Methods of assessment of copper status in humans: a systematic review\textsuperscript{1–5}

Linda J Harvey, Kate Ashton, Lee Hooper, Amélie Casgrain, and Susan J Fairweather-Tait

ABSTRACT
Background: The assessment of dietary adequacy of copper is constrained by the absence of recognized copper status biomarkers.

Objectives: The objectives were to systematically review the usefulness of copper status biomarkers and identify those that reflected changes in status over ≥4 wk.

Design: The methods included a structured search on Ovid MEDLINE, EMBASE (Ovid), and Cochrane databases to October 2007, followed by the use of formal inclusion/exclusion criteria, data extraction, validity assessment, and meta-analysis.

Results: A total of 16 studies (288 participants) were included in the review, with data on 16 possible copper biomarkers. All of the included studies were small and at high risk of bias. Data for serum copper suggested its value as a biomarker, reflecting changes in status in both depleted and replete individuals, although these changes were smaller in the latter. Total ceruloplasmin protein is related to copper status but reflects changes in highly depleted individuals only. Erythrocyte superoxide dismutase and urinary deoxypyridinoline are not useful biomarkers, but there were insufficient data to draw firm conclusions about plasma, erythrocyte, and platelet copper; leucocyte superoxide dismutase; erythrocyte, platelet, and plasma glutathione peroxidase; platelet and leucocyte cytochrome-c oxidase; total glutathione; and urinary pyridinoline. The paucity of data prevented detailed subgroup analysis.

Conclusions: Despite limited data, serum copper appears to be a useful biomarker of copper status at the population level. Further large studies with low risk of bias are needed to explore the effectiveness of other biomarkers of copper status and the relation between biomarker responsiveness, dose, and period of supplementation. Am J Clin Nutr 2009;89(suppl):1S–16S.

INTRODUCTION
The necessity of copper for human health derives from its involvement in myriad biological processes, including iron metabolism, antioxidant defense, neuropeptide synthesis, and immune function (1, 2). The wide-ranging clinical features of nutritional copper deficiency largely derive from perturbations in the activities of various cuproenzymes (3). The diversity of deficiency symptoms means that, although severe deficiency is relatively straightforward to diagnose, identifying marginal deficiency is somewhat problematic. Dietary copper deficiency can result in adverse consequences throughout the life span. In utero, insufficiency 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 copper deficiency has been associated with an increased risk of developing osteoporosis in later life (6, 7) and adverse changes in cholesterol metabolism (8, 9). Conversely, copper toxicity as a result of dietary excess generally is not considered to be a widespread health concern, probably as a result of the homeostatic mechanisms controlling copper absorption and excretion (10).

Despite the widely held view that there is a lack of sensitive and specific biomarkers of copper status, several putative indexes, including plasma copper, ceruloplasmin, and Cu/Zn superoxide dismutase (Cu/Zn SOD), routinely are assayed in human studies. Regardless of the possible futility of these analyses, recently evaluated in a review with subsequent update (3, 11), their use largely continues due to the absence of anything superior. It is, therefore, timely that the present systematic review of copper supplementation and depletion studies was undertaken in an attempt to determine the true validity of both widely accepted and potentially novel biomarkers of copper status in humans. An initial search of the literature suggested that 4 wk was the appropriate minimum period of supplementation for studies to be included in the review. This was based on studies conducted in adult males and females reporting significant responses in copper biomarkers after 3–4 wk of supplementation with a readily absorbable copper supplement (12, 13). Several forms of copper are available in over-the-counter supplements and it was decided to include studies incorporating the most commonly used types,
namely the chloride, sulfate, gluconate, and amino acid chelate forms. Supplementation with cupric oxide was excluded because it is generally accepted to be a much less bioavailable form of copper (14).

The overall aim of the present review was to assess the usefulness of copper status biomarkers in humans and specifically to identify which putative and/or novel biomarkers appropriately reflected changes in status over durations ≥4 wk.

METHODS

The methodology of this review was based on the standard methodology developed for this set of reviews (15) and is provided in an abbreviated version below with differences noted from the main methodology.

Study selection

To be included in the review, studies needed to fulfill all of the following characteristics: 1) human intervention studies involving copper supplementation with copper chloride, copper sulfate, copper gluconate, copper amino acid chelate, or copper depletion; 2) reporting copper status in humans at baseline and after a period of supplementation or depletion; and 3) reporting a change in copper status over ≥4 wk. Study inclusion was not limited by the age of the participants and studies included infants through the elderly. Studies were excluded if they included subjects receiving concomitant therapy for chronic illnesses or nutritional deficiencies other than copper or if subjects had a condition known to affect copper metabolism, eg, Wilson’s disease (genetic disease of copper overload). Studies were excluded if suitable baseline data were unavailable or if information on the statistical variance of the data was not accessible. Four non-English articles were retrieved but subsequently excluded because they contained no relevant data.

Ideally, only randomized controlled trials (RCTs) would have been included in the review; however, during the search process it became apparent that there was a paucity of copper biomarker data from all types of intervention studies. Therefore, data were extracted from all of the RCTs, controlled clinical trials (CCTs), and before-after (B/A) studies meeting the inclusion criteria.

Search strategy

We searched Ovid MEDLINE (www.ovid.com), EMBASE (Ovid; www.ovid.com), and the Cochrane Library CENTRAL (www.thecochranelibrary.com) databases from inception to October 2007 for copper intervention studies using text terms with appropriate truncation and relevant indexing terms. The search was in the form [Copper terms] and [intervention study terms] and [human studies]. The full Ovid MEDLINE search strategy is shown in Table S1 under “Supplemental data” in the online supplement, and the strategies for the other databases were based on this strategy. In addition, the reference list of a recent comprehensive review of copper status biomarkers (3) was consulted to identify any further intervention studies that also may have been relevant for inclusion in this review. Further expertise was contributed to the search process by Harry McArdle, who recently coauthored an update to this earlier literature review with the present copper biomarker systematic review team (11).

Data collection

Titles and abstracts were screened for inclusion by a single reviewer with independent duplicate assessment of 10% by a second reviewer. The full text of all of the articles collected was screened for inclusion using an inclusion/exclusion form by a single reviewer with independent duplicate assessment of random sample of 20% by a second reviewer. Where the 2 reviewers disagreed, the study was discussed and a consensus decision reached.

