Serum Copper Concentration and Coronary Heart Disease among US Adults

Earl S. Ford

Copper, a strong prooxidant, may play a role in atherogenesis. The author examined the association between serum copper concentration and mortality from coronary heart disease using data from the Second National Health and Nutrition Examination Survey (1976–1992). Serum copper concentration was determined using atomic absorption spectroscopy. After various exclusions, 151 deaths from coronary heart disease occurred among 4,574 participants aged ≥30 years. At baseline, the age-adjusted serum copper concentration was about 5% higher among participants who died from coronary heart disease than among those who did not (p = 0.072). After adjustment for age, sex, race, education, smoking status, systolic blood pressure, serum cholesterol, serum high density lipoprotein cholesterol, body mass index, recreational activity, nonrecreational activity, history of diabetes, and white blood cell count, the hazard ratios for death from coronary heart disease for serum copper concentrations in the second, third, and fourth quartiles (versus the first quartile) were 1.84 (95% confidence interval (CI): 0.93, 3.66), 2.14 (95% CI: 1.21, 3.77), and 2.87 (95% CI: 1.57, 5.25), respectively. Several prospective studies, including the present analysis, have found elevated serum copper concentrations to be associated with cardiovascular disease. Whether copper directly affects atherogenesis or is a marker of inflammation associated with atherosclerosis remains to be established. Am J Epidemiol 2000; 151:1182-8.

MATERIALS AND METHODS

From 1976 to 1980, about 28,000 persons aged 6 months to 74 years participated in NHANES II. The
survey used a multistage sampling design so that the data would be representative of the noninstitutionalized civilian population. After participants were interviewed at home, they were invited to have a medical examination during which they completed additional questionnaires, underwent a series of examinations and tests, and provided blood and urine specimens. Survey details have been published elsewhere (25).

A total of 9,252 participants aged 30–75 years who attended the medical examination were followed through 1992, and their vital status was established through computer matching to the Social Security Administration’s Death Master File (1976–1992) and the National Death Index (1979–1992). Participants or surrogates were not contacted. Participants not found to be deceased were assumed to be alive. Causes of death were obtained for 2,103 of 2,145 participants identified as deceased from the Multiple Cause of Death file of the National Center for Health Statistics or from death certificates from offices of state vital statistics. Details of this process have been published elsewhere (26).

If the death certificate contained the International Classification of Diseases, Ninth Revision, Clinical Modification codes 410–414 on the underlying cause of death field, a participant was classified as dying from coronary heart disease. Serum copper concentrations were measured using atomic absorption spectroscopy at the Centers for Disease Control laboratories (27). Coefficients of variation ranged from 2.58 percent to 3.30 percent for copper.

Participants with evidence of heart disease at baseline were excluded from the analysis if they: 1) reported having heart failure, heart attack, any other heart trouble, or hardening of the arteries (“Has a doctor ever told you that you had any of the following conditions, and if so, do you still have it?”); 2) indicated that they had been hospitalized overnight for a cardiac condition (“During the 12 months, how many different times did you stay in a hospital overnight or longer?” “For what condition(s) were you in the hospital?”); 3) had a positive angina questionnaire; or 4) showed electrocardiographic evidence of a probable myocardial infarction (Minnesota codes 1.1.1 through 1.1.7 and 1.2.1 through 1.2.7, together with code 4.1, 4.2, 5.1, or 5.2) or a possible myocardial infarction (Minnesota codes 1.2.1 through 1.2.7, together without code 4.1, 4.2, 5.1, or 5.2, and codes 1.2.8 and 1.3.1 through 1.3.6, together with code 4.1, 4.2, 5.1, or 5.2).

The following covariates were included in the analysis: age (years), sex, race or ethnicity (White, African American, other), education (years), smoking status (never, former, current), systolic and diastolic blood pressure, serum cholesterol concentration, high density lipoprotein cholesterol concentration, body mass index (kg/m$^2$), recreational physical activity (much exercise, moderate exercise, little or no exercise), non-recreational physical activity (very active, moderately active, quite inactive), history of diabetes mellitus, and white blood cell count. The first and second systolic and diastolic readings were averaged. Hypertension was defined as systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or current use of antihypertensive medication.

