Effects of dietary copper on human autonomic cardiovascular function

H. C. Lukaski, L. M. Klevay, and D. B. Milne

United States Department of Agriculture, Agriculture Research Service, Human Nutrition Research Center, Grand Forks, North Dakota 58202, USA

Summary. Heart rate and blood pressure responses during supine rest, orthostasis, and sustained handgrip exercise at 30% maximal voluntary contraction were determined in eight healthy women aged 18–36 years who consumed diets varying in copper and ascorbic acid content. Copper retention and plasma copper concentration were not affected by diet. Enzymatic, but not immunoreactive, ceruloplasmin was lower (p < 0.05) after the low copper and high ascorbic acid diet periods. Diet had no effect on resting supine heart rates, orthostatic responses in heart rate and blood pressure, or standing resting blood pressure. Systolic and diastolic blood pressures were increased significantly (p < 0.05) during the handgrip test at the end of the low copper and ascorbic acid supplementation periods. Also, the ratio of enzymatic to immunoreactive ceruloplasmin decreased significantly during these dietary treatments. The mean arterial blood pressure at the end of the handgrip test was negatively (p < 0.0004) correlated with the ceruloplasmin ratios. These findings indicate a functional alteration in human blood pressure regulation during mild copper depletion.

Key words: Blood pressure — Isometric exercise — Ceruloplasmin

Introduction

There is increasing experimental data suggesting that low dietary copper may be involved in the etiology of ischemic heart disease (Klevay 1984; Klevay 1987a). Glucose intolerance (Keil and Nelson 1934), hypercholesterolemia (Klevay 1973), hyperuricemia (Klevay 1980), electrocardiographic abnormalities (Klevay and Viestenz 1981), and hypertension (Klevay 1987b; Medeiros 1987) were observed in animals consuming diets low in copper. Similarly, hypercholesterolemia (Klevay et al. 1984; Reiser et al. 1987), decreased glucose tolerance (Klevay et al. 1986) and disturbances in myocardial function (Klevay et al. 1984; Reiser et al. 1985) were found among men fed diets with marginal copper content.

The recent finding of elevated resting systolic blood pressure in adult copper-depleted animals suggests that copper may play a role in regulating blood pressure. Although regulation of blood pressure is a complex process, a common control mechanism is baroreceptor function (Zanchetti 1979; Abboud 1982). This function can be assessed safely, easily, and noninvasively using a variety of tests of increasing intensity (Ewing 1983).

We studied autonomic cardiovascular function in healthy adults in whom copper nutrure was altered by feeding diets varying in copper and ascorbic acid content. This dietary plan was selected because of the previously reported interaction between copper and ascorbic acid in animal experiments (Van Campen and Gross 1968; Milne and Omaye 1980; Milne et al. 1981) and some human studies (Finley and Cerklewski 1983; Jacob et al. 1987).

Materials and methods

Eight women aged 18–36 years participated in this study after they received a description of the nature, benefits, and risks of
this investigation, and after they were determined to be free of
metabolic, nutritional, cardiovascular, and psychological dis-
orders. This study was approved by the Institutional Review
Boards of the University of North Dakota and the United
States Department of Agriculture; it followed the guidelines of
the Department of Health and Human Services and the Decla-
ration of Helsinki regarding the use of human subjects.

The women lived on a metabolic unit under close supervi-
sion for 135 days. They consumed constant, weighed diets on a
daily menu rotation. The diets, prepared from conven-
tional foods selected to minimize copper content and variabil-
ity of composition, will be published elsewhere (Milne et al.
1988). The basal diet was low in copper (0.65 mg d−1) and ade-
quate in ascorbic acid (90 mg d−1), and was supplemented as
follows: 0.8 mg d−1 copper for 14 days, control; no supple-
ment for 42 days, low copper; 1.5 g acid d−1 ascorbic for 42
days, ascorbate supplement; 2 mg d−1 copper for 37 days, re-
plication.