Data for each included study were extracted into an Access (Microsoft Corp, Redmond, WA) database file by a single reviewer with independent duplicate assessment of 10% by a second reviewer. Studies were selected for duplicate extraction by identifying every 10th study extracted chronologically by the primary reviewer. The form was piloted by the primary reviewer, and any problems were discussed and resolved among the review team before beginning full data extraction. Data extraction was as discussed in the main methodology article (15).

Data synthesis

The primary question to be answered by this review was to determine which copper status biomarkers appropriately reflected changes in status over a period of ≤4 wk. There also was a series of secondary questions we planned to address if the evidence allowed. These questions included the following:

1) What methods (established and novel) currently are used for measuring copper status in humans?
2) For each biomarker identified,
   a) In which population subgroups (age, ethnicity, and copper status at baseline) do we have evidence that the biomarker accurately reflects change in status in populations?
   b) Over what time scale does the biomarker respond significantly?
   c) What other factors affect the status measure (eg, age, body weight, genotype, illness, and sex)?
   d) What methodologies exist for analyzing this biomarker, and which are preferable in terms of accuracy, reproducibility, and cost?
   e) What evidence do we have of circumstances in which the measure is not helpful?

Data subsequently were synthesized as previously described (15).

RESULTS

The flow diagram for this review is shown in Figure 1. Of the 572 titles and abstracts screened after electronic and bibliographic searches, 81 appeared potentially relevant and all were collected as full-text articles to be assessed for inclusion. Sixty-one potential studies were excluded for a variety of reasons, including an unsuitable study design (eg, nonhuman studies, single-case studies, studies not involving copper supplementation or depletion, or studies not maintaining supplementation or depletion for ≥4 wk). Studies were also excluded if the type of copper supplement was not stated or the study design involved multinutrient supplementation or investigated copper-drug interactions. Nonreporting of baseline and/or subsequent copper status or change data also resulted in exclusion. Sixteen studies (reported in 21 publications) fulfilled all of the inclusion criteria,
and details of the included study characteristics are shown in Table 1. For studies in which data were extracted from more than one article, the main and subsidiary articles are shown in Table 1, but for clarity only the main article is used to reference the study elsewhere.

A total of 16 potential biomarkers of copper status were assessed within the 16 included studies. The quality of the included studies varied; 10 RCTs were included in the review, but in each case the method of randomization was not stated. The remaining studies included 1 CCT and 5 B/A studies. Methods for assessing dose verification were not reported in 14 studies, and methods for checking compliance were reported in only 7 studies. Numbers and reasons for dropouts were reported in only 7 studies. Full information pertaining to the quality assessment of each study is shown in Table 2. Overall, no included studies could be considered to be at low risk of bias.

Total copper concentrations in various blood components

In several included studies, total copper concentrations were measured as potential copper biomarkers in various blood components, including serum, plasma, erythrocytes, and platelets. However, only serum copper met all of the analytic requirements to determine the usefulness of the biomarker (15). Primary outcome data for each biomarker are shown in Table 3.