Two sample comparisons of continuous data were conducted using $t$ tests; comparisons of categorical data used a chi-square test. Tests for linear trend in the association of covariates with quartiles of copper concentration were conducted. Serum copper concentration was analyzed as a continuous variable and as quartiles using the distributions of the analytical sample in proportional hazards analyses. Medians of the quartiles were used to test for a linear dose response. The proportionality assumption was tested by including an interaction term for follow-up time and copper concentration; results showed that the assumption was not violated. All calculations were performed with SUDAAN, a statistical software program that takes into account the complex design of the survey (28).

RESULTS

Of the 9,252 persons who were included in the mortality study, vital status could not be established for two persons. A total of 2,145 participants died during the 12-year follow-up. After various exclusions, 4,574 participants were included in the analyses, of whom 764 died from any cause and 151 died from coronary heart disease (table 1).

Among participants included in the analysis, the serum concentration ranged from 37 to 343 μg/dl (5.8–53.9 μmol/liter). The mean concentration was 122.9 μg/dl (19.3 μmol/liter); median, 117.5 μg/dl (18.5 μmol/liter); and geometric mean, 119.9 μg/dl (18.8 μmol/liter). Among men included in the analysis ($n = 2,113$), the serum concentration ranged from 37

<table>
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<tbody>
<tr>
<td><strong>Action</strong></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Exclude lost to follow-up</td>
</tr>
<tr>
<td>Exclude no copper measurement</td>
</tr>
<tr>
<td>Exclude prevalent heart disease</td>
</tr>
<tr>
<td>Exclude missing data for covariates</td>
</tr>
</tbody>
</table>

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to 329 μg/dl (5.8–51.7 μmol/liter). The mean concentration was 111.4 μg/dl (17.5 μmol/liter); median, 109.4 μg/dl (17.2 μmol/liter); and geometric mean, 109.8 μg/dl (17.2 μmol/liter). Among women included in the analysis (n = 2,461), the serum concentration ranged from 66 to 343 μg/dl (10.4–53.9 μmol/liter). The mean concentration was 111.4 μg/dl (17.5 μmol/liter); median, 109.4 μg/dl (17.2 μmol/liter); and geometric mean, 109.8 μg/dl (17.2 μmol/liter).

Persons who died from coronary heart disease were older and, after adjusting for age, were more likely to be men and more likely to have smoked than were participants who did not die from coronary heart disease (table 2). The mean systolic blood pressure and serum cholesterol were higher and the mean high density lipoprotein cholesterol was lower among participants dying from coronary heart disease than among those who did not. The unadjusted serum copper concentration was 8 percent higher and the age-adjusted concentration about 5 percent higher among persons who died from coronary heart disease than among those who did not.

The associations between serum copper concentration and various covariates are shown in table 3. After adjustment for age and sex, significant linear trends for all variables were found except for high density lipoprotein cholesterol and nonrecreational physical activity.

The serum copper concentration was strongly associated with coronary heart disease mortality in unadjusted, age-adjusted, and multiple-adjusted proportional hazards analyses (table 4). Interaction terms for serum copper concentration and age (p = 0.132) and smoking (p = 0.243) were not significant, suggesting that the associations between serum copper concentration and coronary heart disease mortality did not differ by levels of these factors. There were too few events among persons with a race other than White (nine African Americans, two other) to examine effect modification by race or ethnicity. Hazard ratios were larger among men than women, suggesting that the association between serum copper concentration and coronary heart disease mortality varied by sex (p = 0.023) (table 4).

**DISCUSSION**

Researchers have long thought that copper can influence the pathogenesis of atherosclerosis (29). Over the years, prospective studies, though few, have provided support for this hypothesis (21–24). In one of the largest prospective population-based investigations of this issue to date, the results from the NHANES II Mortality Study add to findings from previous prospective studies by showing a strong association between copper concentration and coronary heart disease mortality. Generally, the size of the hazard ratio increased as the serum copper concentration increased.

