homeostatic range, by assessing classic parameters of copper status in humans at different copper exposure. In adult healthy volunteers that had an estimated daily intake of 0.9 mg Cu/day (approximately 15 μg/kg/d), exposure to additional 50–60 μg of copper/kg/day for three months or up to 150 μg/kg/d for two months resulted in no significant changes of SOD activity in erythrocytes, of copper concentration (in serum, erythrocytes and mononuclear cells) and of serum ceruloplasmin (ANOVA). Neither were found differences by gender or age. As in previous studies in infants, the non-ceruloplasmin copper fraction was positively correlated to serum copper ($r = 0.58$). Assessing variations on copper absorption, infants supplemented/not supplemented with oral copper (80 ug/kg/14 days), at age 1 and 3 months, showed copper absorption close to 80% at both ages; no effect was observed for age or supplementation, suggesting that either these concentrations do not elicit regulatory mechanisms or that at this age down regulation for copper absorption is not efficient. These studies indicate that in the range of the copper homeostasis area the markers tested are not suitable to detect mild changes (within the homeostatic range) of copper metabolism.

Introduction

Copper has both beneficial and adverse effects on human health depending on the dose ingested. Data obtained in animal models and cell lines is extensive and serves as the basis of a large proportion of our knowledge about copper metabolism (Mercer *et al.*, 1994, Schilsky & Sternlieb 1994; Yamaguchi *et al.*, 1994, van de Sluis BJ *et al.* 1999; Peña *et al.* 1999). However, considerable differences among species make it difficult to extrapolate information to humans. In them as in the animal models, severe copper deficiency and severe copper excess are better understood because the genetic conditions Menkes disease and Wilson disease constitute good models of severe changes of copper metabolism (Wilson 1912; Scheinberg & Gitlin 1984; Danks 1988; Cartwright 1950). In contrast, much less is known about relevant biological effects associated with mild to moderate copper deficiency and excess when no mutation is present (Milne *et al.* 1988, 1990; Uauy *et al.* 1985; Cordano *et al.* 1964; Cordano 1998; Danks 1988). Currently available laboratory markers of copper metabolism are mainly used for diagnostic purposes, i.e., to demonstrate changes associated with clinical manifestations already present (Scheinberg & Sternlieb 1996; Kaler 1999; Milne 1999). A pressing challenge today is to identify markers of early changes, with capacity to predict risk before actual tissue or functional damage occur. Several factors make this task difficult. One of the most important is the undefined limits of the homeostatic range (Brewer 1998; IPCS 1998) and, on the excess side of the curve, the fact that detecting early changes associated with increased copper exposure does not necessarily mean

risk of damage; there is a zone in the dose-response curve in which copper status may change, copper may be deposit in the liver, yet this may be a physiological, reversible phenomenon that does not imply risk of damage.

We review here the available data on classic parameters of copper status in humans, mainly serum copper and ceruloplasmin, non-ceruloplasmin fraction and some other potential markers that we have recently evaluated in apparently healthy individuals that underwent controlled copper exposure, at concentrations within the safe recommended limits but separated by up to a 10-fold factor.

Blood markers of copper status

To what extent classic markers of copper status may change in the area within the limits of homeostatic regulation has not been systematically assessed. Serum copper, ceruloplasmin and/or superoxide dismutase activity have been shown to decrease in a proportion of the adults receiving 0.79–1.03 mg copper/d (Food and Nutrition Board, Institute of Medicine 2001; Turnlund *et al.* 1990; Milne *et al.* 1990; Reiser *et al.* 1985). On the excess side, no parameters have been identified as potential early markers of copper excess.

In apparently healthy adults, 60 women aged 20 to 45 years, having an estimated daily intake of 0.9 mg Cu/day, were exposed to a mean of 50–60 μg of additional copper in water/kg/day for three months, which represent a fourfold increase to the basal estimated exposure (Pizarro *et al.* 1999). This study was a first assessment of acute gastrointestinal intolerance (mainly nausea and abdominal pain) after acute, controlled exposure to copper. In this context, symptom report increased significantly among women that ingested 3 or more mg Cu l in drinking water, but no changes were detected in blood parameters, expressed either as mean $\pm$ SD or distribution curves of serum copper and ceruloplasmin values (Figure 1).