Serum copper

Seven supplementation studies (4 RCTs, 1 CCT, and 2 B/A) assessed serum copper. These studies included 99 participants, in studies from 7 participants/arm (44) to 16 participants/arm (26). Overall, serum copper significantly increased by 4.01 μmol/L (95% CI: 0.75, 7.27; P = 0.02) in response to copper supplementation (Table 3), but significant heterogeneity was observed between the studies (P_{heterogeneity} < 0.00001, I^2 = 95.2%). This heterogeneity was entirely eliminated when the data were grouped according to baseline copper status (Figure 2). In 2 B/A studies conducted in severely copper-deficient, enterally fed, bedridden, elderly patients (29, 34), the mean increase in serum copper was 12.11 μmol/L (95% CI: 10.28, 13.94; P_{heterogeneity} = 0.64; I^2 = 0%), whereas in studies involving replete individuals at baseline, the mean increase in serum copper was smaller, 1.11 μmol/L (95% CI: 0.43; 1.79, P_{heterogeneity} = 0.57; I^2 = 0%; Table 3). Overall, these data suggest that serum copper responds to copper supplementation in a copper status–dependent manner, ie, although it responds to copper status in both replete and depleted individuals, there is a much greater response to supplementation in copper-deficient than in replete individuals. Four of the 5 studies in replete individuals were conducted in European countries [UK males (16), Danish females (26), Greek males (50; active), and Greek males (50; hypokinesia)] in healthy young adults (Table 1); although the age range of the male subjects in the United Kingdom was 20–59 y, the mean (±SD) age was 32 ± 11 y (16). The fifth study was conducted in the United States in males and females [mean age: 42 y (SD and range not reported)] with chronic back pain. These studies contrast sharply with those conducted in severely depleted individuals (29, 34), which were both conducted in enterally fed, bedridden, elderly Japanese patients.
<table>
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<tr>
<th>Study</th>
<th>Outcomes reported and analytic methods</th>
<th>Population</th>
<th>Intervention and control</th>
<th>Methodology</th>
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</thead>
<tbody>
<tr>
<td>Baker et al, 1999 (16); subsidiary article (17)</td>
<td>Cp: Immuno-turbidimetry assay kit (Dako, High Wycombe, United Kingdom) SOD: Ransod kit (Randox Laboratories, Crumlin, United Kingdom) GPX: Ransel kit (Randox Laboratories) Serum copper: AAS Urinary pyridinoline and deoxypyridinoline: HPLC method (18–20)</td>
<td>Country: United Kingdom Age range: 20–59 y (mean: 32 ± 11 y) Sex: male Participants: healthy nonsmokers, no medication, supplements. No. of participants: 12</td>
<td>Intervention: 3 × 6-wk phase residential study (1) equilibration phase: 1.6 mg Cu/d (0.7-mg/d low-copper diet plus 0.9 mg/d Cu as sulfate) (2), depletion phase (0.7-mg Cu/d low Cu diet) (3) supplementation phase (0.7-mg/d low-Cu diet plus 5.3 mg/d Cu as sulfate) Latest time: 6 wk No. in study at latest time, intervention: 12 Control: 12</td>
<td>Study design: CCT (nonrandomized, crossover) Study aim: to determine copper absorption, retention and excretion at various intakes of copper</td>
</tr>
<tr>
<td>Baker et al, 1999 (21), males; subsidiary articles (22, 23)</td>
<td>As for Baker et al, 1999 (21), females</td>
<td>Country: United Kingdom Age range: 22–46 y Sex: male Participants: healthy adults without bone or articular disease, no medicine affecting bone/cartilage metabolism No. of participants: 12</td>
<td>Intervention: 6 mg/d Cu as glycine chelate for 6 wk Latest time: 6 wk No. in study at latest time, intervention: 9 Control: 9</td>
<td>Study design: RCT Study aims (1): to investigate the response of putative indicators of copper status in response to copper supplementation (2) To investigate the effects of increasing copper intakes above the usual dietary intake on biomarkers of bone metabolism</td>
</tr>
<tr>
<td>Chen et al, 2007 (29)</td>
<td>Cp: immunodiffusion (NADR) Serum Cu: AAS</td>
<td>Country: Japan Age range: 60–93 y Sex: males and females Participants: bedridden, hospitalized, enterally fed patients No. of participants: 7</td>
<td>Intervention: 1 mg/d Cu as Cu sulfate via tube for 28 d Latest time: 28 d No. in study at latest time: 7</td>
<td>Study design: B/A Study aim: to evaluate serum copper and bone marrow in enterally fed patients pre- and postsupplementation with copper</td>
</tr>
<tr>
<td>Study</td>
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</tbody>
</table>
| Eaton-Evans et al, 1996 | Erythrocyte copper: NADR  
Erythrocyte SOD: NADR                                                                                   | Country: United Kingdom  
Age range: 45–56 y  
Sex: female  
Participants: pre-, peri- and postmenopausal middle-aged women  
No. of participants: 73 | Intervention: 3 mg/d copper as amino acid chelate  
Latest time: 104 wk  
No. at latest time: (erythrocyte copper)  
Intervention: 22  
Control: 29 | Study design: RCT  
Study aim: to determine the effect of copper supplementation on vertebral bone mineral density and copper status |
| Jones et al, 1997 (31)  | Cp: Radial immuno-diffusion plates  
(Binding Site Ltd, Birmingham, United Kingdom)  
SOD: pyrogallol antioxidation assay (32, 33)  
Age range: 31–52 y  
Sex: male  
Participants: healthy volunteers with moderately high cholesterol  
No. of participants: 20 | Intervention: 2 mg/d copper as glycine chelate  
Latest time: 28 d  
No. in study at latest time, intervention: 20  
Control: 20 | Study design: RCT (double-blind, placebo-controlled, crossover)  
Study aim: to determine the effects of copper supplementation on copper status and risk factors for cardiovascular disease in men with moderately raised cholesterol |
| Kawada et al, 2006 (34) | Serum Cu: NADR  
Urinary deoxypyridinoline: Pyrilinks-D ELISA kit (Quidel Corporation, San Diego, CA)  
Copper: NADR  
SOD: NADR  
Plasma DAO: colorimetric assay (25) | Country: Japan  
Age range: 72–94 y  
Sex: males and females  
Participants: enterally fed patients immobilized for ≥12 mo after illness (stable for ≥6 mo), serum Cu ≤2.2 μmol/L, serum Cp ≤0.1 g/L  
No. of participants: 10 | Intervention: 3 mg/d Cu as sulfate  
Latest time: 12 wk  
No. in study at latest time: 9 | Study design: B/A  
Study aim: to determine the effects of Cu supplementation on bone metabolism in long-term, bedridden, elderly patients with total enteral nutrition of low concentration of copper |
| Kelley et al, 1995 (35); subsidiary articles (36, 37) | Cp: radial immuno-diffusion kit (Binding Site, Birmingham, United Kingdom)  
SOD: Method of Marklund and Marklund (38)  
Plasma copper: AAS | Country: United States  
Age range: 21–32 y  
Sex: male  
Participants: healthy nonsmokers not taking medication  
No. of participants: 11 | Intervention: residential Cu depletion study, diet containing 0.66 mg/d Cu for 24 d, followed by 0.38 mg/d Cu for 42 d; study also reports copper-repletion data (2.49 mg/d Cu for 24 d)  
Latest time: 64 d  
No. in study at latest time: 11 | Study design: B/A depletion study  
Study aim: to study the effects of low copper diet on copper status and to identify copper intake at which adequate status can be maintained |
| Milne and Nielsen, 2003 (42) | Cp: Radial immuno-diffusion kit (Behring Diagnostics, Somerville, NJ)  
Erythrocyte SOD: percentage inhibition of nitro blue tetrazolium in presence of riboflavin (40)  
Plasma copper: flame AAS  
Platelet copper: flameless AAS  
Cytosol copper: method of Prohaska and Wells (41) | Country: United States  
Age range: 27–37 y  
Sex: male  
Participants: healthy volunteers  
No. of participants: 12 | Intervention: 0.6 mg/d dietary Cu (48 d) vs 2.6 mg/d Cu (0.6 mg dietary Cu plus 2 mg Cu as sulfate (48 d) with 40.3% dietary energy from starch and 4.1% from fructose)  
Latest time: 48 d  
No. in study at latest time, intervention: 6  
Control: 6 | Study design: CCT  
Study aim: to determine whether increased dietary fructose increases the need for copper |
<table>
<thead>
<tr>
<th>Study</th>
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<th>Intervention and control</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pratt et al, 1985 (44); subsidiary article (45)</td>
<td>Serum copper: AAS</td>
<td>Country: United States; Mean age: 42 y (no range given); Sex: male and female; Participants: patients with chronic back pain</td>
<td>Intervention: 10 mg/d Cu as gluconate; Latest time: 12 wk; No. in study at latest time, intervention: 7; Control: 7</td>
<td>Study design: RCT (double-blind, placebo-controlled); Study aim: to test the efficacy and safety of copper supplementation as part of the management of chronic back pain</td>
</tr>
<tr>
<td>Rock et al, 2000 (46)</td>
<td>Erythrocyte SOD: Ransod kit (Randox Laboratories)</td>
<td>Country: France; Age range: 50–72 y; Sex: males and females; Participants: healthy subjects, no medication or supplements.</td>
<td>Intervention: 6 mg/d Cu as glycine chelate; Latest time: 6 wk; No. in study at latest time, intervention: 26; Control: 26</td>
<td>Study design: RCT (randomized, double-blind); Study aim: to provide data on the significance of increased dietary copper as either a pro- or antioxidant in vivo</td>
</tr>
<tr>
<td>Uauy et al, 1985 (47)</td>
<td>Cp: colorimetric assay (48)</td>
<td>Country: Chile; Age range: 21–32 mo; Sex: male and female; Participants: infants after recuperation from protein-energy malnutrition</td>
<td>Intervention: 80 μg/kg.d copper as sulfate; Latest time: 30 d; No. in study at latest time: 8</td>
<td>Study design: B/A; Study aim: to study erythrocyte SOD activity as a potential index of copper status and as evidence of the biochemical effects of copper deficit</td>
</tr>
<tr>
<td>Zorbas et al, 2004 (50); active</td>
<td>Serum copper: AAS</td>
<td>Country: Greece; Age range: 24.7 ± 4.8 y; Sex: males; Participants: healthy volunteers habitually running 5.3 ± 1.2 km/d</td>
<td>Intervention: residential study, 54.02 μmol/d Cu sulfate; Latest time: 364 d; No. in study at latest time, intervention: 10; Control: 10</td>
<td>Study design: RCT; Study aim: to determine whether copper supplementation affected copper deposition during prolonged hypokinesia vs activity</td>
</tr>
<tr>
<td>Zorbas et al, 2004 (50); hypokinesia</td>
<td>As for Zorbas et al, 2004 (50); active</td>
<td>Country: Greece; Age range: 24.7 ± 4.8 y; Sex: males; Participants: healthy volunteers habitually running 5.3 ± 1.2 km/d</td>
<td>Intervention: residential study, 54.0 μmol/d Cu sulfate; Latest time: 364 d; No. in study at latest time, intervention: 10; Control: 10</td>
<td>Study design: RCT; Study aim: to determine whether copper supplementation affected copper deposition during prolonged hypokinesia vs activity</td>
</tr>
</tbody>
</table>