In a Dutch nested case-control study conducted in a cohort of 10,532 persons aged ≥5 years, 62 persons

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**TABLE 2.** Age-adjusted* means or percentages of selected variables by coronary heart disease status, Second National Health and Nutrition Examination Survey Mortality Study, 1976–1992

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Men (%)</th>
<th>White (%)</th>
<th>Education (years)</th>
<th>Current smoker (%)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum cholesterol (mg/dl)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants dying from coronary heart disease (n = 151)</td>
<td>62.2 (1.0)‡</td>
<td>74.1 (5.5)</td>
<td>94.5 (3.9)</td>
<td>11.3 (0.6)</td>
<td>62.2 (5.8)</td>
<td>137.9 (3.0)</td>
</tr>
<tr>
<td>Participants not dying from coronary heart disease (n = 4,423)</td>
<td>47.2 (0.3)</td>
<td>46.2 (0.7)</td>
<td>89.3 (1.5)</td>
<td>11.8 (0.1)</td>
<td>35.0 (0.8)</td>
<td>128.3 (0.7)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.207</td>
<td>0.451</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum high density lipoprotein cholesterol (mg/dl)†</th>
<th>Body mass index (kg/m²)</th>
<th>Much exercise during recreation (%)</th>
<th>Very active aside from recreation (%)</th>
<th>History of diabetes (%)</th>
<th>White blood cell count (10⁶ cells/μl)‡</th>
<th>Serum copper (μg/dl)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants dying from coronary heart disease (n = 151)</td>
<td>42.2 (2.7)</td>
<td>25.6 (0.6)</td>
<td>12.9 (5.4)</td>
<td>41.3 (11.4)</td>
<td>9.6 (5.0)</td>
<td>7.5 (0.4)</td>
</tr>
<tr>
<td>Participants not dying from coronary heart disease (n = 4,423)</td>
<td>50.4 (0.5)</td>
<td>25.6 (0.1)</td>
<td>17.2 (0.7)</td>
<td>36.8 (0.9)</td>
<td>3.3 (0.4)</td>
<td>7.0 (0.1)</td>
</tr>
<tr>
<td>p value</td>
<td>0.005</td>
<td>0.934</td>
<td>0.442</td>
<td>0.695</td>
<td>0.209</td>
<td>0.172</td>
</tr>
</tbody>
</table>

* Age adjusted to the 1980 US population aged 30–74 years.
† To convert values for serum cholesterol and high density lipoprotein cholesterol concentrations to millimoles per liter, multiply by 0.02586. To convert values for white blood cell count to 10⁶ per microliter, multiply by 1. To convert values for serum copper concentration to micromoles per liter, multiply by 0.157.
‡ Numbers in parentheses, standard error.

<table>
<thead>
<tr>
<th>Serum copper concentration</th>
<th>Age (years)†</th>
<th>Men (%)†</th>
<th>White (%)‡</th>
<th>Education (years attended)</th>
<th>Current smoker (%)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum cholesterol (mg/dl)‡</th>
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</thead>
<tbody>
<tr>
<td>Quartile 1 (n = 1,152)</td>
<td>44.4 (0.4)†</td>
<td>69.9 (1.6)</td>
<td>91.5 (2.4)</td>
<td>12.2 (0.1)</td>
<td>24.4 (1.9)</td>
<td>125.8 (0.8)</td>
<td>214.1 (1.9)</td>
</tr>
<tr>
<td>Quartile 2 (n = 1,157)</td>
<td>46.9 (0.4)†</td>
<td>55.6 (2.2)</td>
<td>91.4 (1.3)</td>
<td>11.9 (0.1)</td>
<td>34.9 (1.6)</td>
<td>128.0 (0.9)</td>
<td>221.1 (1.4)</td>
</tr>
<tr>
<td>Quartile 3 (n = 1,199)</td>
<td>48.1 (0.4)†</td>
<td>39.1 (1.5)</td>
<td>89.2 (1.9)</td>
<td>11.5 (0.1)</td>
<td>40.4 (1.5)</td>
<td>129.1 (1.0)</td>
<td>225.5 (1.8)</td>
</tr>
<tr>
<td>Quartile 4 (n = 1,066)</td>
<td>49.8 (0.4)†</td>
<td>19.3 (1.2)</td>
<td>84.6 (2.0)</td>
<td>11.0 (0.2)</td>
<td>51.2 (2.1)</td>
<td>131.3 (0.8)</td>
<td>227.4 (2.6)</td>
</tr>
</tbody>
</table>