In an effort to better understand the performance of parameters of copper status in individuals whose copper exposure differ but is always within the homeostatic range limit, we recently studied 240 apparently healthy adult volunteers that underwent a two-month controlled exposure receiving waters containing < 0.01, 2, 4 or 6 mg Cu/l (as copper sulfate in water). While the estimated copper intake from the diet was calculated at 0.9 mg Cu/day the additional copper provided by test waters, calculated on the basis of daily volume intake and copper content measured in waters available at households, ranged from 15 to 150 μg/kg/d (groups < 0.01 and 6 mg Cu/l, respectively). Results showed that although customary dietary intake was borderline low prior to the study SOD activity in erythrocytes was not low in the studied individuals receiving the lower intake (group < 0.01 mg Cu/l), and although copper intake from water differed significantly among the 4 study groups there was no significant increase after the two-month controlled exposure period. Expressed as mean values $\pm$ SD in the four study groups, copper concentration (in serum, RBC and mononuclear cells), serum/plasma ceruloplasmin and SOD activity values were within the normal range and were not related to copper intake (ANOVA, NS) (Figure 2). Neither were found differences by gender or age. As in previous studies in infants, the non-ceruloplasmin copper fraction was positively correlated to serum copper ($r = 0.58$). This correlation had been described in patients with high copper load (Wilson disease and other forms of childhood cirrhosis); but, while among apparently healthy adults values were low ($\leqq 1.8$ μM/l), those described in patients were over 8–10 or more μM/l (Eife *et al.* 1999). In our study in infants liver enzyme activities remained within normal limits in all four groups except for 9.2% of the values obtained, which were above the cut off point; however, none of the individuals had clinical evidence suggesting a liver condition, values were somewhat above the cut off point but were of no clinical significance and in all cases the out of range value affected only one of the enzymes measured. One may then conclude that exposure to additional 150 μg Cu/kg/day in water proved safe, however, it is interesting to discuss these results further. It may be possible that changes were mild or remained undetectable because intestinal absorption decreased, or because biliary excretion increased in response to greater copper availability. It is also possible that copper deposit in hepatocytes increased but the magnitude of this increase did not affect the large liver homeostatic capacity, and coarse indicators of liver damage as serum glutamic-oxaloacetic or glutamic-pyruvic transaminases (SGOT, SPGT) and gamma glutamyl tranferase (GGT) did not change.

It is interesting to note that the tails in the distribution curves of copper status indicators showed slight differences among the study groups; unfortunately, because these areas included very few individuals analyzes done did not show significant differences; it would be indeed most interesting in future studies to

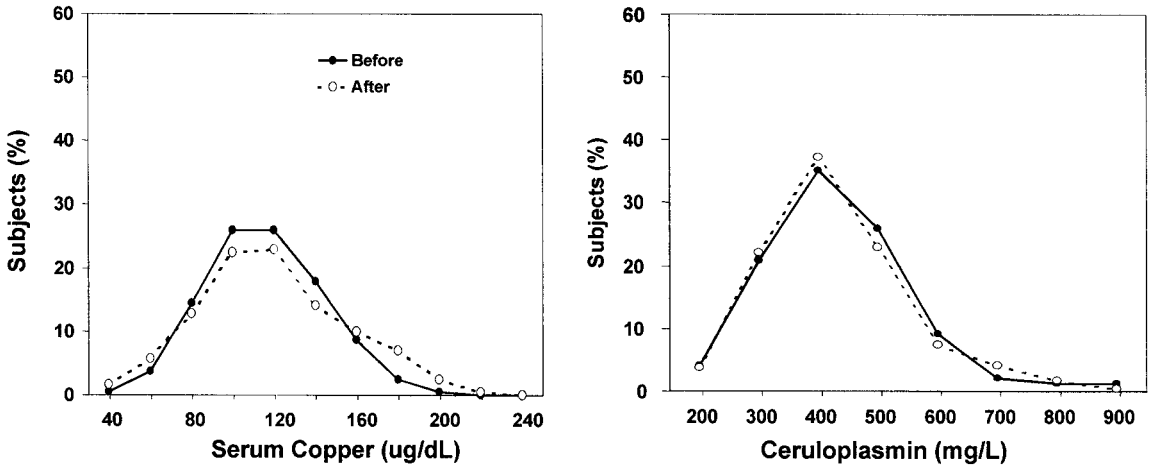

Fig. 1. Serum copper and ceruloplasmin in women supplemented with copper.