Copper retention or chemical balance was determined us-
ing three consecutive six-day periods at the end of each diet
period, except the control period. Retention was calculated as
the difference between intake and excretory losses (urine and
feces) exclusive of whole body surface and menstrual losses.
Chemical analyses of diet and excreta are described elsewhere
(Milne et al. 1988).

Also, at the end of each dietary period, fasting venous
blood samples were obtained without stasis to determine bio-
chemical indices of copper status. Plasma copper was deter-
mined by atomic absorption spectroscopy after dilution with
deonized water (Milne et al. 1984). Ceruloplasmin was as-
sayed both enzymatically as p-phenylenediamine oxidase
(Sunderman and Nomoto 1970) and by radial immunodiffu-
sion (Mancini et al. 1965).

Autonomic cardiovascular function was assessed using
standard tests of increasing stressor intensity as described by
Ewing (Ewing 1983). All tests were performed at the end of
each dietary period. Volunteers were tested in the postabsorp-
tive state after a 30-minute rest during which they were supine
on a bed in a quiet room. Variation in resting supine heart
rate was determined over a three-minute period using the mean
square successive differences of R-R intervals (Gunderson and
Neubauer 1977). Orthostatic responses, upon arising form su-
pine position and standing, were measured for heart rate and
blood pressure. Heart rate response was defined as the ratio of
the R-R interval of the 30th to the 15th beat after standing
(Ewing 1978). Blood pressure was measured as each volunteer
was supine and resting quietly and after one minute upon
standing. Standing heart rate and blood pressure responses
were determined before and during five minutes of sustained
handgrip exercise with the dominant arm at 30% maximal vol-
untary contraction using a calibrated handgrip dynamometer
(Harpenden; England) (Lind et al. 1966). Maximal voluntary
contraction was determined at the end of each diet period.
Heart rate was recorded continuously using a multichannel
electrocardiograph (Quinton model 630A; Seattle, WA) and
standard limb leads. Blood pressure was determined by aus-
cultation on the inactive arm with diastolic pressure defined as
the fourth phase Korotkov sound.

The significance of dietary copper and ascorbic acid ef-
fects were determined by repeated measures analysis of var-
iance (Keppel 1973) followed by Scheffé contrasts for differ-
ences between means (Scheffé 1959). The reliability of the car-
diovascular responses to the autonomic function tests was as-
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may also be suitable.
Table 1. Effect of ascorbic acid (AA), copper (Cu) supplements, and marginal copper intake on copper retention as measured by the intake minus the fecal and urinary losses. The values are means ± SEM measured over three 6-day balance periods on the 8 women.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Copper, mg d⁻¹</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet</td>
<td>Feces</td>
<td>Urine</td>
<td>Retention</td>
</tr>
<tr>
<td>None</td>
<td>0.65 ± 0.02</td>
<td>0.65 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>−0.04 ± 0.03</td>
</tr>
<tr>
<td>AA 1.5 g d⁻¹</td>
<td>0.64 ± 0.02</td>
<td>0.63 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>−0.04 ± 0.03</td>
</tr>
<tr>
<td>Cu 2.0 mg d⁻¹</td>
<td>2.65 ± 0.02</td>
<td>2.57 ± 0.05</td>
<td>0.06 ± 0.01</td>
<td>0.02 ± 0.05</td>
</tr>
</tbody>
</table>

Table 2. Effect of dietary copper (Cu) and ascorbic acid (AA) on plasma copper and enzymatic (ENZ) and radial immunodiffusion (RID) ceruloplasmin concentrations and ratios. The values are means ± SEM for the 8 women. See text for discussion of differences among values.