*p value <0.001 <0.001 0.001 <0.001 <0.001 <0.001


<table>
<thead>
<tr>
<th>No. of events/ no. at risk</th>
<th>Serum copper concentration*</th>
<th>Continuous (per 1 μmol/liter)</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>p value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>151/4,574</td>
<td>1.06</td>
<td>1.03, 1.09</td>
<td>2.47</td>
<td>1.18, 5.17</td>
<td>3.35</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>151/4,574</td>
<td>1.07</td>
<td>1.03, 1.11</td>
<td>1.90</td>
<td>0.94, 3.85</td>
<td>2.08</td>
</tr>
<tr>
<td>Multiple adjusted†</td>
<td>151/4,574</td>
<td>1.10</td>
<td>1.05, 1.14</td>
<td>1.84</td>
<td>0.93, 3.66</td>
<td>2.14</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>86/2,113</td>
<td>1.15</td>
<td>1.09, 1.21</td>
<td>2.98</td>
<td>1.37, 6.51</td>
<td>5.93</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>86/2,113</td>
<td>1.09</td>
<td>1.04, 1.15</td>
<td>2.47</td>
<td>1.15, 5.32</td>
<td>4.53</td>
</tr>
<tr>
<td>Multiple adjusted†</td>
<td>86/2,113</td>
<td>1.10</td>
<td>1.02, 1.19</td>
<td>2.46</td>
<td>1.12, 5.41</td>
<td>4.08</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>65/2,461</td>
<td>1.07</td>
<td>1.03, 1.10</td>
<td>1.87</td>
<td>0.52, 6.77</td>
<td>1.80</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>65/2,461</td>
<td>1.12</td>
<td>1.08, 1.17</td>
<td>1.31</td>
<td>0.38, 4.45</td>
<td>0.94</td>
</tr>
<tr>
<td>Multiple adjusted†</td>
<td>65/2,461</td>
<td>1.12</td>
<td>1.08, 1.17</td>
<td>0.98</td>
<td>0.30, 3.19</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*p Copper quartiles: quartile 1, <106 μg/dl; quartile 2, 106–<120 μg/dl; quartile 3, 120–<137 μg/dl; quartile 4, ≥137 μg/dl.
† Adjusted for age, sex, race, education, smoking status, systolic blood pressure, serum cholesterol, serum high density lipoprotein cholesterol, body mass index, recreational activity, non-recreational activity, history of diabetes, and white blood cell count.

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Finland, 50 persons developed myocardial infarction among 1,666 men aged 42–60 years who were followed from 1 to 5.75 years (23). Participants with serum copper concentrations of 1.02–1.15 mg/liter and ≥1.16 mg/liter had hazard ratios of 3.5 (95 percent CI: 1.3, 9.4) and 4.0 (95 percent CI: 1.5, 10.8), respectively, compared with participants who had serum copper concentrations of <1.02 mg/liter. In a nested case-control study of persons aged 15–69 years attending a multiphasic screening conducted by the Social Insurance Institution in Finland, 230 men who died from cardiovascular disease during the 10-year follow-up were matched with 298 control men (24). In comparison with participants having the lowest serum copper concentrations, the odds ratios for coronary disease were 2.34 (95 percent CI: 0.97, 5.65) and 3.54 (95 percent CI: 1.44, 8.63) for the middle and highest tertiles, respectively. Thus, the four prospective studies have yielded similar estimates of the effect size at the upper end of the copper distribution.

Not all studies have found copper to be directly associated with cardiovascular disease morbidity or mortality. In an early study, the serum copper concentration mean measured on 3 consecutive days after admission in eight patients with myocardial infarction was not significantly higher compared with that of 30 controls (30). In autopsy studies from Canada and the United Kingdom, the copper concentration in heart muscle was lower in persons with ischemic heart disease than in control subjects (31, 32). Although some studies have found small positive associations between copper in drinking water and cardiovascular disease, the sum of the evidence from these studies was not deemed convincing (33). Moreover, in a study of 60 men with angiographically defined coronary artery disease, the plasma copper concentration did not differ significantly from reference values (34). Furthermore, serum copper concentrations in 27 patients undergoing coronary artery bypass surgery were found to be lower than in 64 healthy controls (35).

Elevated copper concentrations may be related to coronary heart disease in at least two ways. Oxidation and free radical formation are two components of atherogenesis. Copper oxidizes low density lipoprotein cholesterol, increasing its atherogenicity (36). Alternatively, copper may be a risk marker for inflammation rather than a risk factor for coronary heart disease directly involved in the pathogenesis of atherosclerosis. Various indicators of inflammation such as the sedimentation rate and acute phase reactants such as C-reactive protein and serum amyloid A protein predict future risk of coronary heart disease (37). Ceruloplasmin, another acute phase reactant, predicted coronary heart disease in at least one study (24). Copper may also fulfill both of these roles.