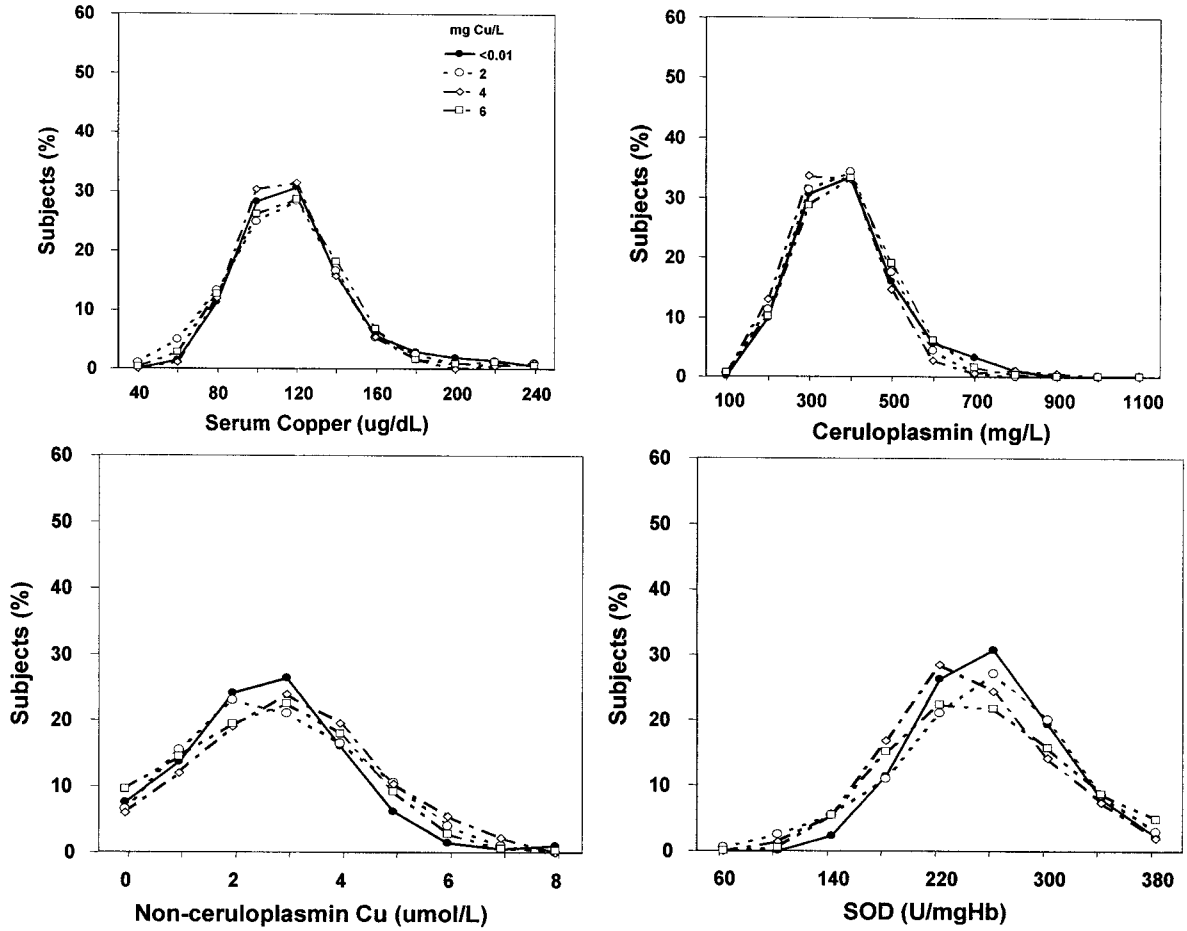

Fig. 2. Serum copper, ceruloplasmin, non-ceruloplasmin fraction and SOD activity in healthy adult volunteers receiving < 0.01, 2, 4 or 6 mg Cu/l (as copper sulfate) in water.