1 AAS, atomic absorption spectrophotometry; Cp, ceruloplasmin; DAO, diamine oxidase; B/A, before-after; ELISA, enzyme-linked immunosorbent assay; GPX, glutathione peroxidase; NADR: no analytic details reported; SOD, superoxide dismutase; CCT, controlled clinical trial.  
2 Study also reports data for 3 mg Cu/d as sulfate and 3 mg Cu/d as glycine chelate.  
3 Study also reports data for 3-mg Cu/d supplementation.  
4 Study also reports data for a 35-d copper repletion phase after depletion.  
5 Study also reports copper data for a diet providing 24.1% of energy from fructose and 20.3% of energy from starch.
Plasma copper

Two supplementation (1 RCT and 1 B/A) and 2 depletion studies (2 B/A) assessed plasma copper as a biomarker in response to copper supplementation (Figure 3, Table 3). These studies included 35 participants, in studies with 6 participants/arm (42) to 11 participants/arm (35). The meta-analysis (Figure 3) showed that overall plasma copper did not respond significantly to copper supplementation. Supplementation increased the mean plasma copper concentration by 1.02 μmol/L (95% CI: −0.92, 2.96), with significant heterogeneity observed between the studies ($P_{heterogeneity} < 0.00001$, $I^2 = 89.2\%$). However, the single B/A supplementation study conducted in severely copper-deficient infants (47) did show a significant response of plasma copper to supplementation, increasing by 10.48 μmol/L (95% CI: 2.35, 18.61). In the case of the 2 depletion studies, there was no significant effect of copper depletion on plasma copper concentration with the mean concentration decreasing by only 0.85 μmol/L (95% CI: −2.08, 3.77). The insufficient number of volunteers (<50) included in this analysis means that no conclusions can be drawn on the usefulness of plasma copper as a status biomarker. However, as with serum copper, it seems likely that plasma copper may be useful in showing copper repletion in depleted individuals after supplementation. However, we have only limited evidence that this marker works with severe depletion at baseline and even less evidence that it works in replete individuals, whereas serum copper appears to respond to supplementation in both replete and depleted individuals.

Other blood components

Only one included study (supplementation, RCT) assessed erythrocyte copper as a status biomarker (39). The study included 52 participants, 29 in the control arm and 23 in the supplementation arm. Erythrocyte copper showed no significant response after copper supplementation [0 mg/mL hemoglobin; 95% CI: −0.04, 0.04; Table 3].

Only one study (depletion, B/A) investigated platelet copper as a status biomarker in (39). Analysis showed no significant effect of copper depletion on platelet copper. The mean platelet copper concentration decreased by 3.8 ng/10^9 cells (95% CI: −4.91, 12.51; Table 3).

Ceruloplasmin protein concentration

The primary outcome data showing the effect of copper supplementation/depletion on ceruloplasmin protein concentration are shown in Table 3. A total of 8 supplementation (5 RCTs, 1 CCT, and 2 B/A) and 2 depletion (B/A) studies assessed serum/plasma ceruloplasmin as a copper status biomarker. These studies included 110 participants, in studies that had 6 participants/arm (42) to 20 participants/arm (31). Meta-analysis showed that ceruloplasmin responded significantly ($P = 0.03$) to changes in copper intake, with mean ceruloplasmin concentration increasing by 0.035 g/L (95% CI: 0.04, 0.65) but with significant heterogeneity observed between the studies ($P_{heterogeneity} < 0.00001$, $I^2 = 97.8\%$; Table 3).

The data for ceruloplasmin protein concentration subgrouped by participant baseline copper status are shown in Figure 4. The majority of these data were generated in copper-replete adults and suggest that total ceruloplasmin protein is not a useful biomarker in these individuals (−0.009 g/L; 95% CI: −0.03, 0.011; Table 4). However, 2 studies conducted in copper-depleted individuals (29, 47) showed a significant increase ($P < 0.00001$) in ceruloplasmin concentration after supplementation (0.258 g/L; 95% CI: 0.225, 0.29), suggesting that ceruloplasmin protein may be a useful biomarker, reflecting increases in copper status in depleted individuals. However, because the analysis was based on <3 included studies and <50 associated participants, no firm conclusions may be drawn from these results. Data for studies subgrouped by ceruloplasmin protein measurements in either serum or plasma also are shown in Table 4. The analysis revealed that in each case there was no significant response to intervention with copper.

Cu/Zn SOD activity

Erythrocyte SOD activity was measured in several studies as a potential biomarker of copper status with a few studies also measuring activity in leukocytes. Six supplementation (4 RCTs, 1 CCT, and 1 B/A) and 2 depletion (B/A) studies assessed erythrocyte SOD activity, and the results of the analyses are shown in Figure 5 and Table 3. These studies included 97 participants and were studies that had 6 participants/arm (42) to 26 participants/arm (46). As shown in Figure 5, erythrocyte SOD activity did not respond significantly to copper intervention. There was no clear effect of intervention on mean SOD activity (51.05 U/g hemoglobin; 95% CI: −54.95, 157.06; $P_{heterogeneity} < 0.00001$, $I^2 = 82.9\%$). These data suggest that erythrocyte SOD is not a useful biomarker of copper status.