The role of copper in atherosclerosis is controversial (38, 39). Klevay (40) has proposed that copper deficiency rather than copper excess is a risk factor for ischemic heart disease and has summarized data about the effects of copper deficiency on various risk factors for ischemic heart disease including cholesterol, blood pressure, glucose tolerance, and electrocardiographic abnormalities. If a high serum copper concentration reflects the inflammatory activity of atherosclerosis, however, the findings from this study and other studies may not be incompatible with Klevay’s postulations (41).

Several aspects of this study deserve comment. This study was a mortality study. Deceased participants were identified by matching information with the National Death Index and Social Security files. While there was a high degree of matching, the possibility remains that some records may have been incorrectly matched or failed to match. Second, the diagnosis of coronary heart disease deaths was based on information from death certificates. Although death certificates may not provide completely accurate information about the cause of death of study participants, many cohort studies have successfully used such information to establish study endpoints. Third, because this was a mortality study, incident cases of coronary heart disease could not be identified. If persons with coronary heart disease did not die from coronary heart disease, they would have been misclassified, likely biasing the results to the null hypothesis of no association.

The serum copper concentration may not reflect actual copper status accurately (38). Copper status can be determined in several ways: serum or plasma copper, ceruloplasmin, erythrocyte superoxide dismutase, hair copper, and tissue or organ copper (42). Superoxide dismutase may provide a better measure of longer term copper status.

A number of factors influence circulating concentrations of copper. Women have higher concentrations than do men (43–45). Circulating concentrations of copper are positively associated with age, white blood cell count, cigarette smoking, serum low density lipoprotein cholesterol, systolic blood pressure, body mass index, and oral contraceptive use or estrogen replacement therapy (9, 23, 43–55). In a couple of studies, copper was inversely associated with high density lipoprotein cholesterol (23, 54). Some studies have failed to find significant associations between serum copper concentration and serum cholesterol concentration, cigarette smoking, blood pressure, alcohol use, physical activity level, and concentrations of plasma glucose and insulin (23, 43, 54). Furthermore, pregnancy, infections, inflammation, and several clinical conditions (rheumatoid arthritis, dilated cardiomyopathy, myocardial infarction) can
increase circulating concentrations of copper to as much as 2-3 times normal values, whereas other conditions such as malabsorptive states, jejunoileal bypass, protein-uric renal disease, parenteral alimentation, and corticosteroids can decrease circulating concentrations of copper (56-58). In comparison, after adjustment for age and sex, the serum copper concentration was positively associated with age, cigarette smoking, systolic blood pressure, body mass index, history of diabetes, and white blood cell count and inversely associated with activity level in NHANES II. In contrast to previous studies, serum high density lipoprotein cholesterol concentrations did not vary much with changes in serum copper concentration in NHANES II.

The relation between dietary intake of copper and circulating concentrations of copper is more complex. Generally, short-term studies have not shown changes in circulating concentrations until the dietary intake of copper falls below 0.8 mg/day nor has a high intake changed concentrations substantially (59-62). Long-term studies of the effect of chronic low or high levels of intake on various copper parameters have not been conducted. Thus, short-term changes in copper intake between 0.8 mg/day and 7.5 mg/day are unlikely to result in substantial changes in circulating concentrations. Major dietary sources of copper include crab meat, fruits, vegetables, nuts, seeds, and legumes (63).

Only a single copper measurement was obtained from participants. Circulating concentrations of copper were relatively stable in short- and longer term studies, however (64-66). Strong homeostatic mechanisms keep circulating concentrations of copper within a fairly tight range (57, 67).

Participants not identified as being deceased were assumed to be alive, thus introducing some misclassification into the analysis. Since nothing is known about the magnitude of this misclassification, its effects on the study results are difficult to predict. Results may have been biased toward the null hypothesis of no association if the misclassified participants who may have died from coronary heart disease had higher serum copper concentrations than did participants who were alive.

Because classical risk factors do not fully explain coronary heart disease incidence and mortality, the search for additional risk factors continues. An important avenue of research has been the roles of prooxidants and antioxidants in atherosclerosis. Several prospective studies have now shown serum copper concentration to predict future risk of coronary heart disease events, suggesting a need for further study. Whether copper contributes to atherosclerosis or whether it is simply a marker for other processes needs to be addressed, since the implications for disease prevention and treatment are substantial.

REFERENCES