<table>
<thead>
<tr>
<th>Diet Supplement</th>
<th>Plasma Copper µg ml⁻¹</th>
<th>Ceruloplasmin ENZ mg L⁻¹</th>
<th>Ceruloplasmin RID mg L⁻¹</th>
<th>Ratio of ENZ to RID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 0.8 mg d⁻¹</td>
<td>98.3 ± 9.9</td>
<td>478 ± 31</td>
<td>267 ± 19</td>
<td>1.81 ± 0.11</td>
</tr>
<tr>
<td>None</td>
<td>89.4 ± 6.9</td>
<td>385 ± 29</td>
<td>266 ± 13</td>
<td>1.44 ± 0.14</td>
</tr>
<tr>
<td>AA 1.5 g d⁻¹</td>
<td>94.3 ± 7.4</td>
<td>386 ± 24</td>
<td>311 ± 17</td>
<td>1.26 ± 0.08</td>
</tr>
<tr>
<td>Cu 2.0 mg d⁻¹</td>
<td>95.5 ± 6.9</td>
<td>484 ± 35</td>
<td>271 ± 19</td>
<td>1.80 ± 0.09</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of dietary copper and ascorbic acid supplements on cardiovascular responses of 8 women during sustained handgrip exercise to 30% maximal voluntary contraction. Mean arterial pressure = diastolic plus ½ (systolic — diastolic). Bars with an asterisk are significantly (p < 0.05) different than bars without an asterisk within a given time.
and ascorbic acid supplementation periods. Similarly, mean arterial pressure was elevated during each minute of the grip test at the end of the low copper and ascorbic acid supplemented periods.

Because both biochemical indices and physiological function appeared to be influenced by diet, we sought to determine how these variables were related. The relationship between individual ceruloplasmin ratios and the calculated mean arterial pressures at the end of the sustained handgrip test is shown in Fig. 2. With the exception of one volunteer in whom this relationship was weak \( r = -0.50 \), the individual relationships had correlation coefficients ranging from \(-0.90\) to \(-0.99\) \( p < 0.01 \). For the entire study sample, the ceruloplasmin ratio was a significant \( p < 0.0004 \) predictor of mean arterial pressure at the end of the handgrip test. This demonstrates a relationship between a biochemical index of nutritional status and a physiological response when nutrient intake is altered.


discussion

The effects of experimental copper deficiency on the development of the known risk factors for ischemic heart disease were described for animals; altered copper status has been associated with the epidemiology of ischemic heart disease (Klevay 1984, 1987a). While much has been written on the influence of copper on hypercholesterolemia, hyperuricemia, glucose intolerance, and myocardial abnormalities, only recently has blood pressure response to copper nutrure been studied.

Studies using rats made copper deficient at different stages of development show specific effects on resting blood pressure. Copper deficiency induced in animals at or prior to weaning results in hypotension associated with mechanical alterations (Prohaska and Heller 1982; Meireira et al. 1984; Fields et al. 1984; Wu et al. 1984), including depressed coronary vascular resistance and heart rate, reduced rate of left ventricular pressure development, and increased myocardial oxygen consumption (Prohaska and Heller 1982). These functional impairments are related to derangements in connective tissue structure due to disturbances in elastin and collagen cross-linking caused by impaired lysyl oxidase, a copper dependent enzyme, activity (Hill et al. 1967; Siegel et al. 1970). In contrast, dietary copper restriction in adult rats is associated with hypertension (Klevay 1987b; Meireira 1987).

The results of the present study indicate no effect of dietary copper restriction with or without ascorbic acid supplementation on resting heart rate and blood pressure, and no effect on cardiovascular responses during mild autonomic function tests. However, distinct and similar magnitude elevations in blood pressure responses during sustained handgrip exercise of moderate intensity were found with both copper restriction and supplementation with ascorbic acid. This is the first finding of impaired human blood pressure response in association with altered copper nutrure.

One explanation for the observed elevations in blood pressure among adult animals and humans consuming diets low in copper may involve an altered sensitivity of arterial smooth muscle to catecholamines precipitated by the stress of the autonomic tests. In vitro studies showed that copper deficiency potentiates both the response of perfused mesenteric arteries (Cunnane et al. 1979) and the contractibility of aortic strips (Kitano 1980) to the stress hormone norepinephrine.

Another possibility is the stabilizing effect of copper on myocardial irritability. In perfused hearts, low concentrations of prostaglandins \( E_2 \) and \( F_2 \alpha \) caused electrocardiographic rhythm disturbances when copper was absent from the perfusates. These disturbances were eliminated when copper was added in physiological concentrations to the perfusion solution (Swift et al. 1978). Thus,
copper apparently exerts an important regulatory role on the myocardium and vascular smooth muscle.