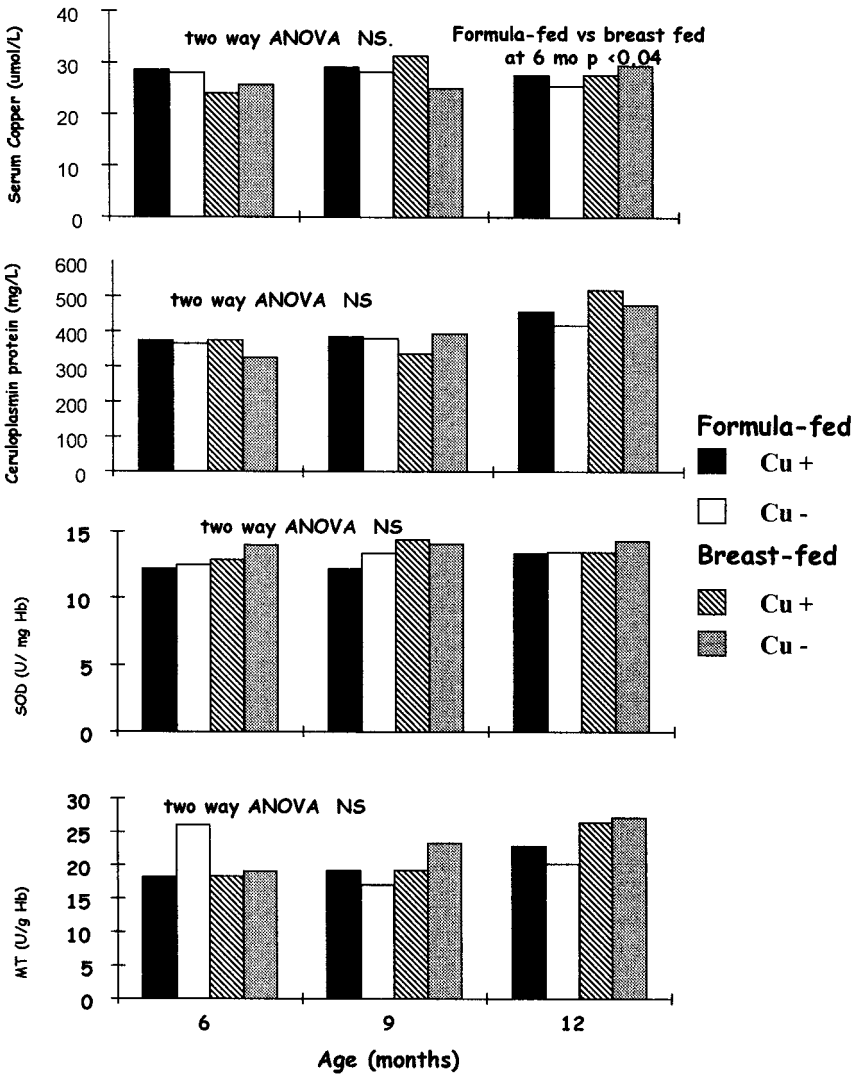

Fig. 3. Serum copper, ceruloplasmin, SOD activity and erythrocyte metallothionein in infants receiving water with/without 2 mg of additional copper.

expand the number of these individuals to better understand what they represent as metabolic responses to copper exposure.

There are few studies on copper exposure in children. Olivares *et al.* (1998) assessed healthy infants ($n = 128$) randomly assigned to receive drinking water with either < 0.01 mg/l ($n = 4$) or 2 mg/l of copper ($n = 80$) from 3 to 12 months of age. At ages 6, 9 and 12 months serum copper, ceruloplasmin, SOD, erythrocyte metallothionein, bilirubin, transaminases and gamma glutamyl transferase were measured. Only minor and not significant differences were observed in biochemical indices of copper nutrition in the groups that received water with low and high copper content (Figure 3). Serum copper positively correlated with the non-ceruloplasmin fraction ($r = 0.86$) (Olivares *et al.* 2000).

Absorption studies

Another approach to assess copper status is by means of assessing variations of copper absorption and defining factors that up/down regulate it. In the classic study by Turnlund *et al.* (1989), young adults received dietary intakes varying from 0.79 mg/d ('low'), to 1.68 mg/d ('normal') and to 7.53 mg/d ('high') and their copper absorption averaged 55.6 $\pm$ 0.9%,

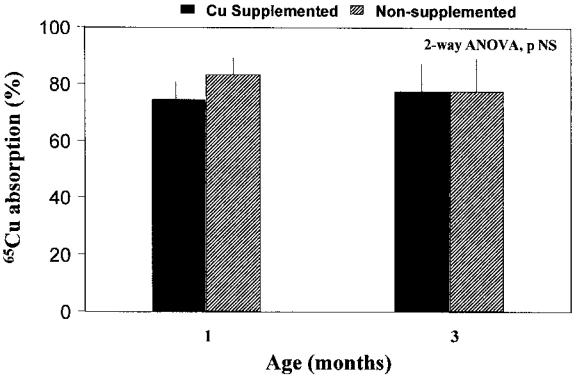

Fig. 4. Apparent copper absorption in infants supplemented/not supplemented with copper.

$36.3 \pm 1.3\%$, and $12.4 \pm 0.9\%$, respectively, indicating that in these range of exposure copper absorption is strongly dependent on dietary Cu.

Infants supplemented/not supplemented with oral copper (80 ug/kg/14 days), aged 1 and 3 months, showed copper absorption close to 80% at both ages; no effect was observed for age or supplementation (Figure 4) (Olivares *et al.* 2002, in press). These results suggest that either these concentrations do not elicit regulatory mechanisms or that at this age down regulation for copper absorption is not efficient. These results stress the need to define markers of relevant biological changes associated with copper exposure.

In summary, studies carried out so far suggest that the markers that somewhat responded to variations in copper exposure in acute designs are not suitable to detect mild/moderate changes of copper metabolism in longer-term designs, such as the ones used in these studies. Given the obvious ethical constraints associated with research in humans future studies should include animal models, in which more intense changes of exposure can be applied. Also of utmost importance is the search for new markers capable of detecting mild changes in both sides of the curve of copper nutrition.

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