Three RCT supplementation studies, including 34 participants, assessed leukocyte SOD activity; these studies had from 9 participants/arm (21; males) to 14 participants/arm (26). As shown in Table 3, leukocyte SOD did not respond significantly to copper supplementation. Supplementation did not significantly alter mean leukocyte SOD activity (0.03 U/g protein; 95% CI: −0.52, 0.58; $P_{heterogeneity} = 0.18$, $I^2 = 42.3\%$). The small number of total participants in the included studies means that no conclusions can be drawn about the usefulness of leukocyte SOD activity as a biomarker of copper status.

Glutathione peroxidase activity

Several studies measured glutathione peroxidase (GPX) activity in various blood components, including erythrocytes, platelets, and plasma, in response to copper supplementation or depletion. As with the majority of potential copper status biomarkers, there was a paucity of data as a result of limited numbers of studies and participants. One CCT and 2 RCT supplementation studies assessed erythrocyte GPX activity. These studies included a total of 32 participants and had from 9 participants/arm (21; males) to 12 participants/arm (16). Erythrocyte GPX activity did not respond significantly to copper supplementation (0.01 U/g hemoglobin; 95% CI: −4.95, 4.96; $P_{heterogeneity} = 0.77$, $I^2 = 0\%$; Table 3). Again, the paucity of data means that it was not possible to draw any conclusions about the usefulness of erythrocyte GPX as a biomarker for copper status.

Only one CCT study (16), which included a total of 12 adult male participants, assessed both plasma and platelet GPX activity in response to copper supplementation (Table 3). Platelet GPX activity was not significantly altered after copper
<table>
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<th>Compliance</th>
<th>Intervention</th>
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<tbody>
<tr>
<td>Baker et al, 1999 (16)</td>
<td>Randomized? No,</td>
<td>Dropouts: NR</td>
<td>Checking method: supplements and meals supervised, volunteers asked re noncompliance</td>
<td>Dose verification: Cu content of meals and supplements analyzed by AAS</td>
</tr>
<tr>
<td></td>
<td>sequential crossover trial</td>
<td></td>
<td>Check results: “excellent compliance”</td>
<td>Actual dose delivered: as reported</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baker et al, 1999 (21); females</td>
<td>Randomized? Yes</td>
<td>Dropouts: NR</td>
<td>Checking method: Questioned at blood sampling</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td>Check results: “excellent compliance”</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Baker et al, 1999 (21); males</td>
<td>As for Baker et al, 1999 (21); females</td>
<td>As for Baker et al, 1999 (21); females</td>
<td></td>
<td>As for Baker et al, 1999 (21); females</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td>Check results: 95% compliance</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Chen et al, 2007 (29)</td>
<td>Randomized? No</td>
<td>Dropouts: NR</td>
<td>Checking method: NR, subjects were enterally fed and bedridden</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td>Check results: “full compliance”</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Eaton-Evans et al, 1996 (30)</td>
<td>Randomized? Yes</td>
<td>Dropouts: 3 control, 14 supplement group</td>
<td>Checking method: verbal questioning</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td>Reasons: constipation, nausea, vomiting, skin rashes, ill health, other medication</td>
<td>Check results: 17 women deemed noncompliant</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: by study pharmacist</td>
<td></td>
<td>Check results: “100% compliance”</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td>Reason: one male died during trial</td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Kelley et al, 1995 (35)</td>
<td>Randomized? No</td>
<td>Dropouts: one</td>
<td>Check method: subjects supervised by study staff in residential facility</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td>Reasons: disruptive behavior</td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Milne and Nielsen, 1996 (39)</td>
<td>Randomized? No</td>
<td>Dropouts: 3</td>
<td>Check method: NR</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td>Reasons: one personal reasons, 2 heart problems</td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Milne and Nielsen, 2003 (42)</td>
<td>Randomized? Yes, double-blind crossover trial</td>
<td>Dropouts: 6, unclear which arm</td>
<td>Checking method: NR</td>
<td>Dose verification: meal duplicates analyzed by ICP-MS; supplement analysis not reported.</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td>Reasons: 2 for protocol nonadherence, 4 for personal reasons.</td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td>Pratt et al, 1985 (44)</td>
<td>Randomized? Yes</td>
<td>Dropouts: 9, unclear which arm</td>
<td>Check method: subjects seen fortnightly to evaluate progress</td>
<td>Dose verification: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: Random dispensing of capsules by pharmacist</td>
<td>Reasons: NR</td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
<tr>
<td></td>
<td>Allocation method: NR</td>
<td></td>
<td>Check results: NR</td>
<td>Actual dose delivered: NR</td>
</tr>
</tbody>
</table>

*(Continued)*
supplementation (40 U/g protein; 95% CI: −48.74, 128.74). Plasma GPX activity also remained unchanged after copper supplementation (0 U/g protein; 95% CI: −1.27, 1.27). No conclusions may be drawn about the potential usefulness of platelet GPX activity because of the limited data.

**Cytochrome-c oxidase activity**

Platelet cytochrome-c oxidase activity was assessed in 2 RCT supplementation studies, with 14 participants in total (reference 21, females; reference 21, males) and one B/A depletion study (39), which could not be included in the analysis because it was not possible to standardize the units. Platelet cytochrome-c oxidase activity responded significantly \((P < 0.00001)\) to copper supplementation with mean activity increasing by 0.95 U/g protein (95% CI: 0.49, 1.41; \(I^2 = 0\%\); Table 3).

Leukocyte cytochrome-c oxidase activity was evaluated in 3 RCTs (reference 26, 34 participants; reference 21, females; reference 21, males) in response to copper supplementation. Activity did not respond significantly to copper supplementation (0.12 U/g protein; 95% CI: −0.84, 1.08; \(P\) heterogeneity \(= 0.36; I^2 = 1.8\%\); Table 3). In the case of both platelet and leukocyte activities it was not possible to draw any firm conclusions about the usefulness of either biomarker because of lack of data.

**Biomarkers of bone turnover**

Several studies assessed the response of urinary markers of bone resorption, namely pyridinoline and deoxypyridinoline, as potential biomarkers for copper status. In each included study, both pyridinoline and deoxypyridinoline measurements were normalized using urinary creatinine measurements by the respective authors.