The neural control of blood pressure requires an integration of afferent input and efferent output in cardiovascular reflexes. Copper was identified in some aspects of this pathway. The locus ceruleus, a copper-containing structure in the brain, is intimately involved in reflex control of blood pressure via noradrenergic neurons (Bannister 1983). According to Owen (Owen 1983), this structure has the "highest concentration of copper in the brain, and possibly the mammalian body".

Ogawa (Ogawa 1978) observed systemic hypertension in rats that received chemically-induced lesions in the locus ceruleus. Furthermore, the hypertension was associated with a disappearance of the locus ceruleus as determined histologically. Interestingly, blood pressure control during isometric handgrip exercise involves sympathetic (noradrenergic) afferents from skeletal muscle neurons (Lind 1983). Whether central or peripheral effects of restricted copper intake are involved in altered cardiovascular function remains to be studied. Under the conditions of this study, the daily copper requirement of these women exceeded 0.65 mg because this intake produced physiological signs of depletion, and reductions in enzymatic ceruloplasmin activity. This daily intake of copper is similar to that found in many contemporary diets in the United States. For example, the geometric mean of daily copper intake in 20 diets was 0.82 mg (Klevay et al. 1979).

Assessment of human copper status is difficult and controversial. Plasma or serum concentration of copper may not be sensitive indices of short-term, marginal copper depletion in humans because of strong homeostatic regulation of this element (Evans 1979), and because of its sensitivity to factors not related to dietary copper intake (Solomons 1979). Another approach is the measurement of the activity of copper-containing enzymes in blood. In the present study, dietary copper had an effect on the enzymatic activity of ceruloplasmin but no influence on ceruloplasmin protein measured by radial immunodiffusion. The ratio of enzymatic ceruloplasmin to immunoreactive ceruloplasmin seems to be a sensitive index of copper status. Discussion on the use of this ratio is presented elsewhere (Milne et al. 1988).

A recent approach to the assessment of human nutritional status is the use of functional tests. The concept of functional testing is quite broad; it includes neuropsychological function, work capacity, immune response, and reproductive competence (Anonymous 1977). Thus, nutritional status is impaired when physiological and biochemical functions are depressed (Solomons and Allen 1985).

In the present study, dietary treatment effects were observed only when physiological control systems were challenged by a moderate stressor. Previously, we showed this approach to be useful by identifying a relationship between diet-induced changes in relative body zinc balance and postexercise changes in plasma zinc concentration corrected for hemocentration (Lukaski et al. 1984). This approach may prove useful in identifying other functional aberrations associated with marginal nutrient depletion.

In contrast to some animal studies (Van Campen and Gross 1968; Milne and O'Nay 1980; Milne et al. 1981), ascorbic acid supplementation exerted no demonstrable effect on whole body copper retention. However, high levels of supplementation (1.5 g d⁻¹ ascorbic acid) depressed the ceruloplasmin ratio by increasing immunoreactive ceruloplasmin concentrations. No change was found in plasma copper concentrations. This contrasts with the data of others (Finley and Cerklewski 1983), who reported reduced serum copper and ceruloplasmin in free-living men consuming 1.8 mg d⁻¹ copper and supplemented with 1.5 g d⁻¹ ascorbic acid. Our data are consistent with those reported by Jacob (Jacob et al. 1987), who observed lower enzymatic ceruloplasmin and no change in plasma copper concentrations in men consuming 2.1 mg copper and 605 mg ascorbic acid daily.

In summary, this study demonstrated altered blood pressure responses during submaximal handgrip exercise and changes in the function of ceruloplasmin, a copper-dependent enzyme, when copper intake was low. Also, these variables were negatively related over the range of copper intake considered to be usual in the western population. These findings indicate that copper may be important in regulation human blood pressure, particularly during acute exposure to stressors. Additional work is necessary to identify the role of copper in the central nervous system and its effect on blood pressure. This information will contribute to our understanding of the biological function of copper, and will be useful in establishing recommendations for copper intake to optimize physiological function and performance.

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References


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