**Urinary pyridinoline**

Four supplementation studies (3 RCTs and 1 CCT), which included a total of 47 participants, assessed urinary pyridinoline concentrations, and the results of the analyses are shown in Table 3. Participants in these studies ranged from 10/arm (21; males) to 16 participants/arm (26). Urinary pyridinoline did not respond significantly to copper supplementation \((-1.31 \text{ nmol/mmol creatinine}; 95\% \text{ CI: } -7.00, 4.38; P_{\text{heterogeneity}} = 0.88; I^2 = 0\%\); Table 3). Once again, the small number of participants means that no conclusions can be drawn about the usefulness of urinary pyridinoline as a biomarker for copper status.

**Urinary deoxypyridinoline**

Five supplementation studies (3 RCTs, 1 CCT, and 1 B/A) assessed urinary deoxypyridinoline concentrations. These studies included a total of 56 participants in studies ranging from 9 participants/arm (34) to 16 participants/arm (26). As illustrated in Figure 6, urinary deoxypyridinoline did not respond significantly to copper supplementation with the mean normalized pyridinoline concentration decreasing slightly by 0.11 nmol/ mmol creatinine (95% CI: −1.99, 1.77), with no significant heterogeneity observed between the studies \((P_{\text{heterogeneity}} = 0.86; I^2 = 0\%\)). This analysis provides sufficient data to suggest that urinary deoxypyridinoline is not a useful biomarker for copper status.
### TABLE 3
Primary outcome data for all biomarkers

<table>
<thead>
<tr>
<th>Analysis (study type) and included studies</th>
<th>Mean effect, Units</th>
<th>Study design</th>
<th>no. of studies (no. of participants) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum copper (supp)</td>
<td>4.01 (0.75, 7.27) µmol/L</td>
<td>RCTs 4 (70), CCTs 1 (12), B/A 2 (17)</td>
<td>95.2 Yes</td>
</tr>
<tr>
<td>Plasma copper (supp/depletion)</td>
<td>1.02 (−0.92, 2.96) µmol/L</td>
<td>RCTs 1 (6), CCTs 0 (0), B/A 3 (29)</td>
<td>89.2 Unclear</td>
</tr>
<tr>
<td>Erythrocyte copper (supp)</td>
<td>0.0 (−0.04, 0.04) mg/mL hemoglobin</td>
<td>RCTs 1 (52), CCTs 0 (0), B/A 0 (0)</td>
<td>— Unclear</td>
</tr>
<tr>
<td>Platelet copper (depletion)</td>
<td>3.80 (−4.91, 12.51) ng/10^9 cells</td>
<td>RCTs 0 (0), CCTs 0 (0), B/A 1 (10)</td>
<td>— Unclear</td>
</tr>
<tr>
<td>Serum/plasma ceruloplasmin (supp/depletion)</td>
<td>0.035 (0.004, 0.065) g/L</td>
<td>RCTs 5 (62), CCTs 1 (12), B/A 4 (36)</td>
<td>97.8 Yes</td>
</tr>
<tr>
<td>Erythrocyte SOD (supp/depletion)</td>
<td>51.05 (−54.95, 157.06) U/g hemoglobin</td>
<td>RCTs 4 (56), CCTs 1 (12), B/A 3 (29)</td>
<td>82.9 No</td>
</tr>
<tr>
<td>Leukocyte SOD (supp)</td>
<td>0.03 (−0.52, 0.58) U/g protein</td>
<td>RCTs 3 (34), CCTs 0 (0), B/A 0 (0)</td>
<td>42.3 Unclear</td>
</tr>
<tr>
<td>Erythrocyte GPX (supp)</td>
<td>0.01 (−4.95, 4.96) U/g protein</td>
<td>RCTs 2 (20), CCTs 1 (12), B/A 0 (0)</td>
<td>0 Unclear</td>
</tr>
<tr>
<td>Platelet GPX (supp)</td>
<td>40.0 (−48.74, 128.74) U/g protein</td>
<td>RCTs 0 (0), CCTs 1 (12), B/A 0 (0)</td>
<td>— Unclear</td>
</tr>
<tr>
<td>Plasma GPX (supp)</td>
<td>0.00 (−1.27, 1.27) U/g protein</td>
<td>RCTs 0 (0), CCTs 1 (12), B/A 0 (0)</td>
<td>— Unclear</td>
</tr>
<tr>
<td>Platelet cytochrome c oxidase (supp)</td>
<td>0.95 (0.49, 1.41) U/g protein</td>
<td>RCTs 2 (14), CCTs 0 (0), B/A 0 (0)</td>
<td>0 Unclear</td>
</tr>
<tr>
<td>Leukocyte cytochrome-c oxidase (supp)</td>
<td>0.12 (−0.84, 1.08) U/g protein</td>
<td>RCTs 3 (34), CCTs 0 (0), B/A 0 (0)</td>
<td>1.8 Unclear</td>
</tr>
<tr>
<td>Total plasma glutathione (supp)</td>
<td>−0.03 (−0.13, 0.07) U/g protein</td>
<td>RCTs 1 (6), CCTs 0 (0), B/A 0 (0)</td>
<td>— Unclear</td>
</tr>
</tbody>
</table>

(Continued)
Other biomarkers

**Plasma glutathione**

Only one supplementation RCT, including 6 participants/arm, assessed total plasma glutathione concentration as a potential biomarker of copper status (42). Plasma glutathione concentration did not respond significantly to copper supplementation (0.05 mmol/L; 95% CI: 0.11, 0.21; Table 3). Consequently, it was not possible to draw any conclusions about the usefulness of plasma glutathione as a biomarker for copper status.

**Diamine oxidase**

Three RCT supplementation studies, including a total of 37 participants, assessed plasma/serum diamine oxidase activity. These studies included from 7 (21; males) to 20 participants/arm (31). Diamine oxidase activity responded significantly \((P = 0.02)\) to copper supplementation with mean activity increasing by 1.13 U/L (95% CI: 0.21, 2.05; Table 3), with no significant heterogeneity observed between the studies \((P_{\text{heterogeneity}} = 0.91, I^2 = 0\%). However, it should be noted that the activity measured in the single study assessing activity in plasma was an order of magnitude greater than that in the serum studies. On the basis of the current evidence, no conclusions can be drawn about the usefulness of diamine oxidase activity as a biomarker for copper status.

### DISCUSSION

A total of 16 relevant studies were included in the review, and these identified a total of 16 potential biomarkers of copper status. However, a significant proportion of the analyses conducted for the individual biomarkers did not meet the minimum criteria for determining their usefulness, ie, they had <3 studies or <50 participants contributing data to the meta-analysis (15).

### TABLE 3 (Continued)

<table>
<thead>
<tr>
<th>Analysis (study type) and included studies</th>
<th>Mean effect, WMD (95% CI)</th>
<th>Units</th>
<th>Study design</th>
<th>RCTs</th>
<th>CCTs</th>
<th>B/A</th>
<th>(I^2)</th>
<th>Biomarker useful?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamine oxidase (supp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baker et al, 1999 (females; 21); Baker et al, 1999 (males; 21); Jones et al, 1997 (31)</td>
<td>1.13 (0.21, 2.05) U/L</td>
<td></td>
<td>3 (37)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0</td>
<td>Unclear</td>
<td></td>
</tr>
<tr>
<td>Urinary pyridinoline (supp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baker et al, 1999 (16); Baker et al, 1999 (females; 21); Baker et al, 1999 (males; 21); Cashman et al, 2001 (26)</td>
<td>−1.31 (−7.00, 4.38) nmol/mmol creatinine</td>
<td>3 (36)</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>0</td>
<td>Unclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary deoxypyridinoline (supp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baker et al, 1999 (16); Baker et al, 1999 (females; 21); Baker et al, 1999 (males; 21); Cashman et al, 2001 (26); Kawada et al, 2006 (34)</td>
<td>−0.11 (−1.99, 1.77) nmol/mmol creatinine</td>
<td>3 (36)</td>
<td>1 (11)</td>
<td>1, 9</td>
<td>0</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(B/A, \text{ before-after studies}; CCTs, \text{ controlled clinical trials}; \text{ Depletion, includes depletion studies only}; \text{ GPX, glutathione peroxidase}; \text{ RCTs, randomized controlled trials}; \text{ supp, includes supplementation studies only}; \text{ supp/depletion, includes a combination of supplementation and depletion studies}; \text{ WMD, weighted mean difference.} \)

### FIGURE 2.

Serum copper response to copper supplementation subgrouped by baseline copper status (µmol/L). Arrowheads indicate direction of response when unable to represent data on most appropriate scale. WMD, weighted mean difference.
Consequently, for the majority of the biomarkers identified, it was not possible to draw any firm conclusions concerning their effectiveness in reflecting changes in copper status. Although this systematic review offers primary outcome data for each biomarker identified, the limited number of included studies has-precluded meaningful subgroup analysis [eg, age, sex, or body mass index (BMI)] by type of study. It also was not possible to draw conclusions about potential relations between biomarker responsiveness and type, dose, or length of supplementation. In addition, the majority of studies were conducted in copper-replete adults with relatively few volunteers; therefore, there was no capacity within the data to conduct subgroup analysis for a range of population groups, eg, infants, adolescents, or pregnant women.

Sufficient data were available in the case of serum copper to suggest its value as a biomarker, because it appeared to reflect changes in copper status in both copper-depleted and copper-replete individuals, with smaller changes in copper-replete individuals. On the other hand, ceruloplasmin total protein may be copper status dependent, effectively reflecting copper status changes only in highly depleted individuals (although evidence for this effect comes from only 2 studies). There were adequate data to suggest that erythrocyte SOD and urinary deoxypyrinoline are not useful biomarkers of copper status. There were insufficient studies or participants to draw firm conclusions about plasma, erythrocyte and platelet copper, leukocyte SOD, erythrocyte, platelet and plasma GPX, platelet and leukocyte cytochrome-c oxidase, total glutathione, di-amine oxidase, and urinary pyridinoline as biomarkers of copper status.

The results of this review suggest that serum copper may be the most useful biomarker of copper status and appears to be effective in both replete and depleted individuals. However, it was not possible to perform other subgroupings, so it is not clear under what circumstances it can be relied on. Data are too sparse to draw firm conclusions about most other potential biomarkers, but we may eliminate erythrocyte SOD and urinary deoxypyrinoline from future consideration.

Study
or sub-category
N Intervention
Mean (SD) N Control
Mean (SD) WMD (random)
95% CI WMD (random)
95% CI Order
01 Supplementation RCT
Mire (42) 6 12.40 (0.50) 6 12.50 (0.50) -0.10 [-0.67, 0.47] 7
Subtotal (95% CI) 6 -0.10 [-0.67, 0.47] 4
Test for heterogeneity: not applicable
Test for overall effect: Z = 0.35 (P = 0.73)
02 Supplementation before / after
Mire (47) 8 21.70 (5.20) 8 15.30 (10.47) 10.49 [2.35, 18.61] 4
Subtotal (95% CI) 8 10.49 [2.35, 18.61] 10.49 [2.35, 18.61] 4
Test for heterogeneity: not applicable
Test for overall effect: Z = 2.33 (P = 0.01)
03 Depletion before / after
Kelley (35) 11 -12.60 (0.99) 11 -14.80 (0.99) 2.20 [1.97, 3.03] 6
Mire (39) 10 -18.10 (2.00) 10 -17.30 (2.20) -0.80 [-2.82, 1.22] 14
Subtotal (95% CI) 21 0.85 [-2.08, 3.77] 1.02 [-0.92, 2.96]
Test for heterogeneity: CH2 = 7.27, df = 1 (P = 0.007), P = 86.2%
Test for overall effect: Z = 0.57 (P = 0.57)
Total (95% CI) 35 1.02 [-0.92, 2.96]
Test for heterogeneity: CH2 = 27.78, df = 3 (P = 0.00001), P = 89.2%
Test for overall effect: Z = 1.03 (P = 0.30)

FIGURE 3. Plasma copper response to copper supplementation or depletion (µmol/L). Arrowheads indicate direction of response when unable to represent data on most appropriate scale. WMD, weighted mean difference; RCT, randomized controlled trial.

FIGURE 3. Plasma copper response to copper supplementation or depletion (µmol/L). Arrowheads indicate direction of response when unable to represent data on most appropriate scale. WMD, weighted mean difference; RCT, randomized controlled trial.

Study
or sub-category
N Supplementation
Mean (SD) N Control
Mean (SD) WMD (random)
95% CI WMD (random)
95% CI Order
01 Depleted at baseline
Chen (20) 7 1.00 (0.40) 7 0.40 (0.20) 2.60 [2.27, 2.93] 4
Usay (47) 0 1.00 (1.40) 0 2.30 (2.60) 1.70 [-0.36, 3.76] 4
Subtotal (95% CI) 15 2.60 [2.25, 2.90] 2.60 [2.25, 2.90] 4
Test for heterogeneity: CH2 = 0.72, df = 1 (P = 0.39), P = 0%
Test for overall effect: Z = 15.44 (P = 0.00001)
02 Replete at baseline
Baker: females (21) 11 2.90 (0.70) 11 2.30 (0.70) 0.50 [-0.39, 1.39] 6
Baker: males (21) 9 1.80 (0.30) 9 1.90 (0.30) -0.10 [-0.98, 0.78] 6
Cashman (20) 16 2.00 (0.70) 16 2.20 (0.50) -0.20 [-0.62, 0.22] 4
Jones (31) 20 4.60 (1.00) 20 4.60 (1.00) 0.10 [-0.46, 0.66] 4
Mire (42) 6 2.30 (0.03) 6 2.60 (0.03) -0.30 [-0.93, -0.27] 7
Subtotal (95% CI) 74 0.50 [-0.30, 0.33] 0.30 (-0.11, 0.71) 7
Test for heterogeneity: CH2 = 89.17, df = 5 (P = 0.00001), P = 93.8%
Test for overall effect: Z = 0.88 (P = 0.38)
Total (95% CI) 89 0.30 [0.03, 0.67]
Test for heterogeneity: CH2 = 359.94, df = 7 (P = 0.00001), P = 99.1%
Test for overall effect: Z = 2.13 (P = 0.03)

FIGURE 4. Serum/plasma ceruloplasmin response to copper supplementation subgrouped by baseline status [g/L (×10)]. WMD, weighted mean difference.
The tight homeostatic regulation of copper concentrations in the bloodstream and other tissues generally restricts major perturbations in concentration to the extremes of dietary intake, thus potentially restricting biomarker responses to deficiency or overload states. This is supported within this review with the most significant changes in ceruloplasmin and serum copper occurring in studies involving participants who have extremely low copper intakes and status before supplementation (29, 34, 47). It should be noted that ceruloplasmin total protein and serum copper concentrations are likely to respond in a similar fashion because the majority (60–95%) of serum copper is ceruloplasmin bound (51). Plasma copper concentrations consistently are reported to be lower than serum values (52), which possibly contributed to lack of response with this biomarker in copper-replete individuals (Figure 3).

The primary outcome data for ceruloplasmin are strongly influenced by 3 studies conducted in copper-depleted individuals. Although these data appear highly significant, it should be remembered that, as shown in Table 1, they were generated in individuals with a range of health problems. It is well accepted that the responsiveness and concentration of ceruloplasmin can be affected by a range of nondietary factors. Concentrations tend to increase with age and generally are higher in females than in males throughout the life span (53). The latter is exacerbated in premenopausal women as a result of oral contraceptive use (53), estrogen-dependent ceruloplasmin synthesis, and secretion by the liver (54). Ceruloplasmin also is an acute-phase protein regulated by inflammatory hormones and, consequently, in individuals with chronic inflammatory conditions, eg, rheumatoid arthritis, have raised levels. Therefore, measurements of ceruloplasmin should be viewed with extreme caution. Subgroup analysis of the data by baseline copper status showed no significant response of total ceruloplasmin protein concentration but again it is essential to be aware that serum copper concentrations are affected by similar nondietary factors as is ceruloplasmin.

Erythrocyte SOD activity is commonly used as a biomarker of copper status in intervention studies, but this review suggests that this probably is inappropriate. However, to a certain extent, the primary outcome data (Table 3, Figure 5) are affected strongly by 2 separate studies: first, a depletion study undertaken in postmenopausal women (39), which showed a dramatic and significant decrease in activity, and second, a copper repletion study undertaken in severely copper-deficient infants. In each case, these were B/A studies that lacked a control group, which is not an ideal study design.

Studies investigating human copper metabolism claim to be hindered by the lack of useful indicators of copper status. However, the present review has revealed that serum copper is a potentially useful biomarker in both replete and depleted individuals, with plasma copper and ceruloplasmin concentrations showing potential promise in depleted individuals. However, in these cases, further studies need to be undertaken to identify the boundaries of their use, eg, age range, sex, or BMI.

The main problems associated with studies excluded from this review related to poor study design with lack of a control group identified as a major flaw coupled with inappropriate data reporting. In many instances the citing of study data often was unclear or even absent, with common omissions, eg, baseline or control group data, numbers of subjects, and measures of variance. Many studies did not clearly report type of dose, subject dropouts, compliance, or randomization methods. The reporting of analytic methodologies also was vague in many cases. The primary aim of many studies included in this review was not to evaluate the usefulness of copper status biomarkers, which may account for study designs that were unsuitable for this purpose.

Recent literature reviews of copper status biomarkers (3, 11) have highlighted the apparent ineffectiveness of so-called traditional status indicators, eg, ceruloplasmin and serum copper concentrations, which this review has shown is not the case in all circumstances. However, the earlier reviews also suggested that several novel biomarkers may be useful in certain instances, eg, lysyl oxidase, peptidylglycine α-amidating monoxygenase, and CCS (the copper chaperone for SOD). Unfortunately, the present
review has highlighted a complete absence of suitable data to evaluate these potentially novel biomarkers. Consequently, this underlines a pressing need to undertake well-designed intervention RCTs in healthy individuals across all copper intakes and statuses in order to evaluate the responsiveness of putative biomarkers of copper status. Subsequently, further studies then would need to be undertaken in various population subgroups, e.g., infants, pregnant women, and the elderly, to assess effectiveness. In addition, it ultimately will be necessary to undertake studies investigating the effect of form of dietary copper intake, thus providing data on the bioavailability of both food and supplemental copper forms. In due course, these data would contribute to the development of evidence-based dietary recommendations for copper across all population groups, which to date have proved elusive. (Other articles in this supplement to the Journal include references 15 and 55–61.)

The authors’ contributions were as follows—SJF-T, LH, LJH, and KA: developed the study concept and design; LH: wrote the study protocol; KA: conducted the electronic searches; LJH, LH, AC, and KA: assessed the studies for inclusion, extracted data, and assessed validity; LJH: conducted meta-analyses and tabulated data; LJH and LH: wrote the first draft of the manuscript; and all other authors contributed to writing the manuscript and approved the final version. None of the authors had a personal or financial conflict of interest.

FIGURE 5. Erythrocyte SOD response to copper supplementation and depletion (U/g hemoglobin). Arrowheads indicate direction of response when unable to represent data on most appropriate scale. RCT, randomized controlled trial; CCT, controlled clinical trial; WMD, weighted mean difference.

FIGURE 6. Urinary deoxypyridinoline response to copper supplementation (nmol/mmol creatinine). Arrowheads indicate direction of response when unable to represent data on most appropriate scale. RCT, randomized controlled trial; CCT, controlled clinical trial; WMD, weighted mean difference.